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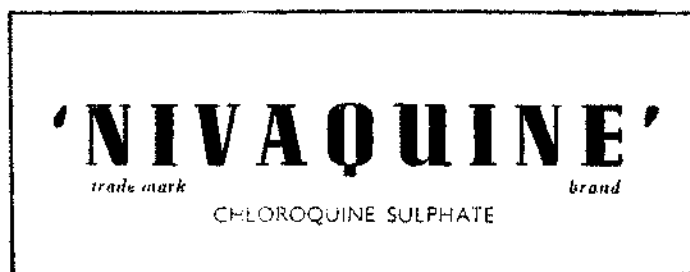
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EDITOR :—LIEUT.-COLONEL JASWANT SINGH, M.B., CH.B., D.P.H.,
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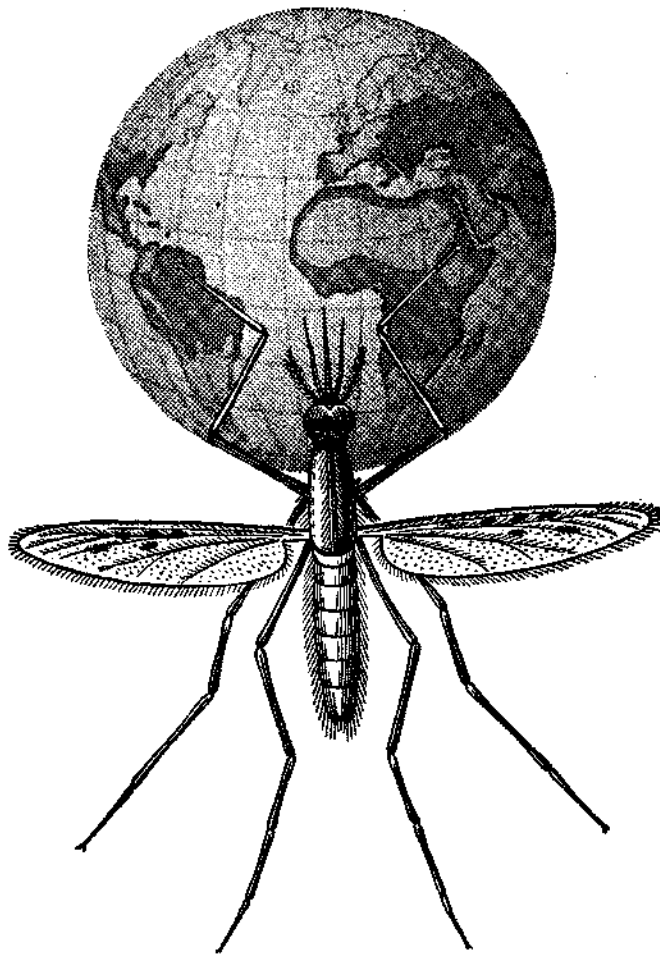
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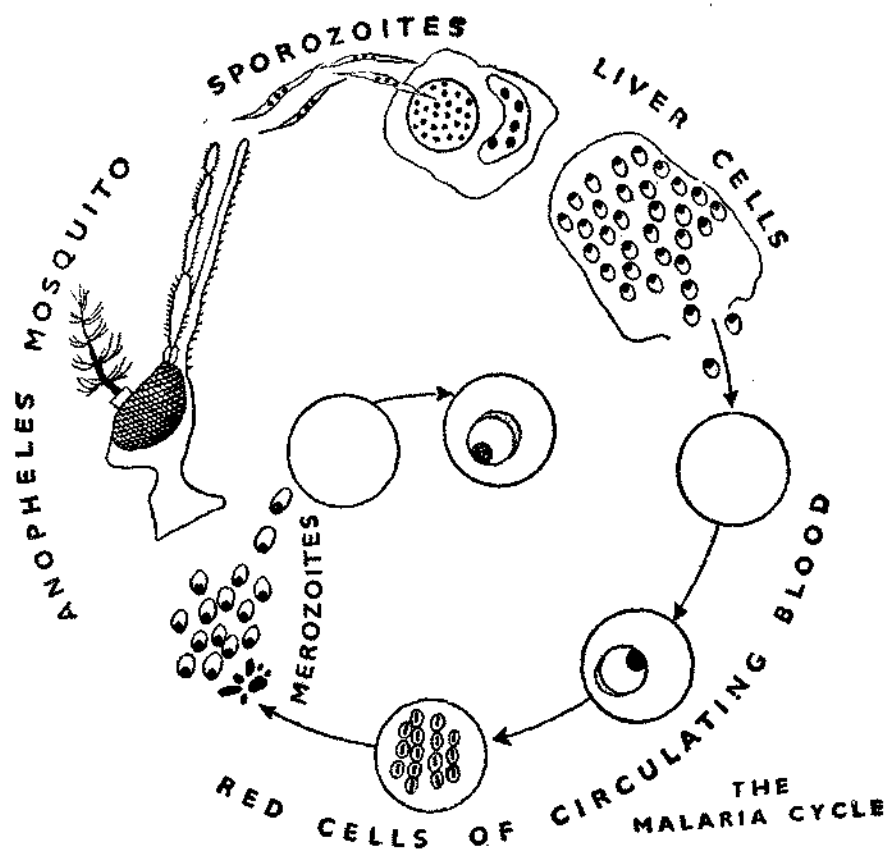
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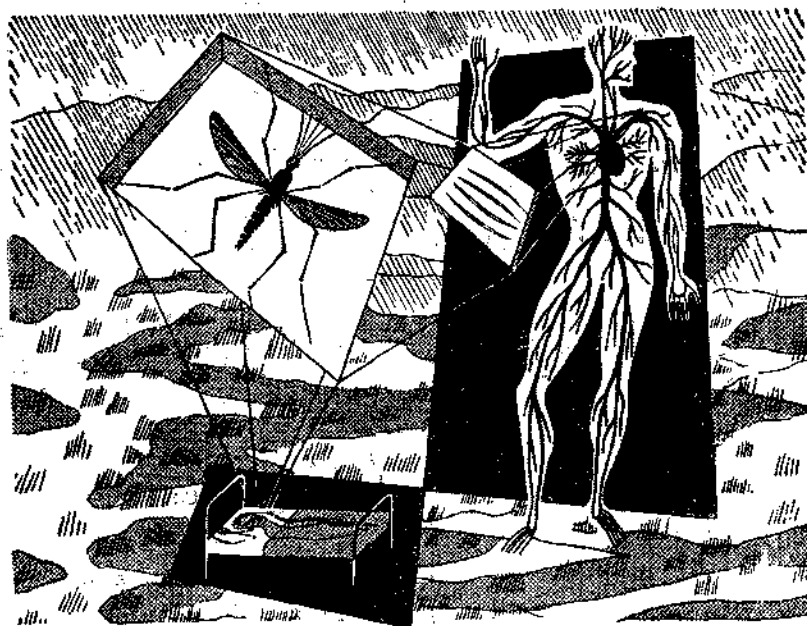
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Ind. J. Mal.
Ind. J. Med. Res.
J. Mal. Inst. Ind.
Jl. R. A. M. C.
J. Trop. Med. Hyg.
Meded. Volk. Ned.-Ind.
Rec. Mq. Surv. Ind.

Rev. App. Ent.
Riv. Malariol.
Trans. Roy. Soc. Trop. Med.
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Tables, charts, etc., should be numbered, and alluded to in the text as 'Table I', etc. and not as 'the following table', etc.

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ICP 167

COURSES IN MALARIOLOGY AT THE MALARIA INSTITUTE OF INDIA, DELHI.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., Ch.B. (Edin.),
D.P.H. (Eng.), D.T.M. & H. (Lond.)
(*Director, Malaria Institute of India*).

[May 15, 1951.]

BESIDES training technical subordinate personnel, such as Laboratory Assistants, Laboratory Attendants and Insect Collectors, three types of courses in malariology are held at the Institute every year :—

- (1) A post-graduate course for medical officers,
- (2) a short course for engineers, and
- (3) a four weeks' course for malaria inspectors.

COURSES IN MALARIOLOGY FOR MEDICAL OFFICERS.

The first course of instruction in malariology for medical officers in India was conducted by Captain (now Colonel Sir Rickard) Christophers, I.M.S. (retired), at Amritsar, Punjab, in 1910, and three further courses were held there by 1912. During the period 1912-1914, four more were held in Delhi. There was then a hiatus during the period of the First World War (1914-1918) and it was not until 1925 that these courses were revived at Kasauli by Major (now Brigadier) J. A. Sinton, V.C., I.M.S. (retired). When the Malaria Survey of India (the previous designation of the Malaria Institute of India) was founded in 1927, these courses formed an important part of its many functions. Up to the year 1940, courses were held annually, three at Saharanpur, U.P., where a bungalow was hired temporarily for the purpose, and the following 11 at the Ross Field Experimental Station for Malaria, Karnal, Punjab. In 1939, the field station was transferred from Karnal to Delhi. To meet the increasing demand for antimalaria personnel during the Second World War, the courses began to be held more frequently. Thus in 1941, two courses were held but in the years 1942-1945, 22 such courses were arranged. In 1946, 1947 and 1948, an additional course was held in autumn each year. Only one course, each of 3 months' duration, was held in 1949, 1950 and 1951.

2 *Courses in Malariology at the Malaria Institute of India, Delhi.*

The course is designed for post-graduate training of medical officers in the basic principles and advanced aspects of malariology. The subjects taught include identification and dissection of mosquitoes and their larvæ, bionomics of mosquitoes, especially the malaria-carrying anophelines, hæmatology, immunology, pathology, protozoology and epidemiology of malaria, modern methods of investigating and measuring the extent of malaria incidence, chemotherapy and chemoprophylaxis and other principles and practice of control measures. The course of instruction has been considerably modified since 1925 as a result of the experiences gained in teaching. The tendency during the last two decades has been to make it more and more practical in character, a considerably larger proportion of time being given to actual field work and to the application of practical control measures.

Up till 1929, the duration of the course was 4 weeks but was increased to 6 weeks from 1930 onwards. During the period 1942-1945, the course was primarily meant for military medical officers and for obvious reasons it was made more intensive but shorter in duration, viz. 4 weeks. In 1946, with the termination of the war, the usual duration of 6 weeks was resumed. As a result of the developments that have taken place in the technique of malaria control, scope of the course was enlarged in 1949 and it now lasts for 12 weeks. It is held during the months of February, March and April and it consists of 78 lectures (each of 1 hour) and about 250 hours of practical work in the laboratory and in the field and tutorial classes. Besides, the programme includes at least three practical demonstrations of field work in hyperendemic areas in the Uttar Pradesh and the Punjab (I) and visits to irrigation and multi-purpose projects where malaria problems exist.

In October each year, a circular announcing dates of the course to be held during the following spring and inviting nominations, is issued by the Ministry of Health to all State Governments and administrations.

No tuition fee is charged but students are expected to make their own arrangement for board, lodging and conveyance. Visits to areas outside Delhi, however, involve an expenditure of about Rs. 200/- per student. This is met either by the nominating authorities or the students themselves.

Each student is provided with a comprehensive set of laboratory and field equipment for use during the course, a list of which is given in Appendix IV. No general textbook is used, the place of this being taken by Malaria Bureau Bulletin Nos. 1, 2, 3, 5, 6, 7, 8, 9, 13 and 14 (Appendix V). New editions of these are issued at frequent intervals, so that the information contained in them is continually being brought up to date. Besides, a number of pamphlets are distributed (Appendix VI).

The lectures are delivered by senior members of the staff. They are assisted in conducting laboratory and field demonstrations by the sub-professional members of the staff.

The lectures are illustrated by series of diagrams and charts prepared in the Institute as also by films. Diagrams and charts along with models of mosquitoes, antimalaria apparatus, pathological specimens, epidemiological graphs, photographs, etc., are placed on permanent exhibit in the museum, where they may be studied by the students at their leisure.

In recent years the material obtained from the study of mammalian and bird malaria has been found most useful for instructional purposes.

A practical, written and *visu voce* examination is held at the end of the course and certificates are issued to those successful in the examination.

Since 1925, one thousand and sixty-six officers including 33 from U.S.A., China, Thailand, Ceylon, Afghanistan, Nepal, Portuguese India, besides a few from Burma and 197 British Officers attached to the Indian Armed Forces have received training. Of these over 550 belonging to the Armed Forces were given intensive training during the World War II.

The number of students in relation to the space available in the laboratories is limited to 26. The students are drawn from the Public Health Departments of States, from the Defence Services, Railways, local bodies, industrial concerns in India and countries of the South-East Asia Region, viz. Afghanistan, Burma, Ceylon, China, Nepal, Portuguese India, Thailand, etc. As will be seen from the syllabus (Appendix I), the course is comprehensive and students who have passed through it are in a position themselves to train subordinate antimalaria personnel in practical field methods.

This Institute has been internationally recognized as a training centre in malariology and during recent years nominees of the South-East Asia Region of the World Health Organization have been trained.

MALARIA COURSE FOR ENGINEERS.

In 1937, an International Conference on Rural Hygiene was held in Java under the auspices of the League of Nations. One of its resolutions dealt with the effect of construction works on rural malaria, and drew attention to the harmful effects of improper siting of houses; indiscriminate aggregation of labourers; uncontrolled jungle clearing, excavations such as borrowpits, brickfields and quarry-pits; obstruction of natural drainage by road, railway and canal embankments with culverts too few and too high; impounding of water without due regard to leakages, seepages and raised water-table levels; and irrigation without the provision of adequate drainage. The conference recommended the institution of courses of instruction in the fundamental principles of antimalarial sanitation for engineers, prior to their assumption of executive responsibilities in the tropics, and the issue of suitable pamphlets for their subsequent guidance.

As a result of these recommendations, a combined course in malariology for doctors and engineers was held in Malaya in 1939, under the auspices of the League of Nations Health Organization. About 25 engineers attended and Lieut.-Colonel (now Major-General Sir Gordon) Covell, C.I.E., I.M.S. (retired), took part in the conduct of the course. A second course was held in the following year. Both proved successful.

As a result of his experience, Sir Gordon Covell took steps to organize similar courses in malariology for engineers, at Delhi. The first two of these were held in September 1940 and were attended by 61 engineers of all branches. A third, attended by 26 engineers, was held in 1941, but from then onwards on account of insistent demands for training military medical officers during

4 *Courses in Malariology at the Malaria Institute of India, Delhi.*

World War II, these courses were held in abeyance. On the termination of hostilities, a course each was held in the years 1946, 1947, 1948, 1949 and 1951. In all, 212 engineers have benefited by these courses.

The object of this course is to give a general idea of the factors concerned in the transmission of malaria and of the principles underlying modern methods of malaria control. It is endeavoured to show how certain engineering details of trivial nature are important to the malariologists and how slight modifications in the construction of major works may make all the difference to the success or failure of a scheme from the antimalaria point of view.

In the national programme of construction of highways, railways, water and sewage works, hydro-electric, irrigation, water utilization and other multi-purpose projects, it is considered highly essential that all engineers before taking up their duties in this country, should receive adequate training at the Malaria Institute and become fully conversant with the fundamental principles of prevention and control of malaria that have such an important bearing on public health. In these fields, engineers have ample opportunities of analysing problems, utilizing their ingenuity in devising solutions and applying all possible methods at the appropriate time with due regard to economy and needs of the malariologist.

A general circular announcing the dates of the course and inviting nominations is issued by the Ministry of Health.

The duration of the course is only 10 days and it is usually held during the period July–October. Additional courses are arranged whenever required.

As will be seen from the syllabus (Appendix II), the course consists of 16 lectures (1 hour each) and 28 hours' practical demonstration in the laboratory and in the field.

No tuition fee is charged but the engineers have to make their own arrangement for board, lodging and conveyance.

As a result of this course, the top-ranking engineers have become malaria-conscious and appreciate the need of preventing malariogenic conditions in the construction of railways, roads and buildings and in the execution of other multi-purpose water utilization projects. The question of starting a suitable course in malariology for engineers in the engineering colleges is under the active consideration of the authorities. The University of Roorkee invited the Malaria Institute to arrange a series of lectures and demonstrations on malariology to the final year civil engineer class students in 1951.

Lectures are based on Malaria Bureau Bulletin Nos. 1, 3, 5, 13 and 14 (Appendix V). A number of pamphlets (Nos. 1 to 8 and 14 to 19 of Appendix VI) are also issued. Unlike the courses for medical officers and inspectors, no examination is held at the end of this course.

MALARIA COURSE FOR INSPECTORS.

The Health Survey and Development Committee in their report recommended that the training of malaria inspectors, supervisors and overseers should be undertaken by the provincial malaria organization but till these organizations are fully equipped to take up this function, the Malaria Institute of India should continue

to perform this important function. One to three courses have been held every year. Training is given in elementary principles of malaria and more stress is laid on practical work rather than on detailed theoretical knowledge of the subject.

The necessity for organizing such courses was felt as far back as 1937, when malaria inspectors were first trained at Karnal. The fourth regular course was held in July and August in 1940. During the World War II, this course was replaced by short courses for Indian Hospital Corps personnel and 383 of them were trained. Regular annual courses have been held since 1946 during July and August each year. In view of the increased number of candidates two courses were held each in 1948 and 1949 and three courses each in 1950 and 1951. So far a total of 327 inspectors have undergone training.

The duration of the course is 4 weeks. As will be seen from the syllabus (Appendix III), the course usually consists of about 30 lectures (1 hour each) and 80 hours of laboratory and field work and demonstrations. Malaria Bureau Bulletins Nos. 1, 2, 3, 6, 9 and 14 (Appendix V) and certain pamphlets (Nos. 1, 2, 3, 6, 9, 10, 20 and 21 of Appendix VI) form the textbooks of the course.

A circular giving the dates and inviting nominations is issued to the health authorities of different states about three months ahead and 26 students are usually selected. As usual no fees are charged but the students have to make their own arrangements for board, lodging and conveyance. Delhi is comparatively an expensive place and the authorities nominating candidates are advised to bear in mind while making nominations that the allowances ordinarily admissible, are not sufficient to meet the extra cost of living.

A comprehensive set of laboratory and field equipment (Appendix IV) for use during the class is issued to each student. Full use is made of the central museum containing models and illustrative charts, etc., and the library maintained at the Institute.

A practical and *viva voce* examination is held on the last day of the course and the students who pass it are issued certificates.

The course has gained popularity and in some of the states it is insisted upon as an essential qualification for appointment as Malaria Inspectors.

APPENDIX I.

*PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—First week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00		LECTURE III. Haematology 2. Formed blood elements—erythrocytes and platelets.	LECTURE V. Protozoology 1. Classification of protozoa.	PRACTICAL 5. Laboratory. Study of human malaria parasite (<i>P. vivax</i> in thin film).	PRACTICAL 6. Laboratory. Study of human malaria parasite (<i>P. malariae</i> in thin film).	PRACTICAL 7. Laboratory. Study of human malaria parasite (<i>P. falciparum</i> in thick and thin films).
11.00 to 12.00	LECTURE I. Introductory lecture. LECTURE II. Haematology 1. Preparation of blood films, staining and staining technique.	PRACTICAL 1. Laboratory. Preparation of blood films.	PRACTICAL 3. Laboratory. Preparation of stains and staining of blood films.	PRACTICAL 5. (Contd.)	PRACTICAL 6. (Contd.)	PRACTICAL 7. (Contd.)
12.00 to 13.00	Taking over of equipments.	PRACTICAL 2. Laboratory. Staining of blood smears (Giemsa, Leishman and J.S.B.).	PRACTICAL 3. (Contd.)	LECTURE VII. Protozoology 3. <i>P. falciparum</i> , <i>P. tenuis</i> and <i>P. ovalis</i> .	LECTURE VIII. Protozoology 4. Cycle in mosquito.	LECTURE IX. Protozoology 5. Factors influencing the development of the parasite in mosquito.
13.00 to 14.30	L	U	N	C	H	
14.30 to 16.00		PRACTICAL 2. (Contd.)	PRACTICAL 4. Laboratory. Enumeration and differential blood counts.	PRACTICAL 5. (Contd.) (<i>P. vivax</i> in thick and thin films.)	PRACTICAL 6. (Contd.) (<i>P. malariae</i> in thick and thin films.)	
16.00 to 17.00		LECTURE IV. Haematology 3. Leucocytes and their development. Hemopoietic system.	LECTURE VI. Protozoology 2. <i>P. vivax</i> and <i>P. malariae</i> .	PRACTICAL 5. (Contd.)	PRACTICAL 6. (Contd.)	HALF HOLIDAY.

* Subject to adjustment every year.

APPENDIX I--contd.

PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS--Second week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	LECTURE X. Protozoology 6. Tissue phase of malaria parasite.	LECTURE XI. Protozoology 7. Tissue phase.	PRACTICAL 10. Laboratory. Study of avian malaria parasites.	PRACTICAL 12. Laboratory. Study of rodent and other plasmodia of lower animals.	PRACTICAL 14. Laboratory. Observation of living parasite and study of tissue forms of malaria parasites.	PRACTICAL 16. Laboratory. Study of human malaria parasites and demonstration of alides (revision).
11.00 to 12.00	PRACTICAL 8. Laboratory. Study of human malaria parasites (mixed infection).	PRACTICAL 9. Laboratory. Study of human malaria parasite.	PRACTICAL 10. (Contd.)	PRACTICAL 12. (Contd.)	PRACTICAL 14. (Contd.)	PRACTICAL 16 (Contd.)
12.00 to 13.00	PRACTICAL 8. (Contd.)	PRACTICAL 9. (Contd.)	LECTURE XIII. Protozoology 9. Evolution of malaria parasite.	LECTURE XIV. Protozoology 10. Maintenance of strains, transmission experiments.	LECTURE XV. Protozoology 11. Enumeration and culture of malaria parasites.	LECTURE XVI. Protozoology 12. Physiology of malaria parasites.
13.00 to 14.30						
14.30 to 16.00	PRACTICAL 8. (Contd.)	PRACTICAL 9. (Contd.)	PRACTICAL 11. Laboratory. Study of simian malaria parasites.	PRACTICAL 13. Laboratory. Study of exflagellating gametocytes, zygotes and ookinetes.	PRACTICAL 15. Laboratory. Enumeration of leucocytes and malaria parasites.	
16.00 to 17.00	PRACTICAL 8. (Contd.)	LECTURE XII. Protozoology 8. Malaria parasites of animals and birds.	PRACTICAL 11. (Contd.)	PRACTICAL 13. (Contd.)	PRACTICAL 15. (Contd.)	HALF HOLIDAY.

APPENDIX I—contd.
PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—Third week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	LECTURE XVII. Entomology 1. Arthropods in the transmission and production of disease. Classification and general characters.	LECTURE XIX. Entomology 3. Morphology of adult mosquitoes, head and mouth parts.	LECTURE XX. Entomology 4. Morphology of adult mosquitoes, thorax and legs.	LECTURE XXI. Entomology 5. Morphology of adult mosquitoes, abdomen and genitalia.	LECTURE XXII. Entomology 6. Morphology of adult mosquitoes, wings.	PRACTICAL 24. Field. Collection of larvae.
11.00 to 12.00	PRACTICAL 17. Laboratory. Demonstration of important arthropods transmitting human disease.	PRACTICAL 19. Laboratory. General morphology of adult mosquitoes.	PRACTICAL 20. Laboratory. Morphology of adult mosquitoes, mouth parts.	PRACTICAL 21. Laboratory. Morphology of adult mosquitoes, genitalia.	PRACTICAL 22. Laboratory. Morphology of adult mosquitoes, wings.	PRACTICAL 24. (Contd.)
12.00 to 13.00	LECTURE XVIII. Entomology 2. Classification and general characters of mosquitoes.	PRACTICAL 19. (Contd.)	PRACTICAL 20. (Contd.)	PRACTICAL 21. (Contd.)	PRACTICAL 22. (Contd.)	PRACTICAL 24. (Contd.)
13.00 to 14.00		L	U	N	O	H
14.00 to 15.30	PRACTICAL 18. Laboratory. General characters of mosquitoes.	PRACTICAL 19. (Contd.)	PRACTICAL 20. (Contd.)	PRACTICAL 21. (Contd.)	PRACTICAL 23. Laboratory. Revision morphology of adult mosquitoes.	
15.30 to 16.30	PRACTICAL 18. (Contd.)	PRACTICAL 19. (Contd.)	PRACTICAL 20. (Contd.)	PRACTICAL 21. (Contd.)	LECTURE XXIII. Entomology 7. Collection of larvae and pupae.	HALF HOLIDAY.

APPENDIX I—contd.

PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—Fourth week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	LECTURE XXIV. Entomology 8. General characters of mosquitoes, larvae and chaetotaxy.	LECTURE XXV. Entomology 9. Internal anatomy and feeding habits—important identification—characters and difference between anophelines and culicines.	PRACTICAL 26. (Contd.)	PRACTICAL 27. (Contd.)	PRACTICAL 28. (Contd.)	PRACTICAL 29. Field. Collection of adult mosquitoes.
11.00 to 12.00	PRACTICAL 25. Laboratory. General characters of larvae.	PRACTICAL 26. Laboratory. General characters of larvae and internal anatomy.	PRACTICAL 26. (Contd.)	PRACTICAL 27. (Contd.)	PRACTICAL 28. (Contd.)	PRACTICAL 29. (Contd.)
12.00 to 13.00	PRACTICAL 25. (Contd.)	PRACTICAL 26. (Contd.)	LECTURE XXVI. Entomology 10. Structure differentiation of anopheline and culicine pupae.	LECTURE XXVII. Entomology 11. Eggs of mosquitoes.	PRACTICAL 28. (Contd.)	PRACTICAL 29. (Contd.)
13.00 to 14.00		L	N	C	H	
14.00 to 15.30	PRACTICAL 25. (Contd.)	PRACTICAL 23. (Contd.)	PRACTICAL 27. Laboratory. General characters of pupae.	PRACTICAL 28. Laboratory. Examination of eggs of anophelines and culicines.	PRACTICAL 28. (Contd.)	
15.30 to 16.30	PRACTICAL 25. (Contd.)	PRACTICAL 26. (Contd.)	PRACTICAL 27. (Contd.)	PRACTICAL 28. (Contd.)	LECTURE XXVIII. Entomology 12. Collection of adult mosquitoes and their distribution.	HALF HOLIDAY.

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10 Courses in Malariology at the Malaria Institute of India, Delhi.

APPENDIX I—contd.

PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—*fifth week*

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	LECTURE XXIX. Entomology 13. General characters and identification of Indian anopheline mosquitoes.	LECTURE XXXI. Entomology 18. Bionomics of mosquitoes—density, range of dispersal, oviposition, etc.	LECTURE XXXIII. Entomology 17. General characters and identification of Indian anopheline larvae.	LECTURE XXXIV. Entomology 18. Dissection of gut and gland of mosquitoes.	LECTURE XXXVI. Entomology 20. Dissection of mosquitoes, examination of ovaries, etc., in relation to age of specimens.	LECTURE XXXVIII. Epidemiology 1. History of malaria.
11.00 to 13.00	PRACTICAL 30. Laboratory. Mounting of adult mosquitoes, general characters and identification.	PRACTICAL 31. Laboratory. Identification of adult mosquitoes.	PRACTICAL 32. Laboratory. Identification of larvae.	PRACTICAL 33. Laboratory. Dissection of mosquitoes, gut and gland.	PRACTICAL 35. Laboratory. Examination of ovaries.	PRACTICAL 37. Laboratory. Cure of laboratory animals and visit to animal house.
13.00 to 14.00						
14.00 to 15.30	PRACTICAL 30. (Contd.)	PRACTICAL 31. (Contd.)	PRACTICAL 32. (Contd.)	LECTURE XXXV. Entomology 19. Method of making permanent mount of gut and gland.	LECTURE XXXVII. Entomology 21. Other mosquito-borne diseases.	
15.30 to 16.30	LECTURE XXX. Entomology 14. Bionomics of mosquitoes (general).	LECTURE XXXII. Entomology 16. Bionomics of mosquito biting and feeding habits—known habits of vector species in India.	PRACTICAL 32. (Contd.)	PRACTICAL 34. Laboratory. Permanent mount of gut and gland.	PRACTICAL 36. Laboratory. Demonstration of vector species.	HALF HOLIDAY.

APPENDIX I—contd.
PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—Sixth week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 12.00	PRACTICAL 38. Field. Recording data for map making.	PRACTICAL 39. Field.	PRACTICAL 40. Field. * Malaria survey of villages	PRACTICAL 41. Field.	PRACTICAL 42. Field.	PRACTICAL 43. Field.
12.00 to 13.00	LECTURE XXXIX. Epidemiology 2. Map making.	LECTURE XL. Epidemiology 4. Basic etiology.	LECTURE XLIII. Epidemiology 6. Endemic malaria.	LECTURE XLV. Epidemiology 8. Epidemic malaria. Irrigation and forecast of malaria epidemics.	LECTURE XLVI. Epidemiology 9. and	PRACTICAL 43. (Contd.)
13.00 to 14.00	L U M N O H					
14.00 to 15.30	PRACTICAL 38. (Contd.) Laboratory. Preparation of maps enlarging, copying, etc.	PRACTICAL 39. (Contd.) Laboratory. Study and recording of data.	PRACTICAL 40. (Contd.) Laboratory. Study and recording of data.	PRACTICAL 41. (Contd.) Laboratory. Study and recording of data.	PRACTICAL 42. (Contd.) Laboratory. Study and recording of data.	HALF HOLIDAY.
15.30 to 16.30	LECTURE XL. Epidemiology 3. Malariometry. Methods of collection of data, etc.	LECTURE XLII. Epidemiology 5. Statistics. Introduction. Role of statistics in malariology.	LECTURE XLIV. Epidemiology 7. Simple statistical methods.	PRACTICAL 41. (Contd.) Laboratory. Study and recording of data.	PRACTICAL 42. (Contd.) Laboratory. Study and recording of data.	

* Students have to prepare essays on such surveys and field demonstrations in hyperendemic areas and irrigation projects.

APPENDIX I—*contd.*PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—*Seventh week.*

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	Visit to irrigation and other projects in the Punjab (I).					
11.00 to 12.00						
12.00 to 13.00						
13.00 to 14.00						
14.00 to 15.30						
15.30 to 16.30						
					LECTURE XLVII. Epidemiology 10. Climate and malaria.	PRACTICAL 43. Laboratory. Demonstration of statistical methods.
					PRACTICAL 42. Laboratory. Study and recording of data collected at Nangal and Indri.	PRACTICAL 43. (<i>Contd.</i>)
					PRACTICAL 42. (<i>Contd.</i>)	LECTURE XLIX. Epidemiology 12. Other mosquito-borne disease— <i>filariasis (i)</i> .
					LUNCH	
					PRACTICAL 42. (<i>Contd.</i>)	
					LECTURE XLVIII. Epidemiology 11. Statistics—Collection and presentation of data.	HALF HOLIDAY.

APPENDIX I--contd.
PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS--Eighth week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	PRACTICAL 44. Laboratory. Identification of microflariae and staining.	PRACTICAL 45. (Contd.)	PRACTICAL 47. Field. Demonstration of oiling in the field.	PRACTICAL 49. Field. Demonstration of application of paris green (dry and dustless methods).	PRACTICAL 51. Laboratory. Demonstration of space and residual sprays.	LECTURE LIX. Control 8. Personal prophylaxis.
11.00 to 12.00	PRACTICAL 44. (Contd.)	PRACTICAL 45. (Contd.)	PRACTICAL 47. (Contd.)	PRACTICAL 49. (Contd.)	PRACTICAL 51. (Contd.)	PRACTICAL 53. Laboratory. Demonstration of antimalaria sprays and equipment.
12.00 to 13.00	LECTURE L. Epidemiology 13. Filariasis (ii).	LECTURE LIH. Control 1. General principles.	LECTURE LIV. Control 3. Chemicals--adulticides--vegetable origin.	LECTURE LVI. Control 5. Control by chemicals--residual sprays.	LECTURE LVIII. Control 7. Drainage (i).	PRACTICAL 53. (Contd.)
13.00 to 14.00						
14.00 to 15.30	PRACTICAL 45. Laboratory. Dissection of infected mosquitoes showing micro-filaria.	PRACTICAL 46. Laboratory. Demonstration of vectors, micro-filariae, filariasis.	PRACTICAL 48. Laboratory. Demonstration of methods of testing efficacy of different oils.	PRACTICAL 50. Laboratory. Demonstration of methods of testing, etc. Space and residual efficacy of sprays.	PRACTICAL 52. Laboratory. Demonstration of methods of preparing formulation of residual sprays.	
15.30 to 16.30	LECTURE LI. Epidemiology 14. Dengue and yellow fever.	LECTURE LIIL. Control 2. Control by chemicals and larvicides.	LECTURE LV. Control 4. Control by chemicals--adulticide--synthetic origin.	LECTURE LVII. Control 6. Formulation of different sprays and discussion on spraying equipment.	PRACTICAL 52. (Contd.)	HALF HOLIDAY.

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14 *Courses in Malariology at the Malaria Institute of India, Delhi.*

APPENDIX I—*contd.*

PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—*Ninth week.*

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	Visit to hyperendemic areas of U.P. Terai to study special malaria problems in foothill areas.			Return to Delhi.	LECTURE LXI. Immunology 1.	LECTURE LXIII. Epidemiology 15. Special malaria problems in foothill area.
11.00 to 13.00					Discussion on the visit to Terai.	Discussion on the visit to Terai.
13.00 to 14.00					L	U
14.00 to 15.30				Examination of material collected in Terai.	Discussion on the visit to Terai.	
15.30 to 16.30				LECTURE LX. Control 9. Malaria control in Bombay State.	LECTURE LXII. Immunology 2.	HALF HOLIDAY.

APPENDIX I--contd.

PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS--Tenth week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 11.00	PRACTICAL 54. Field. Tour of Delhi anti-malaria works.	PRACTICAL 56. Field. Tour of Delhi anti-malaria works.	PRACTICAL 55. Field. Visit to control organization around Delhi (Rural).	PRACTICAL 59. Laboratory. Demonstration of precipitin test.	LECTURE LXVIII. Pathology 2.	LECTURE LXXI. Control 13. Malaria control in coalfields.
11.00 to 12.00	PRACTICAL 54. (Contd.)	PRACTICAL 56. (Contd.)	PRACTICAL 58. (Contd.)	PRACTICAL 59. (Contd.)	LECTURE LXIX. Epidemiology 16. Ceylon epidemic. Forecast of epidemics.	TUTORIAL. Discussion on special problems in different States.
12.00 to 13.00	LECTURE LXIV. Control 10. Drainage (ii).	LECTURE LXV. Control 11. Naturalistic measures.	LECTURE LXVI. Control 12. Control organization.	LECTURE LXVII. Pathology 1.	LECTURE LXX. Pathology 3. Black-water fever.	TUTORIAL. (Contd.)
13.00 to 14.00						
14.00 to 16.30	PRACTICAL 55. Laboratory. Revision. Entomology.	PRACTICAL 57. Laboratory. Revision. Protozoology.	TUTORIAL. Discussion on Delhi antimalarial organization.	PRACTICAL 60. Laboratory. Elementary histopathology.	TUTORIAL. Discussion--pathology and clinical malaria.	HALF HOLIDAY.

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APPENDIX I—*contd.*PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—*Eleventh week.*

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 12.00	Discussion on special problems in different states, industrial areas, tea gardens, railways, etc.	TUTORIAL. Protozoology.	TUTORIAL. Entomology.	TUTORIAL. Other mosquito-borne diseases.	TUTORIAL. Chemotherapy.	PRACTICAL 65. Laboratory. Demonstration screening of anti-malarial drugs.
12.00 to 13.00	LECTURE LXXII. Control 14. Malaria control and organization in the Army, by Director, Army Medical Services.	TUTORIAL. Control measures.	LECTURE LXXIII. Chemotherapy 1. Chemoprophylaxis.	LECTURE LXXIV. Chemotherapy 2. Clinical cure.	LECTURE LXXVI. Chemotherapy 4. Methods of testing anti-malarial drugs.	TUTORIAL. Insecticides.
13.00 to 14.00		L	N	C	H	
14.00 to 15.30	PRACTICAL 61. Laboratory. Conducted tour—library and museum.	TUTORIAL. Statistics.	PRACTICAL 63. Laboratory. Entomology.	PRACTICAL 64. Laboratory. Other mosquito-borne diseases.	TUTORIAL. Epidemiology.	HALF HOLIDAY.
15.30 to 16.30	PRACTICAL 61. (<i>Contd.</i>)	PRACTICAL 62. Laboratory. Demonstration of protozoology.	PRACTICAL 63. (<i>Contd.</i>)	LECTURE LXXV. Chemotherapy 3. Radical cure.	TUTORIAL. (<i>Contd.</i>)	

APPENDIX I—concl'd.

PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—Twelfth week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
10.00 to 12.00	TUTORIAL. Discussion on special problems connected with neighbouring countries.	TUTORIAL. Epidemiology.	Discussion on all aspects of malaria.	Practical examination.	Written and viva voce examination.	Demonstration of films and film strips.
12.00 to 13.00	LECTURE LXXVII. Address by Director-General of Health Services.	LECTURE LXXVIII. Role of World Health Organization in combating malaria by Regional Director, W.H.O., South-East Asia.	Discussion on malaria.	Examination.	Examination.	Demonstration of films.
13.00 to 14.00						
14.00 to 16.30	PRACTICAL 66. Laboratory. Revision. Protozoology.	PRACTICAL 67. Laboratory. Revision. Entomology.	Discussion on malaria.	Handing over equipment.	Examination.	

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APPENDIX II.

*PROGRAMME OF MALARIA COURSE FOR ENGINEERS—First week

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
8.00 to 9.00		LECTURE II. Life history of malaria parasite in human body.	LECTURE V. Antifilarial measures.	LECTURE VII. Malaria problem in Delhi.	PRACTICAL 8. Field. Field demonstration — Timarpur area.	PRACTICAL 10. Field. Field demonstration — Metcalf House Nallah.
9.00 to 10.00		PRACTICAL I. Laboratory. Demonstration of malaria parasites in man and mosquito.	PRACTICAL 3. Laboratory. Demonstration of equipment and larvicides.	PRACTICAL 6. Field. The Delhi Anti-malaria Campaign.	PRACTICAL 8. (Contd.)	PRACTICAL 16. (Contd.)
10.00 to 11.00	LECTURE I. INTRODUCTION.— Object and scope of the course. History of malaria and its social and economic importance.	LECTURE III. Life history of mosquito — anopheline and culicine.	LECTURE VI. Anti-mosquito measures.	LECTURE VIII. Malaria associated with engineering projects.	PRACTICAL 8. (Contd.)	PRACTICAL 10. (Contd.)
11.00 to 12.00	Film.	PRACTICAL 2. Laboratory. Demonstration of eggs, larva, pupae and adult mosquitoes.	PRACTICAL 4. Laboratory. Demonstration of spraying equipment and fumigations of insecticides.	PRACTICAL 7. Laboratory. Demonstration of film strips.	LECTURE X. Malaria associated with irrigation projects.	
12.00 to 13.00		LECTURE IV. Life history of the malaria parasite in mosquito. How malaria is transmitted? Factors which influence transmission.	PRACTICAL 5. Laboratory. Exhibits in museum.	LECTURE IX. Irrigation and malaria in general.	PRACTICAL 9. Laboratory. Film on Malaria.	HALF HOLIDAY.

*Subject to adjustment every year.

APPENDIX II—contd.
PROGRAMME OF MALARIA COURSE FOR ENGINEERS—Second week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
8.00 to 9.00	PRACTICAL 11. Field. Field demonstration —Delhi Electric Power House to Lodi Road area.	PRACTICAL 12. Field. Field demonstration —ridge pits and canal irrigated area.	LECTURE XIII. Tropical aggregation of labour.	LECTURE XVI. Other mosquito-borne diseases.		
9.00 to 11.00	PRACTICAL 11. (Contd.)	PRACTICAL 12. (Contd.)	PRACTICAL 13. Laboratory. Exhibits in the museum.	PRACTICAL 14. Laboratory. Demonstration of vectors and para-sites.		
11.00 to 12.00	PRACTICAL 11. (Contd.)	PRACTICAL 12. (Contd.)	LECTURE XIV. Treatment and suppression of malaria.	PRACTICAL 15. Seminar.		
12.00 to 13.00	LECTURE XI. Malaria control by T.V.A., Panama and other engineering projects including Pontine Marshes.	LECTURE XII. Malaria associated with large stream impoundments. Hydro-electric reservoirs, etc.	LECTURE XV. Prevention of man-made malaria.	PRACTICAL 15. (Contd.)		

APPENDIX III.

*PROGRAMME OF MALARIA COURSE FOR MALARIA INSPECTORS—*First week.*

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
9.00 to 10.00		LECTURE III. Entomology 1. Morphology of mos- quitoes.	LECTURE IV. Entomology 2. Morphology of adult mosquitoes.	PRACTICAL 3. Field. Collection of larvae and pupae.	PRACTICAL 5. Field. Collection of adult mosquitoes.	LECTURE VIII. Entomology 6. Dissection of mos- quitoes.
10.00 to 11.00	LECTURE I. Introductory.	PRACTICAL I. Laboratory. Demonstration of microscopes and other equipment.	PRACTICAL 2. (<i>Contd.</i>)	PRACTICAL 3. (<i>Contd.</i>)	PRACTICAL 5. (<i>Contd.</i>)	PRACTICAL 7. Laboratory. Identification of adult mosquitoes.
11.00 to 12.00	LECTURE II. Care of microscope and other equip- ment.	PRACTICAL 2. Laboratory. Morphology of adult mosquitoes.	PRACTICAL 2. (<i>Contd.</i>)	PRACTICAL 3. (<i>Contd.</i>)	PRACTICAL 5. (<i>Contd.</i>)	PRACTICAL 7. (<i>Contd.</i>)
12.00 to 14.00						
14.00 to 15.00		PRACTICAL 2. (<i>Contd.</i>)	PRACTICAL 2. (<i>Contd.</i>)	PRACTICAL 4. Laboratory. Examination of larvae and pupae. Preparation of permanent slides of larvae.	LECTURE VII. Entomology 5. Identification of adult mosquitoes.	
15.00 to 16.00	Taking over of equipment.	PRACTICAL 2. (<i>Contd.</i>)	PRACTICAL 2. (<i>Contd.</i>)	PRACTICAL 4. (<i>Contd.</i>)	PRACTICAL 6. Laboratory. Pinning, mounting and storing of adult mosquitoes.	HALF HOLIDAY.
16.00 to 17.00		PRACTICAL 2. (<i>Contd.</i>)	LECTURE V. Entomology 3. Collection of larvae and pupae.	LECTURE VI. Entomology 4. Collection of adult mosquitoes, bio- monics, etc.	PRACTICAL 6. (<i>Contd.</i>)	

* Subject to adjustment every year.

APPENDIX III—contd.

PROGRAMME OF MALARIA COURSE FOR MALARIA INSPECTORS—Second week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
9.00 to 10.00	PRACTICAL 7. (Contd.)	LECTURE X. Malaria control 1. General principles.	PRACTICAL 10. Field. Larvæ control.	PRACTICAL 12. Field. Adult control.	PRACTICAL 13. Field. Larvæ control.	LECTURE XVI. Epidemiology 2. Epidemic and endemic malaria.
10.00 to 11.00	PRACTICAL 7. (Contd.)	PRACTICAL 9. Laboratory. Revision. Entomology.	PRACTICAL 10. (Contd.)	PRACTICAL 12. (Contd.)	PRACTICAL 13. (Contd.)	PRACTICAL 14. (Contd.)
11.00 to 12.00	PRACTICAL 7. (Contd.)	PRACTICAL 9. (Contd.)	PRACTICAL 10. (Contd.)	PRACTICAL 12. (Contd.)	PRACTICAL 13. (Contd.)	PRACTICAL 14. (Contd.)
12.00 to 14.00						
14.00 to 15.00	LECTURE IX. Epidemiology 1. Factors influencing transmission of malaria.	LECTURE XI. Malaria control 2. Antilarval measures. Oil and chemicals.	LECTURE XII. Malaria control 3. Adult control.	LECTURE XIII. Malaria control 4. Drainage.	LECTURE XV. Malaria control 6. Personal prophylaxis.	
15.00 to 16.00	PRACTICAL 8. Laboratory. Dissection of mosquitoes.	PRACTICAL 9. (Contd.)	PRACTICAL 11. Laboratory. Demonstration of equipment, stores and maintenance.	Film 20 minutes.	PRACTICAL 14. Laboratory. Stores and equipment for adult control and their maintenance.	HALF HOLIDAY.
16.00 to 17.00	PRACTICAL 8. (Contd.)	PRACTICAL 9. (Contd.)	PRACTICAL 11. (Contd.)	LECTURE XIV. Malaria control 5. Naturalistic methods.	PRACTICAL 14. (Contd.)	

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APPENDIX III—contd.
PROGRAMME OF MALARIA COURSE FOR MALARIA INSPECTORS—Third week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
9.00 to 10.00	PRACTICAL 15. Field. Larva control.	LECTURE XVIII. Other mosquito-borne diseases 1.	LECTURE XIX. Other mosquito-borne diseases 2.	PRACTICAL 19. Laboratory. Revision. Parasitology.	LECTURE XXII. Parasitology 1. Malaria parasites in man.	PRACTICAL 21. Laboratory. J.S.B. stain preparation.
10.00 to 11.00	PRACTICAL 15. (Contd.)	PRACTICAL 17. Laboratory. Other mosquito-borne diseases.	PRACTICAL 18. Laboratory. Revision. Entomology.	PRACTICAL 19. (Contd.)	PRACTICAL 20. Laboratory. Preparation of blood films and staining.	PRACTICAL 21. (Contd.)
11.00 to 12.00	PRACTICAL 15. (Contd.)	PRACTICAL 17. (Contd.)	PRACTICAL 18. (Contd.)	PRACTICAL 19. (Contd.)	PRACTICAL 20. (Contd.)	PRACTICAL 21. (Contd.)
12.00 to 14.00						
14.00 to 15.00	LECTURE XVII. Malaria control 7. Introduction to animal life.	PRACTICAL 17. (Contd.)	PRACTICAL 18. (Contd.)	LECTURE XX. Epidemiology 3. Map reading.	PRACTICAL 20. (Contd.)	
15.00 to 16.00	PRACTICAL 16. Laboratory. Inspection and maintenance of stores and supplies for transport.	PRACTICAL 17. (Contd.)	PRACTICAL 18. (Contd.)	PRACTICAL 19. (Contd.)	LECTURE XXIII. Malaria control 8. Organization—supervision of control measures, etc.	HALF HOLIDAY.
16.00 to 17.00	PRACTICAL 16. (Contd.)	PRACTICAL 17. (Contd.)	PRACTICAL 18. (Contd.)	LECTURE XXI. Stores, record, equipment and maintenance equipment.	LECTURE XXIV. Parasitology 2. Stains and staining.	

APPENDIX III--concl'd.

PROGRAMME OF MALARIA COURSE FOR MALARIA INSPECTORS--Fourth week.

Time (hours).	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
9.00 to 10.00	LECTURE XXV. Parasitology 3. Malaria parasites in man.	LECTURE XXVII. Parasitology 4. Malaria parasites in mosquitoes.	PRACTICAL 22. Field. Malaria survey.	LECTURE XXXI. Malaria control 10. Preparation for malaria season. Action in epidemic.	PRACTICAL EXAMINATION. Entomology.	VIVA-VOCE EXAMINATION.
10.00 to 11.00	PRACTICAL 21. Laboratory. Staining and identification of mosquitoes.	PRACTICAL 21. (Contd.)	PRACTICAL 22. (Contd.)	PRACTICAL 24. Laboratory. Dissection of infected mosquitoes and examination of gut and glands.	EXAMINATION. (Contd.)	EXAMINATION. (Contd.)
11.00 to 12.00	PRACTICAL 21. (Contd.)	PRACTICAL 21. (Contd.)	PRACTICAL 22. (Contd.)	PRACTICAL 24. (Contd.)	EXAMINATION. (Contd.)	EXAMINATION. (Contd.)
12.00 to 14.00						
14.00 to 15.00	PRACTICAL 21. (Contd.)	LECTURE XXVIII. Symptomatology of malaria.	PRACTICAL 23. Laboratory. Revision. Parasitology.	Film for 20 minutes. (Contd.)	PRACTICAL EXAMINATION. Parasitology.	
15.00 to 16.00	PRACTICAL 21. (Contd.)	PRACTICAL 21. (Contd.)	PRACTICAL 23. (Contd.)	PRACTICAL 25. Laboratory. Conducted tour museum.	PRACTICAL EXAMINATION. Parasitology. (Contd.)	HALF HOLIDAY.
16.00 to 17.00	LECTURE XXVI. Malaria control 9. Antimalarial drugs.	LECTURE XXIX. Malaria survey.	LECTURE XXX. Other mosquito-borne diseases 3.	PRACTICAL 25. (Contd.)	Handing over charge of equipment.	

Jaswant Singh.

APPENDIX IV.**MALARIA COURSE LABORATORY EQUIPMENT.**

Name.....

Class Number.....

1.	Bag, hand, cloth	...	...	...	...	1
2.	Blocks, glass, with excavation and cover	...	...	...	...	2
3.	Bottle, for cedar oil	...	...	...	...	1
4.	Bottle, hollow-stoppered (breeding jar), with gauze and rubber ring	...	...	...	...	1
5.	Bowls, china, with glass covers	...	...	...	...	6
6.	Box of matches	...	...	...	...	1
7.	Bristol board, piece	...	...	...	...	1
8.	Cage, pocket, complete	...	...	...	...	1
9.	Canada balsam	...	...	...	...	1 glass tube.
10.	Cardboard, pieces	...	...	...	...	4
11.	Cork sheet	...	...	...	...	1
12.	Cotton-wool, packet	...	...	...	...	1
13.	Cover-glasses	...	...	...	...	Supply.
14.	Cup, enamelled	...	...	...	...	1
15.	Duster	...	...	...	...	1
16.	Electric torch	...	...	...	...	1
17.	Filter papers, packet	...	...	...	...	1
18.	Forceps, entomological	...	...	...	...	1 pair.
19.	Forceps, straight, fine-pointed	...	...	...	...	1 pair.
20.	Gater's fluid	...	...	...	...	1 glass tube.
21.	Globe lantern, with gauze and rubber ring	...	...	...	...	1
22.	Handkerchief, cotton	...	...	...	...	1
23.	Handkerchief, silk	...	...	...	...	1
24.	Holder, wooden, for 4 specimen tubes	...	...	...	...	1
25.	Hone (one per two students)	...	...	...	...	1
26.	India-rubber	...	...	...	...	1
27.	Jar, stoppered, with gauze and rubber ring, 6 inches × 2½ inches	...	...	...	...	1
28.	Jar, stoppered, 4 inches × 2 inches	...	...	...	...	4
29.	Knife, utility, with blade	...	...	...	...	1
30.	Magnifier, pocket	...	...	...	...	1
31.	Measuring cylinder, glass, 10 c.c.	...	...	...	...	1
32.	Microscope No. with 3 objectives, 2 oculars, condenser and mechanical stage	...	...	...	...	1
33.	Microscope, dissecting, with one lens; two/three lenses	...	...	...	...	1
34.	Needle, Hagedorn No. 7, in specimen tube with spirit	...	...	...	...	1
35.	Needles, mounted, No. 6, for dissecting	...	...	...	...	3
36.	Needle, mounted, coarse	...	...	...	...	1
37.	Net, hand, for larvæ	...	...	...	...	1

APPENDIX IV—*concl'd.*

38. Pencil, grease ...	...	...	...	...	1
39. Petri dish, with cover ...	...	...	...	...	1
40. Pins, entomological, double-pointed, nickel, No. 3 ...	...	...	...	...	Supply.
41. Pins, entomological, white, No. 16 ...	...	...	...	...	Supply.
42. Pins, ordinary, white ...	...	...	...	...	Supply.
43. Pipettes, with teats ...	...	...	...	...	2
44. * Pipette with throttle ...	...	...	...	...	1
45. Pipette for larvæ, with teat ...	...	...	...	...	1
46. Pith, piece ...	...	...	...	...	1
47. Plasticine, piece ...	...	...	...	...	1
48. Rack, staining, glass ...	...	...	...	...	1
49. Racks, wooden, for specimen tubes ...	...	...	...	...	2
50. * Ring, iron, with capsule ...	...	...	...	...	1
51. Ring, wooden, coated with paraffin ...	...	...	...	...	1
52. Rule, steel, 1 foot ...	...	...	...	...	1
53. Scissors, sharp-pointed, 5 inches ...	...	...	...	...	1 pair.
54. Slide-box ...	...	...	...	...	1
55. Slides, glass, 3 inches $\times$ 1 inch ...	...	...	...	...	1 doz. (Supply).
56. Specimen tubes, 3 inches $\times$ 1 inch, with corks ...	...	...	...	...	1 doz. (Supply).
57. Specimen tubes, 2 inches $\times$ $\frac{1}{2}$ inch, with corks ...	...	...	...	...	6
58. Spirit lamp ...	...	...	...	...	1
59. Spoon, tin ...	...	...	...	...	1
60. Stand, wooden, with 12 drop-bottles, and 2 N.M. stoppered 4 oz. bottles ...	...	...	...	...	1
61. Store-box, entomological ...	...	...	...	...	1
62. Tape measure, with safety pin ...	...	...	...	...	1
63. Test tubes ...	...	...	...	...	1 doz. (Supply).
64. * Tray, cardboard, for 20 slides ...	...	...	...	...	1
65. Tray, photographic, enamelled, 5 inches $\times$ 4 inches ...	...	...	...	...	1
66. Tray, photographic, enamelled, 10 inches $\times$ 8 inches ...	...	...	...	...	1
67. Tube, sucking ...	...	...	...	...	1
68. Wash-bottle, with rubber cork and glass tubing ...	...	...	...	...	1
69. Watch-glasses ...	...	...	...	...	4

* Not issued to inspectors.

APPENDIX V.

MALARIA BUREAU BULLETINS.

- No. 1. Lectures on malaria. By G. Covell. 4th edition. 1949. *As. 5.*
- No. 2. Synoptic table for the identification of the anopheline mosquitoes of India. By S. R. Christophers, J. A. Sinton, G. Covell and P. J. Barrand. 4th edition, revised, enlarged and illustrated by I. M. Puri. Reprint 1950. *As. 5.*
- No. 3. Antimosquito measures with special reference to India. By G. Covell. 7th edition. 1946. *As. 8.*
- No. 4. Table for the identification of Indian fresh-water fishes, with description of certain families and observations on the relative utility of the probable larvivorous fishes of India. 2nd edition, revised and enlarged by S. L. Hora and D. D. Mukerjee. 1938. *As. 7.*
- No. 5. Instructions for collecting and forwarding mosquitoes. By J. A. Sinton. 4th edition, revised by I. M. Puri. 1949. *As. 3.*
- No. 6. How to do a malaria survey? By S. R. Christophers, J. A. Sinton and G. Covell. 5th edition. Revised by G. Covell. 1945. *Rs. 1/12.*
- No. 7. Synoptic table for the identification of the full-grown larvae of the Indian anopheline mosquitoes. By I. M. Puri. 6th edition. 1949. *Rs. 1.*
- No. 8. The distribution of anopheline mosquitoes in India. By G. Covell. 3rd edition, revised and brought up to date by I. M. Puri. 1948. *As. 8.*
- No. 9. A practical entomological course for students of malariology. By P. J. Barrand. 5th edition, revised by I. M. Puri. 1951. *Rs. 3/6.*
- *No. 10. Man-made malaria in India. By J. A. Sinton and Raja Ram. 3rd edition, revised by G. Covell. 1949. *As. 4.*
- No. 11. Handbook of common water and marsh plants of India and Burma. By C. C. Calder and K. Biswas. 1937. *Rs. 2/12.*
- *No. 12. Prevention of malaria incidental to engineering construction. By H. W. Mulligan and M. K. Afridi. 1941. *As. 7.*
- No. 13. What malaria costs India? By J. A. Sinton. 1951. *As. 14.*
- No. 14. Malaria incidental to engineering construction. By Jaswant Singh and I. M. Puri. 1951. *As. 12.*

Note. These bulletins are obtainable from the Manager of Publications, Government of India Central Publication Branch, Civil Lines, Delhi.

* Replaced by No. 14.

APPENDIX VI.

PAMPHLETS AND LEAFLETS.

1. Note on D.D.T. and other synthetic insecticides.
2. Principal mosquito vectors of India and neighbouring countries.
3. Classification of antimalaria measures.
4. Malaria map of India.
5. Investigation of malaria.
6. Basic aetiology of malaria.
7. Stages in the course of malaria epidemic.
8. Malaria problems in Delhi.
9. Malaria parasites of man.
10. Rapid staining of malaria by a water-soluble stain (J.S.B. stain).
11. Parasitology.
12. A simple methylene blue-eosin substitute for Leishman and Giemsa stains.
13. Exo-erythrocytic cycle in *P. gallinaceum* in the chick.
14. Prevention of malaria on field service.
15. What malaria costs India ?
16. Report of the committee convened to consider the measures to be taken to prevent the creation of conditions favourable to the spread of malaria during the construction of roads and railways.
17. Canal irrigation and water-logging in India.
18. Malaria and irrigation.
19. Drainage and malaria.
20. Forms for examination of spleens.
21. Instructions for the use of microscopes.
22. Standard abdominal chart.
23. The tissue phase of *P. gallinaceum* in the chick.
24. Staining and restaining of oöcysts and sporozoites from infected mosquitoes.

STUDIES ON THE BIONOMICS OF *ANOPHELES* *AQUASALIS* CURRY, 1932 (concl.).

Part III.*

BY

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[April 12, 1951.]

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* Parts I and II of this article have appeared in the September and the December 1951 issues respectively.

SECTION VIII.

ADULT FEEDING HABITS.

(I) PRECIPITIN RESULTS.

BEFORE proceeding to Trinidad, the author was a signatory of a memorandum to Professor Buxton by several entomologists working during 1947 at the London School of Hygiene and Tropical Medicine on the need for workers under the auspices of the Colonial Office being furnished with the same facilities for identifying bloods in insects as had long been available to Indian workers through the Malaria Institute of India. This proposal fructified in the appointment of Mr. B. Weitz of the Lister Institute of Preventive Medicine for this purpose, and all the results to be now set forth derive entirely from his work on stomach smears made in the field locally.

It was well that from the start the author most carefully trained his field staff on the essentialness of using a fresh spreader (tooth pick or *Acronia* palm spine) for every specimen, or the phenomenon of multiple feeding to be described later would otherwise have been suspect as an artefact. The discovery of the daytime resting places of *aquasalis* forthwith led to the collection of stomach content smears for subjection to precipitin tests. Originally, the Lister Institute tested for man, ox, goat, horse, dog and fowl. After some months pig was added to the anti-sera used, and subsequently the marsupial *Didelphis marsupialis insularis* (*vern.* 'manicou'), in the hope of elucidating the origin of the considerable percentage of 'unknowns' that appeared, originating from smears which were apparently in as early a stage of digestion as the 90 per cent of those submitted which gave a positive reaction. So few dog and fowl reactions were obtained that the use of these anti-sera was abandoned from the end of 1948. Testing for dog alone was resumed in June 1950, and the results indicate that dog is quite an important pabulum. *Didelphis* was abandoned when the anti-serum prepared from one specimen was finished, as it had only yielded two positive reactions.

In the first year's (1948) work, 960 smears were collected of which 867 (90.3 per cent) gave positive reactions. Table XXXVIII sets out the results. With the amount of multiple feeding during the last meal that was revealed, the best way to express the percentage of individual animal species bitten was to count each multiple feed as 2, 3 or more bites, and to calculate the percentages from the Σ bites, and not the actual number of specimens.

During the non-inclusion of anti-pig in the testing of the first sending of 362 smears, the percentage of unknowns was 10.6, in the second sending of 598 smears, the unknowns amounted to 8.2 per cent. In this sending pig + was 2.9 per cent. It is therefore concluded that the unknowns of the first sending were artificially raised by the omission of pig from these tests. Likewise, not testing for dog in the first five months of 1950 artificially raised the unknowns. The second six months of 1950 showed that in some areas there is much dog biting.

This first year's general results require consideration in the light of the general conditions of the areas in which they were collected.

Success is a closely built-up hill-side suburb of Port of Spain, containing a population of city workers. Almost all the anopheline production affecting it is from a narrow marshy strip along the railway between the Laventille mangrove

and the lowest houses, though the mangrove fringe [Section VI (i)] is not as unproductive as was first thought. There are naturally but few domestic animals. Here an anthropophilic index (A.I.) of 30 per cent was found. Now this suburb has been under regular residual D.D.T. application for several years. The sex ratio of the captures (Table XLIX) shows an excessive proportion of males, indicating the amount of female destruction within houses that occurs, yet there is still this high outdoors anthropophilic index. This can only be indicative of the amount of human biting that goes on out of doors, including house verandahs, in the early evening, and indicates that *aquasalis* as a species is not completely amenable to residual insecticide control. Giglioli (1949) is also of this opinion.

Barataria is a well-planned suburb of urban workers out on the Caroni Plain, with more garden space between the houses than Success and much good resting scrub around it. It has also much more prolific breeding grounds south of it around the cross-cultivation beds than has Success. There are rather more domestic animals, but again the anthropophilic index found is quite high.

El Socorro is also largely a suburban area, but the houses are largely a ribbon development along two roads. There is not much local breeding, the *aquasalis* population infiltrating the locality from the already described cross and vegetable areas, and the saline reed beds lying south and south-west of them. Anthropophilic index is low. The animal population of New la Paille, south of it, probably acts as a diversion barrier.

New la Paille, Glendon, Old la Paille and Streatham Lodge are Indian agricultural villages, the last two in the irrigated rice area where D2 trap figures (Table XXXIII) show that *aquasalis* density is greatly reduced. Anthropophilic index is low in all of them owing to the amount of animal deviation that occurs (*vide infra*).

Bamboo Village, in 1948, was a just-commencing resettlement of Indians displaced from their homes in the marshy lands south of the Caroni owing to the inclusion of these in an irrigation scheme. In 1948, the inhabitants were living in rather temporary huts, and had not yet brought up all their domestic animals. The anthropophilic index was then, therefore, unusually high for an Indian-inhabited rural area. Conditions in this village in later years are described subsequently.

The Government Farm at Yalsayn provided too few specimens to dogmatize. There are numerous cattle and other animals but a certain number of watchmen are on night duty. It will be seen that the only human bite was a multiple one.

Bordenal Savanah has scattered houses of Indian cultivators, who are almost completely protected by their domestic animals. The solitary (multiple) human meal was almost certainly taken near their houses, as dogs do not go far afield. Conditions in the experimental quadrat on this savanah are shown in Table XLVII.

Curepe Savanah and quadrats.—This savanah contains the buffalo and mule stables of the Curepe Division of the Caroni Sugar Estates, housing over 100 bovines and about half as many mules. There is a night watchman on these stables, who protests that he is never bitten, and the anthropophilic index of 1.5 per cent for these two subdivisions of the savanah is doubtless derived from specimens which have fed in Spring Village, a ribbon development bounding the savanah on

the east along the Southern Main Road. The savannah generally is tabulated separately from the rather distant experimental catching quadrats.* To show the result of furnishing a human blood supply in a normally quite innocuous area the following data are of value. Each October the Hindu Ramhla festival is held here. The festival attracts, during the week it lasts, an afternoon audience of about 2,000 persons, who disperse towards dusk. Though they are thus exposed to only the first moments of *aquasalis* activity, collections in the same scrub as during the rest of the year show an anthropophilic index temporarily increased from 1 to 15, an interesting example of 'tropical aggregation of labour' and its dangers, even under circumstances of slight and waking, exposure to a crepuscular anopheline.†

The *Nelson Bamboo* data were obtained during the few weeks that the dry cows described in Section VI were corralled here. There were two watchmen on the locality, but whence came the goat and horse bloods it is difficult to say, unless from animals passing along the 'Churchill-Roosevelt' highway that cuts straight through the Bamboo Plantation from west to east. After the very varying anthropophilic index found the most interesting phenomenon revealed by these first year's figures is the numbers of specimens which, during one meal, had fed on more than one species of animal, culminating in the single specimen from New la Paille which had fed on man, goat, horse and dog! Nothing like this has been revealed by precipitin tests on any other species of *Anopheles*. Rice and Barber (1937) used 5 anti-sera in their work in Greek Macedonia. They do not refer to natural multiple feeding at all, though they induced it experimentally. With the exception of the few specimens tested by Rice on material from a locality in the Jeypore Hills (Senior White, 1938) in which 3 anti-sera were used, all the work in India at the Malaria Institute has been carried out with human and bovine anti-sera only. In recording (Senior White, 1947) the results of 8,456 positive reactions obtained from 16 species in India, using these 2 anti-sera only, though double reactions were found, they were so few as not to merit any special mention in that paper. Thus the very high amount of multiple feeding recorded in *aquasalis* is a completely new phenomenon. Possibly it would not have shown up under a less delicate technique than that of Mr. Weitz and it may not, therefore, be a phenomenon peculiar to *aquasalis* only. The results in Table XXXVIII may be further analysed to show the proportion of pure and mixed feeds on the four principal domestic animals and man:

		Man.	Ox.	Goat.	Horse.	Pig.
Pure feeds	...	23	624	40	56	14
Mixed feeds	..	27	90	54	39	5
Mixed, per cent	...	54	13	57	43	26

It is at once apparent that the ox affords a much higher percentage of 'full' single meals than any other species. As will be shown later the goat is very

* Situated in the south-west angle of the savannah close to the only nearby breeding water.

† The phenomenon was not marked in 1950. *A. aquasalis* was very sparse in September-October 1950 in this locality. Two catches before and one ten days after the festival gave a Σ of 23 feeds, with an anthropophilic index of 4.3. One catch during the festival, with a Σ of 15 bites, showed an anthropophilic index of 6.7. On such small numbers the difference is insignificant.

intolerant of attack, and presumably man is also. Equines are in an intermediate position. The figures for pig are too small for discussion. The animal is not commonly kept in the Caroni Valley.

So widely variant were the anthropophilic indices in the 16 localities studied in this first year's work that it was felt that thenceforward selected areas should be taken for continued study, *pari passu* with censuses of their human and animal populations, so far as could be ascertained by periodical house-to-house questioning. Dogs and fowls are too motile to be included in such censuses.

One exception to this policy of no further random sampling was made: an all-island coastal areas circuit (a) to discover whether outdoor resting of *aquasalis* was locally a universal phenomenon, and (b) to obtain simultaneously random samples of precipitin reactions from all the districts visited. In Table LII are set out the combined results of two all-coastal catching circuits in the dry season and the rains respectively. Censuses were taken on both circuits, and averaged, but densities for the two circuits are separately recorded to indicate seasonal fluctuations. The biting results are combined and averaged under three human population subdivisions.

Predominantly Indian villages have a man : ox + horse ratio of 7.0 : 1, against more than twice this figure for predominantly negro villages. The mixed population areas show intermediate values, nearer that of the Indian than the negro. The man : all animals ratio does not greatly differ between the three subdivisions, largely due to the numbers of pigs kept by negroes, but, as will be shown in Section VIII (ii), the pig is not a good diverter of *aquasalis* from man, though the pig populations of the negro villages get thrice as many bites as do the pigs in Indian areas. Again the pig biting in the mixed areas is intermediate. The all-important figure in this table is the anthropophilic index column, and the differences are quite insignificant as between Indian and negro villages. A much closer comparative experiment is required, based on regular collections in one or two purely negro villages to compare with the Indian villages on the Caroni Plain. Unfortunately, such villages are very hard to find. Nearly all such are not easily accessible for regular catches by men based on Port of Spain. Comparisons of the suburban Laventille area with any villages would be definitely misleading. Generally, however, the results tend to suggest that the oxen-less negro is more exposed to *aquasalis* attack than the cattle-keeping Indian, for all that the former appears to live chiefly in areas of lower *aquasalis* production. Compared with the Caroni Plain, of which the first place in the table, Caccandee, might almost be considered a part, the catching densities recorded are very low except at Chatham and Icacos, on the south-west peninsula of the island, in the dry season, while in the rains only Icacos shows a high density. The census figures show man : ox + horse ratios varying from 4 : 1 to 48 : 1, and study of the table as divided according to the predominant ethnological element shows that generally the ratio is highest (i.e. cattle are least numerous) where the negro element in the population predominates. The West Indian negro in the main originated from the coastal areas around the Bight of Benin, and seems to have lacked the cattle-keeping proclivities of the tribes of the West African hinterland and of the Bantu races of East Africa. He has brought this disinclination to animal husbandry with him to the New World, hence his villages lack the considerable cattle population.

of the Indian villages, with the outstanding capacity of the ox for diverting *aquasalis*.

Equines,—horses, mules and donkeys,—though also highly attractive to *aquasalis*, are everywhere only maintained in small numbers that suffice for local transport. Pigs are, as stated above, much more commonly kept by negroes than by Indians (to whom, whether Muslim or Hindu in origin, the animal is wholly or to the higher castes more or less 'unclean'), and the census figures show very considerable pig populations in many places around the coasts. The percentage of pig bites found during the first tour is 15.4, as against 1.9 found on the Caroni Plain as in Table XXXVIII. Unfortunately, as this table shows, the pig is not a favoured host of *aquasalis*, and though it doubtless causes some deviation from man, it is much less effective than the ox or the equines. In this respect *aquasalis* differs greatly from the *maculipennis*-group in Europe.

(II) EXPERIMENTS ON BLOOD PREFERENCES.

The relative attraction of the various animals for *aquasalis* was accordingly examined by setting up a series of dawn traps baited with man, ox, goat, horse and pig. The traps were located about 100 ft. apart, to minimize cross-attraction odours. Goat was not introduced into the series until Mr. Whiteside demonstrated the interesting part played by this animal in the feeding of *aquasalis* (*vide infra*). The total captures over the 11 months of this experiment cannot therefore be compared, but the average daily catch for seven and a half months in the variously baited traps was :

Man.*	Ox.	Goat.	Horse.	Pig.
31	345	24	270	25

giving ratios of

Man.	Ox.	Goat.	Horse.	Pig.
1	14	1	11	1

or expressed as percentages of the average daily total catch of 695

Man.	Ox.	Goat.	Horse.	Pig.
4.5	49.6	3.5	38.8	3.6

88 per cent of the total biting is on the ox and the horse, and, therefore, in Table LII, the man : ox + horse ratio in the various localities from which precipitin test material has been collected is recorded separately from the man : 'all animals' ratio. Bovines and equines alone effectively serve to deviate *aquasalis* from man, whom the above figures show is 1 per cent more a preferred host than the goat and the pig, which therefore afford little protection to him.

The food preference and the amount of multiple feeding found in *aquasalis* was related by Mr. Weitz to Mr. Whiteside of the East African Tsetse Research Organization during a visit paid by the former to East Africa in 1948. Mr. Whiteside was so interested in these facts that during a leave in England he studied the

* Though this man attracted and was presumably bitten by 1,039 *aquasalis* in 34 weeks, he did not contract malaria. No drug prophylaxis was given to him.

Trinidad protocols on file at the Lister Institute, and produced an elaborate statistical analysis thereof, from which, on a study of the first 362 results, he deduced that the order of host preference in *aquasalis* is ox : goat : horse : man : 30 : 5 : 4 : 1, a ratio very different from that found by the dawn trap catches described above. Mr. Whiteside was not acquainted with the diverse conditions of the various localities of the results of which are given in Table XXXVIII and it was therefore decided to conduct a further experimental trial of these ratios, using the precipitin test and not trap numbers.

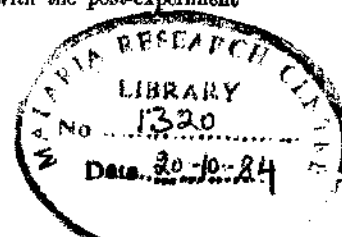
On a part of Bordenal Savanah well distant from the nearest habitations an area of bush, mostly *Cordia*, *Flemingia* and *Acroceras*, was squared into a quadrat of 78×48 ft. The grass surrounding the bush was kept as short as possible to discourage any resting. On one side of the bush a ricefield not in occupation of the Malaria Division, Port of Spain, runs up to the bush, but the amount of resting in this is also very low. It is cultivated in vegetables after the rice harvest.

As the results in Table XLVII (i) show, there was normally a considerable *aquasalis* population in the quadrat. This is created (a) by breeding in small irrigation channels passing nearby, and the ricefield whilst wet, and this breeding furnished the males and unfed Stage I's of the collections; (b) by specimens which have fed by day on vegetable cultivators and their transport donkeys working in the vicinity, and odd cows tethered out to feed on the short grass and on the road verge nearby; and (c) by late digesters drifting southwards from the drier, inhabited area, for oviposition in the wetter areas of the savanah. The food source (b) is strictly diurnal. A few cultivators certainly work until dusk, and they and their animals are then subject to attack, but when the quadrat was thoroughly caught-out up to 16.00 hours, it was felt that by evening it must have been fairly well emptied of all save recent emergences and late-stage drifters of the early evening movement. From June 15 to December 28, 1949*, 28 searches, averaging 10.9 man-hours each, which is equivalent to no more than 343 sq. ft. searched per man-hour, yielded the results shown in the first part of Table XLVII.

This is a normal sex distribution for an area containing much breeding water, and the densities and stage distributions are also normal. The experimental material was furnished by the 372 Stage II females. Of these, 134 were tested for precipitin reactions with results as in part (iii) of Table XLVII.† This shows a higher anthropophilic index than was expected and indicates the extent to which *aquasalis* feeds by day, when alone men, usually working, but at times having a 'lie back' for their midday meal, etc., are present in the immediate vicinity of the quadrat, as the road is too lonely at night for passers-by to linger. Goats have not been noticed tethered here to pasture, and the presence of their blood is not easy to explain. It will be observed that all of the goat feeds were multiple. Of 129 positive feeds tested, in all 13.2 per cent were multiple, a figure close to that shown for the valley generally. The 4.5 per cent of Stage II's which gave negative

* A search on May 25 (just before the first rain after the long drought) was negative, and the *aquasalis* population was small until mid-July.

† Only 20 Stage II were obtained in the four pre-experiment nights' catches. Other normal days' catches had to be included to obtain a reasonable size figure for comparison with the post-experiment catches.



reactions had probably derived their blood from birds or small rodents. A monthly watch on birds settling in this bush after a line of men had driven them out in the late afternoon showed, in the next hour before darkness, an average of 36 birds belonging to up to seven species coming in to roost.

1950-51, Month:	3	4	5	6	7	8	9	10	11	12	1	2
<i>Columbigallina (?) rufipennis</i> ...	24	15	33	10	15	2	9	6	20	10	17	7
<i>Thraupis episcopus slateri</i> ...	11	1	3	2	4	1	...	...	3	...	5	2
<i>Troglodytes musculus albicans</i> ...	5	5	2	3	2	2	...	3	...	3	4	...
<i>Spermophila grisea</i> ...	2	...	...	1	...	2	3	2	...	1	4	...
<i>Pitangus sulphuratus trinitatis</i> ...	1	1	6	5	14	...	2	1	2	4	1	...
<i>Icterus nigrogularis</i> ...	1	1	...	2	...	...	...	...	...	2	2	...
<i>Holquigualis lugubris</i> ...	...	...	4	...	3	1	2	...	1	1	...	...
<i>Crotophaga ani</i> ...	...	...	...	4	...	...	...	...	...	...	...	...

The actual experiment consisted of the tethering about 10 ft. out from the bush on the grass along the quadrat face, of an ox, a goat, and a horse. A thatched hut was built about 20 ft. out on the grass, and in this two fieldmen slept (two were necessary for company in this rather lonely spot). The hut had a broad gap under the eaves by which mosquitoes could enter or leave at will, but actually the door was also open whilst other observations (to be described later) were made on the first attack by men who were all protected from biting by 6-12 repellent fluid. The sleeping bait men were, of course, not so protected.

As the bush quadrat had been thoroughly searched during the days preceding the four experimental exposures, it was hoped that at the start of these the bush would be as nearly void of mosquitoes as possible and that all the fresh feds captured in the following morning searches which ensued, would be of mosquitoes which had fed on the men and animals exposed experimentally during the night, and which then would have entered from the vicinity of their hosts either on immediate repletion or (from the hut) at dawn.

The following morning catches after the four experimental nights give as part (ii) of Table XLVII.

This shows an enormous change from the average of the 28 normal searches tabulated above: (i) the male percentage of the catch is halved; (ii) the percentage of unfeds is nearly halved; (iii) the percentage of fresh feds is quadrupled; (iv) the late digesters are reduced from 34.6 to 4.8 per cent of the catch; and (v) the total female per man-hour density is trebled.

From these figures it is deduced:—

- (a) that the males and unfeds caught on those post-experiment mornings represent the previous night's eclosion, the latter not yet ready to

feed: the δ density is unchanged from normal values, but the sex ratio has greatly altered;

- (b) that the previous day's catching, resulting in an eightfold reduction of late digesters, really indicates how nearly, just before the experiments started, the bush was empty of its previous population, which was, as regards Stage II's, a contaminant of the experimental precipitin data;
- (c) that the trebling of the total female per man-hour density, and the fact that the fresh-fed percentage of the female captures increases fourfold, is clearly all attributable to the presence of the various baits throughout the night, and it may be accepted that the precipitin results found in these post-experiment captures [Table XLVII (iv)] truly reflect the preference of *aquasalis* for various hosts, though it has to be remembered, in comparing these results with those of the trap experiments, that here two men were being compared with one animal instead of one man, as in the trap series. From these data it is seen: (i) man biting reduced fourfold; (ii) ox biting nearly halved; (iii) goat biting completely unchanged; and (iv) horse biting trebled.

But ox + horse, 85.1 per cent of the normal series, is only raised to 94.7 per cent of the experimental. These two animals again attract the greater part of the bites. The fall in the human percentage (anthropophilic index) is due to the very nearly complete protection furnished to two men by one ox and one horse, compared to the chance deviation by odd grazing and draft animals of the normal series, under which circumstances their masters are quite severely attacked, far more so than in their villages by night (cf. Table XXXVIII). The experimental set-up slightly reduces the percentage of bites which are multiple, though the triples are increased.

The hut in which the two bait men slept was also searched during the post-experiment catches (at other times it was unoccupied). As was to be expected from the long-recognized habit in *aquasalis* of vacating buildings at dawn the catches of stragglers remaining after daylight were not large, but they showed such extraordinary variations in the source of the blood meals that they are set forth separately for each of the four runs:

Morning of	M	O	G	H	M O	M H	POSITION OF BAIT ANIMALS.		
							West.	Centre.	East.
1949									
Aug. 10	3	0	0	0	1	0	*H	G	O
" 17	0	10	0	0	0	0	*O	H	G
" 24	2	0	0	0	5	1	*G	O	H
Nov. 16	15	0	0	0	0	0	G	*O	H

M = man.

O = ox.

G = goat.

H = horse.

* Received the first bite on each occasion.

Thus on November 16, the stragglers in the hut consisted entirely of specimens which had fed there. On August 10, one specimen had double fed on man and ox : on August 24, though all eight but captures contained human blood, six of them had double fed. Seeing that it is improbable that a specimen partially fed on man would go out and complete its meal on the ox or horse, *and then return to the hut rather than go on to the bush*, it is probable that these seven double-fed specimens, having fed on the animals, had entered the hut rather than the bush to shelter, but finding themselves in the presence of a further blood supply, had managed to find room for 'just another small drink', perhaps late in the night, for, as is shown in Section VII (i), there is some evidence from the periodically closed traps that it is those which are only open to entrance after 01 hour that on being 'flitted' to drive stragglers from the animal compartment to the trap compartment, yield the largest percentage of such, and these are insects which had perforce fed later in the night, as earlier entrance to the bait animal was debarred. An early feeder may clear part of its serum load and have space for a little more blood late in the night. A late feeder appears to be somewhat less subject to the dawn exit tropism than an early one, which is quite comprehensible.

The catch of August 17 was extraordinary. On this day all the human feeders had left the hut, and its population consisted of purely ox-feds which should have entered the bush for shelter.

The position of the three bait animals was changed at each run. The animal receiving the first bite on each occasion is denoted by an asterisk. It will be seen that, with a single exception* when the goat was passed over, the westernmost animal received the first bite, irrespective of species. The hut position was of course fixed. It was located between the west and the centre tethering pickets. Anopheles feeding on the animals at those pickets were therefore nearest to the men in the hut if the latter was used, instead of the scrub, for shelter, and for taking a second (human) feed. As regards the double feeds, on August 10 and 24, the most distant animal figured in the double feeds. On August 17 and November 16, the hut was equally near the ox, yet on the first occasion ox-fed specimens entered the hut, on the second, none did so. The position of the individual animals on the various experimental nights therefore appears to have no relation to double-feds found in the hut the following morning. The results in Table XLVII have been statistically analysed by Mr. Whiteside. His deductions are :—

- (a) *Preferences before and after bait host exposure are significantly different* : $\sum \chi^2 = 84.5$. $n = 3$. $P = < 0.001$. (i) Exposing a goat had no effect on preferences (which confirms the author's crude percentage figure deductions); (ii) preference for man was markedly lowered after the experiment. This is attributed to the deviation from man caused by the three animal hosts; and (iii) after the experiments, horse was favoured above, and ox below, expectation. (Whiteside considers this to show that horse is quite a favoured host, and the small numbers of horse feeds recorded in ordinary conditions

* On September 5, during an evening exposure of bait animals for height resting observations, the animal on the east of the line, the ox, received the first bite, but a full moon was rising simultaneously with the setting sun.

are due to the actual paucity of their numbers, which is an absolute fact.)

- (b) *Preferences in multiple feeds before and after the experiment are significantly different.* $\sum \chi^2 = 15.76$. $P = < 0.01$. The only considerable contribution to χ^2 comes from man. Thus in recognizable multiple feeds* (i) preferences for ox, goat and horse are unaffected by exposure of the four hosts, but (ii) man appears in multiple feeds too much before and too little after the experiments. (This is a statistical demonstration of the different circumstances under which man is bitten pre- and during experiment, as explained above.)
- (c) *Analysing preferences among mosquitoes caught in the hut against preferences among those caught in the bush.* The catching place (bush or hut) makes no difference in the number of man feeds (as would be expected, there are more in the hut) and a small but still significant difference in the number of horse feeds; there are fewer than expectation in the hut. $\sum \chi^2 = 202.97$. $P = < 0.001$. [Whiteside, who is not a malariologist, thus independently arrives statistically at the conclusion brought out by the author's work in India (Senior White, 1947) that in precipitin work it is essential to state where the mosquitoes caught were taken. This applies *a fortiori* to dissection results.]
- (d) As shown from the author's own crude percentages *exposure of the four experimental hosts has no effect on the amount of multiple feeding occurring.* Whiteside concludes his argument on this subject by stating 'it looks as though multiple feeding is a characteristic of the mosquito population itself rather than a consequence of the hosts with which it is in contact'. (But if propensity to multiple feeding is genetically controlled, this is hard to understand in results obtained over a few miles of country through which one would expect there to be uninterrupted gene-flow.)
- (e) The last question posed by Whiteside was: *does the amount of multiple feeding change during the period in which these experiments were carried out?* As the change is obviously the same in pre- and post-experimental catches, the figures are pooled for statistical estimation. $\sum \chi^2 = 13.60$; χ^2 for early August 0.75; for late August 8.32; for November 4.53; $P = < 0.1$.

Multiple feeding, therefore, is low in late July and early August: about doubled in late August, and back to its original level in November. The rise in late August is quite significant. Whiteside concludes 'it seems that multiple feeding varies at different times, possibly quite quickly'. The causation of this is quite obscure.

As Whiteside had shown that there were rapid changes in the amount of multiple feeding between various months, it was decided to devote much of the field work of 1950 to a study of possible monthly changes in feeding preferences,

* i.e. feeds on two species of animal. A feed on two animals of the same species is unrecognizable with the technique used.

with special reference to fluctuations in the anthropophilic index. Three localities were taken for study :—

- (a) the suburban Success-Laventille area, in which monthly catches were made at four levels from the houses adjacent to the Laventille mangrove and the open swampy land between this and the house-line across the railway to the highest houses up the hill-side. There is no breeding above the lowest line of houses, all specimens from above this area have originated from either the mangrove or the open swampy land just north of it. For the present purpose the four levels are combined ;
- (b) New la Paille Village, in which a general monthly catch was made ;
- (c) Bamboo Village, where fortnightly catches were made (i) in the houses themselves, (ii) in the scrub surrounding the village and interspersed between the houses and the vegetable cultivation, (iii) for three months only in some clumps of uncut bamboo immediately adjacent to the southernmost houses, the remains of the Nelson plantation from which the village area was cleared in 1948.

The results are set forth in Tables XXXII, XLIX, L and LI.

Success area.—The anthropophilic index of 30 shown in Table XXXVIII was found on material collected in September to November 1948. An examination of the anthropophilic index month by month, Table XLIX, shows variation from 5.3 in December to 36.4 in June, and the combined September to November results of 1950 do not approach the corresponding 1948 value. Not only therefore is there monthly variation in anthropophilic index, but also, apparently, variation between the same months of different years. Human feeders in this D.D.T'd area that survive to be collected in the morning must be specimens that have fed out of doors, and this introduces a further variable, the extent to which humans are out of doors between dusk and bedtime being to some extent dependent upon fine evenings. But the data show that in the absence of adequate animal deviation, residual D.D.T. alone does not prevent *aquasalis* human feeding to a dangerous extent. Where larval control is economically feasible, this is a far safer alternative.

New la Paille.—The 1948 data, collected in March and October, showed an anthropophilic index of 3.6 only. It was therefore concluded that the ox-equine population adequately diverted *aquasalis* from man, and from 1949 onwards D.D.T. application was suspended. It is believed that all residual toxicity had vanished from this area by January 1950, and that year's data (Table L) represent natural conditions. But July showed a sudden and transient increase in anthropophilic index to 14.2. Female density was maximal in that month, 53.6 per man-hour, and though one would naturally expect the patient ox to suffer the increased attack, the data would suggest that man is more attacked at high densities were it not that the August figures show a return to a normally low anthropophilic index at a female density of 45.4 per man-hour, an inconsiderable decrease from July.

Bamboo Village.—The data (Table LI) from the outside resters here do not at all show this transient rise in anthropophilic index with the onset of rains breeding. Density was again maximal in July, though much lower than at

New la Paille, being 16.8 per man-hour only. Anthropophilic index for that month (3 collections combined) was one of the lowest found during the year.

The significance of the variations in anthropophilic index shown in Tables XLIX, L and LI has been examined by Mr. Simmonds. The analysis shows :—

Success : $\chi^2 = 33.4112$. $P = < 0.001$. The significant rises in anthropophilic index are mainly caused by July and September.

New la Paille : $\chi^2 = 33.6977$. $P = < 0.001$. If the July figures are removed, $\chi^2 = 9.6242$. $P = < 0.3$ to 0.5 . Thus, almost the whole discrepancy is due to the extremely high anthropophilic index figure for July.

Bamboo Village : $\chi^2 = 16.0677$. $P = 0.1$ to 0.2 . Monthly differences in anthropophilic index are therefore not significant, and in this village alone the percentage of human biting is constant, and lower than in either of the other two localities.

Chart 4 illustrates the fluctuations in months when these are based on significant numbers.

Taking first New la Paille, where results are not complicated by D.D.T., it is seen that the unique rise in July corresponds with the maximal monthly density, but the relationship is obviously not direct, as at the next highest density value, in August, the anthropophilic index is below the mean of the year. Conditions at Success are complicated by D.D.T. The amount of female destruction here is clearly shown by the mean male percentage of the catch being nearly double that at New la Paille, situate in the midst of heavy breeding, and thrice that at Bamboo, much breeding as there is around that village. Hence female density at Success is only 10 per cent of that at New la Paille, and in July, again the maximal month, is only 16.8 against 53.6 in the latter village. In September, density is reduced to nearly half the July value, yet in both months the analysis proves that the increases shown in the anthropophilic index column are significant.

What then happens in both Success and New la Paille in August? In both areas that month's density is intermediate between those of July and September, yet at neither is there any significant difference in anthropophilic index from the mean for the locality. Just as Whiteside's analysis of the Bordenal experiment indicates that multiple feeding varies in amount at different times, 'possibly quite quickly', so Simmonds' analysis of fluctuations in the amount of human feeding in Success and New la Paille reveals monthly fluctuations in the amount of human biting. In neither case are the changes in tropism correlatable with changes in density. Neither are there marked climatic changes over those three months to evoke an explanation.

It is tempting to speculate whether trophic changes thus revealed may not be genetic, but such an idea must remain a pure speculation until chromosome maps of *aquasalis* can be constructed, a remote contingency for a species which it is not possible to cagebreed at all.

The fact remains that a sudden increase in anthropophily, such as is here shown to be a real fact both in a D.D.T'd suburb and in an uncontaminated village, between which there are the widest differences in man : ox ÷ horse ratios, must have an immediate effect on the intensity of malaria transmission. But this problem

requires for its study investigations into similar changes in a vector of sufficient potency to cause such changes to be at once visible in infection rates in man and the vector, and in the amount of dispensary attendances.

CHART 4.
From Tables XLIX, L and LI.

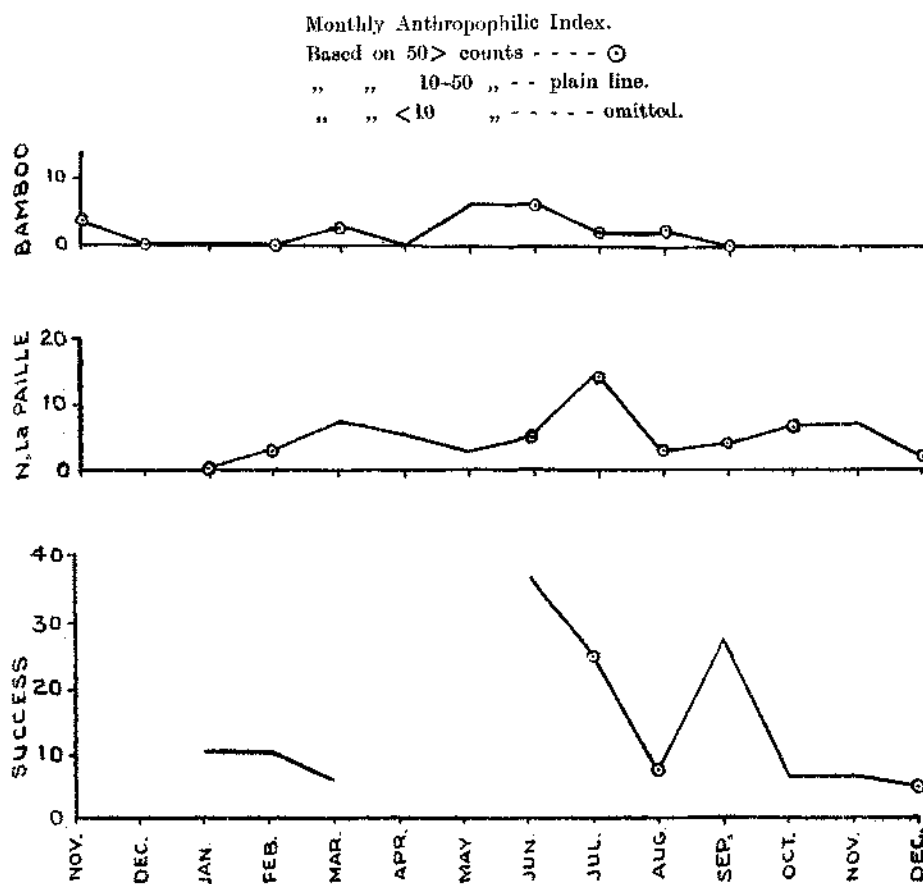


Chart 1 has appeared with Part I of this paper in the *Indian Journal of Malaria*, Vol. 5, No. 3, September 1951, p. 293; and Charts 2 and 3 have appeared with Part II of this paper in the *Indian Journal of Malaria*, Vol. 5, No. 4, p. 465.

Indoor resting.—In Section IX it is shown that an anthropophilic index of 84.9 for September 1949 was found on specimens remaining in houses in the morning. It was therefore decided to amplify the data on this by collections made in houses between June and December 1950. The results are set out in Table XXXII. They show, as is only to be expected, a much higher anthropophilic

index than any in outside resters, but (as might also be expected) a high percentage of dog feeding, as this animal frequently shares the house with man. What was quite unexpected is the finding of ox, goat, and horse bloods in the house. This can only mean that a certain number of specimens which have fed out of doors do not rest there, but enter the house by morning, and presumably remain there all day.

Immediately south of Bamboo Village is an area of still uncleared Nelson Bamboo, which faces the southernmost houses of the village like a wall. Finding that there were considerable numbers of *aquasalis* to be found in these clumps, when searched by men besmeared with 6-12 repellent, in contradistinction to the main uncut area of these bamboos, where fed resters are very sparse, 100 fresh-fed specimens from clumps fairly close to the last houses were smeared. All the usual animal bloods appeared except that of dog, with man positive averaging 10 per cent, higher than shown in Table LI for local scrub-resters during the months of September to November, when these bamboo clump collections were made. Numbers in the clumps were also then higher than in the usually searched scrub in and around the village.

The outcome of the 1950 precipitin work is to raise more problems than it settles! Certainly, the New la Paille data show that testing for dog from June onwards reduces the percentage of unknowns from 7.1 to 4.2. But during the same months dog positive formed 6.7 per cent of the total Σ +, and if dog feeding is constant, the reduction in unknowns should therefore have been greater. It is obvious, however, that dog should not be omitted from tests. But the very wide variation in anthropophilic index in New la Paille in July is without any explanation, even maximal density in that month, as Bamboo Village does not show the same phenomenon. The man : ox + horse ratio is probably never constant anywhere, animals are born and bought and sold as well as humans born and dying, or leaving the village temporarily. Censuses are incomplete, and cannot be undertaken monthly. Density per man-hour varies likewise throughout the year. It is shown in the tables, but affords no clue to the anthropophilic index fluctuations.

One fact is clear, and that is our ignorance of the bionomics of adult female *aquasalis*. This is *a fortiori* even more true for the house-haunting species of the Old World, which when they are studied as total populations in their natural habitats and not in houses alone will probably reveal quite as startling variations in behaviour as does *aquasalis*. Whilst this work was in progress, Deane, Vernin and Damasceno (1949) published on the subject of blood preferences in *A. darlingi* and *A. aquasalis*. Their material was of two kinds, specimens captured in houses and specimens released in a large cage, 19.5 $\times$ 6.5 ft., in which a man slept and the following animals were confined : ox, sheep, horse, pig, dog, cat and fowl. With house captures they showed that the anthropophilic index of both species was high, in *aquasalis* 71.8, which compares well with the 75.5 found in house captures in Bamboo Village over eight months in 1950 (Table XXXII). Multiple feeds in house-caught *darlingi* were 6.7 per cent (921 specimens), whilst in 510 *aquasalis* such were 1.6 per cent only. In the cage experiment results were very different. The anthropophilic index of *darlingi* was here 45.7 per cent, whilst that of *aquasalis* fell to 10.9 per cent. Multiple bites were 3.1 per cent in *darlingi* and 11.2 per cent in *aquasalis*. The conditions of the experiment were artificial, in that the animals

were individually confined in 'special frames' or in wire cages, in which their freedom of movement must have been greatly restricted. The Brazilian authors' work, however, serves to confirm the natural results reported in the present paper regarding the high percentage of *aquasalis* bites which are multiple when an immediate choice of hosts is available.

(III) MULTIPLE FEEDING IN ONE GONOTROPHIC CYCLE.

The amount of partial feeding that can be deduced from the amount of multiple biting found by precipitin tests was subjected to direct observation, during the first hour's attack on the bait animals used in the Bordenal quadrat experimental feedings described above.

Observations were made on seven evenings. The results below are totals.

Each of the experimental animals was observed by two men protected by 6-12 repellent fluid, whilst the unprotected bait men, in the hut, observed one another. The observers fixed their attention on one specimen at a time, and so far as could be discerned by torchlight without capturing or artificially disturbing the specimen, recorded the abdominal state in which it alighted and in which it departed from the animal. As only one specimen at a time could be watched the total numbers recorded are not large, but the results show, in percentages of the totals:—

Bait.	Number observed.	Empty. Full.	Empty. Half.	Empty. Empty.	Half. Full.	Half. Half.	Full. Full.
Man ...	133	41.4	33.1	23.3	2.3	0.0	0.0
Ox ...	140	33.6	22.8	41.6	2.0	0.0	0.0
Goat ...	180	5.5	21.1	72.6	0.0	0.6	0.0
Equine ...	164	24.4	32.3	40.2	1.8	0.0	1.2

The two bait men were instructed to exercise volitional control and not disturb attacking specimens. Even so, less than half the empty mosquitoes that attacked fed full on them. The percentage of half-fed specimens which attacked and completed their meal on man was apparently a little higher than with any other animal. This observation related interestingly to the precipitin results on the hut captures described above. More empty specimens half engorged on man and on the equine than on the ox or the goat.

The behaviour of the goat was very instructive as to why [Table XLVII (iv)] 60.5 per cent of the bites on goat should be multiple. Under mass attack the goat was seen to be extremely intolerant, not only twitching its skin muscles continuously but also using its hooves to brush off attackers. The percentage of simple goat feeds recorded above (5.5) is close to the percentage of goat-only feeds recorded in Table XXXVIII (4.6).

The goat is evidently a very intolerant host so long as attack is heavy, and it is suspected that the majority of full feeds on goat are obtained at periods of the

night when activity is low, when the odd bite may be better tolerated. In the above observations no more than a quarter of the attackers of goat obtained at most a trace of its blood before the attack was repelled. Perhaps under a less delicate technique than Weitz' this trace would not show in the results. Comparing the single (presumably) full feeds recorded above with the single host precipitin reactions found in the post-experimental tests on the quadrat plus the hut catches no good agreement is found. The percentage of single feeds found by precipitin tests after all night bait exposure is very much larger than the percentage observed in the first hour of mass attack after dusk. This suggests that much of the successful single-host feeding found by precipitin reactions is carried out at periods of lower *aquasalis* density than occurs during first activity after dusk.

Single feeds.

		OBSERVED.		PRECIPITIN.	
		Number.	Percentage.	Number tested.	Percentage of positive.
Man	...	55	41.4	15	72.9
Ox	...	50	33.6	216	78.3
Goat	...	10	5.5	8	57.1
Horse	...	40	24.4	331	83.6

Multiple feeds could not be directly observed. The moment a specimen moved from the torch beam it was lost to record. It may have merely moved further round the same animal, it may have deserted that particular animal for another host. The restless feeding that goes on under mass attack is well brought out by the observational figures.

(IV) FLOWER FEEDING.

It has long been known that the males of mosquitoes appear to be exclusively nectar feeders. Females found in the same act seem to have attracted little attention until Mattingley (1949) suggested that sugar is required in both sexes for periods of great activity. In the females, such will undoubtedly include mating.

Since the review of the literature on this subject by Howard, Dyer and Knab (1913), only four more papers on this subject seem to have been published. The author is indebted to the London School of Hygiene and Tropical Medicine for photostat copies of those not locally accessible. But these latter papers all refer to culicines, and the literature on flower feeding by anophelines is very scanty.

Using local, barely literate, fieldmen it is difficult to train them to distinguish between actual feeding in flowers, and mere resting on them. All observations now recorded have perforce been made by torchlight, and it is fortunate that *aquasalis* in particular is so tolerant of this. Unlike *neomaculipalpis*, however,

it is not attracted to light, though Shannon (Weekly Report, February 13 to 17, 1944) records that artificial light, though not attracting, makes *aquasalis* restless, even *p.c.* This observation is not confirmed though it may be of great importance in studying multiple feeds in the field.

Observations on specimens with the proboscis actually inserted in flowers have yielded the following results:*

N.O.	Plant.	Times observed.	<i>aquasalis</i> .	
			♂	♀
Boraginaceæ ...	<i>Cordia macrostachya</i>	8	40	26 (1 blood in gut).
Rubiaceæ ...	<i>Borreria verticillata</i> ...	2	2	7
	<i>Hamilea patens</i> ...	2	0	5
	<i>Hamilea erecta</i> ...	3	0	7
Scrophulariaceæ ...	<i>Capraria biflora</i> ...	1	0	3
Compositæ ...	<i>Eupatorium inulifolium</i> †	3	264	37 (1 blood in gut).
	<i>Eupatorium odoratum</i>	3	19	1
	<i>Bidens pilosa</i> ...	3	0	3
Leguminosæ ...	<i>Mimosa pudica</i> ...	1	0	1
TOTAL ...			319	85

Flowers watched but no feeding seen :—

N.O.	Species.
Malvaceæ ...	<i>Sida rhombifolia</i> .
Aristolochiaceæ ...	<i>Aristolochia</i> sp. indet.
Verbenaceæ ...	<i>Lantana camara</i> .
Solanaceæ ...	<i>Solanum stramonifolium</i> .
Compositæ	<i>Parthenium hysterophorus</i> .
	<i>Syndrella nodiflora</i> .
	<i>Malanthera nivea</i> .
	<i>Flemingia strobilifera</i> .

* 'Discussing these results with Dr. Wigglesworth at Cambridge after this MS. had been despatched, he pointed out that the sugar found in the mosquitoes might not be of floral origin, but be the result of the hydrolysis of glycogen carried over from the larva, or, in the case of females, derived from a blood meal. The matter can only be settled by a detailed research, and Dr. Wigglesworth hopes to interest one of his students in this problem.'

† On September 27, 1950, 5·3 man-hours' observation on this species yielded 75♂, 47♀.

Mr. Simmonds of the Botanical Division of the Imperial College informed the author that the flowers of most of the Malvaceae close at night. So also do those of *Mimosa pudica*, which, large as are the numbers of Hymenoptera, etc., frequenting them by day, are 'closed' to night-flying insects as a nectar source. *Flemingia strobilifera* flowers in January-February, when *aquasalis* density is often low, and the bracts occlude the flower almost completely.

From the above figures it is obvious that *Eupatorium inulifolium* furnished much the greatest number of the specimens, of both sexes, attracted. From the point of view of the nectar-seeking insect, however, this species is not a good source, as it only flowers around October. *E. odoratum* only flowers in December. An all-the-year-round supply of nectar essential for a continuously breeding insect such as *aquasalis* is locally only furnished by *Cordia macrostachya*, the inconspicuous cymes of which are continuously in flower, and *Bidens pilosa*, which the figures show is not very attractive.

In the first place, the author therefore attempted to find out whether the nectar of *Cordia* flowers would yield a Benedict reaction to sugar. Macerating several flowers together this reagent was obviously affected, though no measurable precipitate of CuO_2 was obtained. Obviously, a much more delicate test for sugar than Benedict's solution was required. It was therefore decided to attempt chromatographic methods, and the help of Dr. Forsyth of the Microbiological Institute was sought, who most kindly gave some elementary instruction in qualitative tests with the paper method, from which the following data were obtained:—

Object tested.	Number tested.	Sugar positive, per cent.		
Flowers of <i>E. inulifolium</i>	103	100		
„ „ <i>C. macrostachya</i>	105	96.2		
	♂	♀	♂	♀
<i>A. aquasalis</i> : newly emerged lab. bred*	69	95	5.8	15.8
„ : outdoor resting by day	16	...	12.5	...
„ : caught in evening swarm	110	3	53.6	33.3
„ : feeding at <i>E. inulifolium</i>	113	68	53.1	30.9
„ : feeding at <i>C. macrostachya</i>	2	26	100	3.8
„ : unfeds from dawn trap	...	104	...	25.0
<i>A. osculdoi</i> : outdoor resting by day	137	...	4.4	...

* Reaction very slight, difficult to be certain of amid general dark colour resulting from smear debris.

Sugar is therefore undoubtedly present in both species of flowers tested. Some of the *Cordia* flowers may have been hardly opened buds or just developing fruits, for all stages are found on the same cyme, the individual flowers of which do not appear to develop in regular order from apex to base. The procedure with mosquitoes was to crush the abdomens out on to the test paper, exactly as is done for precipitin work, though much more widely spacing the smears. The matter expressed is far less in extent than the blood content of a female stomach, but it is usually sufficient if sugar is present to give an orange-brown spot when developed with aniline hydrogen oxalate. Apparently, the newly emerged adult of both sexes gives a feeble reaction for sugar. If more delicate tests prove this correct, an interesting matter of physiology requires investigation. Only outdoor resting males have been tested, as so many apparently empty (abdomen Stage I) females show traces of residual blood when crushed, indicating that they have undergone one gonotrophic cycle, and are awaiting the evening to refeed, and the possibly by-products of the metabolism of blood may complicate the crude findings of which we are alone capable. The age of outdoor resting males is undiscoverable, but the fact that only one-eighth of the small numbers tested show sugar suggests that many such are newly emerged awaiting evening to flower feed. About half the males and one-third of the very few females caught in swarms give a sugar reaction. So far as the observations go females appear to go to *Cordia* flowers after full darkness has fallen, when the swarms have either dispersed or been lost to view. On this hypothesis the female sugar feeds after swarming, for the exact time of capture of females at flowers has not been noted, but appears to be commoner after the swarm has disappeared. The dawn trap used for capturing hungry females was one specially modified, a screen being interposed between the entrance slits fitted with traps, and the bait animal. The modification has so far not been particularly successful. Baulked of blood the hungry females largely manage to make their way out of the traps again.* But a quarter of the would-be biters have proved positive for sugar, though there is neither *Cordia*, nor in its season *Eupatorium*, in the immediate vicinity of the two traps concerned, near which there is no breeding. The *A. oswaldi* captured outdoor resting came from specimens sitting on fallen leaves within the Nelson Bamboos near the village. Nectar would only be obtainable from the scrub around the houses, as there is no flowering undergrowth beneath these bamboos.

SECTION IX.

TRANSMISSION.

(I) DENSITY AT WHICH *AQUASALIS* REMAINS IN HOUSES AFTER DAWN.

The dissection catches set out in Table XLVIII show that after November 1949 there was such a rapid fall-off in stragglers found resting after daylight in houses, that from December collections for this purpose were too few to be worth while

* It was hoped by this modification to collect large numbers of hungry ♀♀ for staining and subsequent release for flight experiments, but a trap so modified yields very small numbers in comparison with a normal trap nearby, and much further experimenting is required.

continuing. Study of Table LIII setting out the dissections of 1941-42 shows that none was made between December and June; so Shannon evidently encountered the same phenomenon, which is of great epidemiological importance, as though precipitin tests in D.D.T'd suburban areas reveal how much out-of-doors human feeding occurs, none the less, and for infants and young children especially, biting within the house is almost certainly more important. Were it not, the spleen rate reductions found in Trinidad since residual D.D.T. application commenced could not have been achieved, though the arguments presented under precipitin reactions indicate that with *aquasalis* no more than the expectation of a lower level of malaria can be looked for by residual D.D.T. alone.

During 1950, therefore, density in the scrub surrounding, and that within the un-D.D.T'd houses of Bamboo Village, has been recorded in Tables XXXII and LI. Comparison is not directly possible, scrub densities being measured 'per man-hour' of catching, whilst house densities are measured on a 'per house' basis. There appears to be some indefinite correlation, as the following figures abstracted from these tables show:

	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Outside resting density, per man-hour.	6.2	16.8	9.2	4.9	0.7	0.6	1.2
House resting, per house	1.4	1.7	0.8	0.8	0.6	0.2	0.2

At least the figures suggest that transmission within the house, excluding any consideration of longevity, is a matter related to the maximal outside densities of the early rains and ensuent first heavy breeding.

(II) ANALYSIS OF PREVIOUS WORK IN TRINIDAD.

During the Rockefeller Foundation's period of work in Trinidad, between 1941 and 1943, two record books of dissections appear to have been filled. Of these only one can now be found, covering only the first 13 months from September 1941.

This is peculiarly unfortunate, since it is only by going back to the original protocols that it would be possible to break down by localities and seasons the gross dissection totals given in the annual reports and summarized in Section I. Unless this is done, it is impossible to distinguish particularly dangerous areas, or the local seasons of malaria transmission. In Trinidad, it does not seem to have been sufficiently appreciated that, like everywhere else in the world, malaria transmission must certainly at least vary in intensity locally and seasonally, even if the detailed study of earlier data does not show that there are seasons during which transmission by *aquasalis* ceases altogether.

The dissections of all the species so far examined in Trinidad are set forth in Table XLVI. It shows that, of the 13 species known from the island, only seven have been dissected at all, and five of these together have only been examined to the number of 199 specimens! This figure is, of course, completely inadequate

for the study of one, let alone five species, and for all one knows, among these five or the altogether un-examined remaining six local species, a greater culprit than either of those so far incriminated may be lurking.* Two of those not dissected locally, *A. punctimacula* and *A. brasiliensis*, are known, if minor vectors in the high Andes and Amazonia respectively. But this work will involve detailed studies of the habits, and initially the discovery of the resting places, of all these species, and so far it is only of *A. eiseni* that the author has succeeded in finding the latter. The peculiar resting place of *A. nimbus*, on cobwebs on tree trunks, has long been known. *A. albitarsis* is not found in outdoor scrub catches in numbers commensurate with the intensity of breeding often found.

Thus, only *aquasalis* and *bellator* have been at all adequately dissected, and the table shows that the hundreds of thousands of dollars expended locally, both by the Local Government and the U.S.A. Forces, on their control rests for its protozoological justification on the finding of one infected gland in each of these two species. From Tobago there has been no dissection work done at all, yet a costly *aquasalis*-eradication scheme is there in progress on no surer basis than analogy with the Trinidad findings and the existence of a high malaria incidence.

When the author thinks of the waste of money and energy that he himself was guilty of in India by for many years accepting Christophers' (1933) dictum on *A. culicifacies*, 'a vector wherever found', whereas there are vast tracts in that sub-continent where, as was later discovered by dissection, that species is of no public health importance, one is inclined to 'blink' a bit at the epidemiological and financial faith placed on the dissections carried out in Trinidad.

For the 13 months covered by the one surviving dissection book the *aquasalis* dissections break down as in Table LIII. The book does not contain the record of the single gland infection found, but from a remark in Downs, Gillette and Shannon (1944, p. 36) this must have been in a dissection from the Success-Laventille suburban area between September and December 1942.

Taking the oöcyst rate by months, the results for the 13 months of 1941-42 are as under :

Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1.0	1.0	5.4	8.7	1.6	0.8	1.0

This strongly suggests that August and September are the months of maximal transmission on the Caroni Plain, but the fact that the single sporozoite finding occurred after September suggests, as might well be expected, that longevity may

* In this regard the story of McArthur's (1950) incrimination of *A. leucosphyrus* as the vector of North Borneo may be profitably considered. In larval collections yielding over 44,000 specimens of 7 spp., only 74 larvae were those of *leucosphyrus*. Adult catches, using house searches (almost completely negative), animal and human bait trapping and catching, and jungle resting searches, together produced nearly 12,000 ♀♀ of 9 spp., of these barely 7 per cent were *leucosphyrus*. But, of 960 specimens of these catches, comprising 7 spp., taken attacking or attracted to man, 67 per cent were *leucosphyrus*. Turning next to the 4 most likely suspects, 3 of these spp. showed a nil anthropophilic index, but *leucosphyrus* showed 100 per cent human blood. Armed with these 2 pointers McArthur carried out 2,715 dissections of 9 spp., in which of 761 *leucosphyrus* 2.4 per cent showed sporozoites, the other 8 all being negative. Thus was discovered a vector, apparently rare as both a larva and an adult, on which, once incriminated, intensive investigations revealed the specialized breeding places and peculiar house-entrance habits, leading to a simple method of biological control.

be greatest at seasons of reduced density and actual transmission may be autumnal. The evidence from relict egg findings set out in Section VII (x) should also be taken into consideration.

Table LIII shows the quite considerable all-over house oöcyst rate of 3.4, but, though the author is prepared to go further than Hodgkin (1950) in stating that 'the oöcyst rate is only indicative of the infectiveness of the human community to mosquitoes',—for it is considered that in comparison with the sporozoite rate it affords valuable data on the relative and the seasonal longevity of individual species*,—yet the author is entirely in accordance with Hodgkin, and with all malaria investigators since Stephens and Christophers (1902), that the incrimination of a species as a vector must rest solely on the finding of sporozoites at a rate commensurate with the amount and seasonal distribution of the local malaria occurring. But if this criterion be applied to the Neotropics, Table 104 in Boyd (1949) (admittedly a condensed summary, and with grave omissions) records data on only ten of the 66 known species, and only in three of these do the numbers dissected amount to four figures. As compared with India, the dissection work is considered to be quite insufficient to evaluate the relative importance of the various Neotropical species found in any locality.

On what is now known (Section VIII) of the preponderant zoophily of *aguasalis* the 304 dissections recorded in Table LIII from stables were quite unlikely to have afforded even a gut infection. Yet now that almost every malarious area in which *aguasalis* occurs within the island is plastered with residual D.D.T., the carrying out of a really adequate dissection programme in Trinidad must henceforth rest, not on house-caught stragglers, but on fed outdoor resters captured around houses. In Tobago, where the *aguasalis* eradication programme includes larviciding and drainage, dissection is no longer feasible.

Dissection of outdoor captures is, however, likely to be extremely disappointing unless really large numbers are dealt with.† Whilst examining the Nelson Bamboo captures for ovarian condition the guts were *pari passu* examined for oöcysts. Numbers examined were:

Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.
...	150	157	78	180	104	162
Aug.	Sep.	Oct.	Nov.	Dec.	Total.	
430	344	681	75	117	2,487	

Not a single gut infection was found. But Table XXXV shows that a considerable proportion of these were in ovarian Stages I and IIA and would be most unlikely to show even gut infections.

Table LIV shows the areal and monthly breakdown of dissections carried out in 1945-46, after D.D.T. application had been commenced on all three of the

* The very high oöcyst : sporozoite ratio, 49 : 1, recorded in Table XLVI, is definitely indicative of the very short average life of the female *aguasalis*.

† Adequate dissections, in Trinidad, should in future amount to 10,000 monthly. This number would be quite feasible to collect, but impossible to get dissected or to examine unaided. Evaluation of the vectorial status of *A. culicifacies* in India rests on the dissection of 67,894 specimens.

areas tabulated. The finding of one gut infection in February is interesting, indicating that transmission is climatically possible after December. The figures in Table XXXIII for trap A1 show that in 1946 February was productive of a high percentage of the year's total in the Laventille area, and it is probable that the quality of the D.D.T. application work was not then of the standard subsequently achieved.

(III) RECENT DISSECTIONS.

Table XLVIII shows the results of five months' dissections carried out in 1949 on house captures in the then new Village of Bamboo. The Bamboo Village bush resting A.I. was, on collections between August and December 1948, at the locally high value of 19. The child parasite rate of the village (53 children examined) in April 1949 was 20.8, and at the author's earnest solicitation, the village was left un-D.D.T'd for purposes of investigations. The Division's Public Health Nurse visited the village regularly, and an ample supply of paludrine was placed at the disposal of the ladies operating the voluntary Child Welfare Clinic at Curepe, the only proviso to the gift being that the drug was not to be distributed except on parasitological findings from blood films.

Table XLVIII shows that, instead of the instructive little transmission period that was hoped for, only 2 gut infections (0.1 per cent) were found in 2,336 dissections carried out between August and December. A second parasite examination on 64 children in December was nil. In other words, malaria had died out in this population a year after their transfer from their former location without the use of either D.D.T. or drugs. As a further check on transmission in this village, the blood of all newborns was examined monthly, commencing from one month after birth, when any immunity carried over from the mother in the placental circulation can be assumed to have died out even in a hyperendemic locality, such as does not exist in Trinidad. Eleven children undoubtedly born in this village subsequent to its first settlement late in 1948 were examined. Of these, one born in the dry season of March 1949 showed *falciparum* at its second examination in July. This must be accepted as a locally acquired infection, though Trinidad villagers are not nearly as static as their counterparts in India and the Bamboo villagers probably frequently visit relatives in the less salubrious areas south of the Caroni from which they were transferred.

During the house dissections tabulated above, the author became so worried by the lack of positive findings that it occurred to him that house captures might not have actually fed on human blood at all! However, a series of 56 smears from houses submitted to the Lister Institute in September showed 84.9 per cent positive for man* (other animals were not tested for, some of the man + may actually have been multiple). As these Bamboo villagers settled in and brought in their animals, the man : ox + horse ratio fell to 5 : 1 in January 1950, and with it the anthropophilic index of the local outside resting *aquasalis*, which was

* Mr. Weitz transmitted these results by cable, for which the author is extremely grateful. Failing this dissections might have been stopped, thinking that even house resters in Indian villages have animal fed, as Table XXXII actually shows to be, in a certain extent, the case. All examinations of dissections have had to be carried out by the author himself personally, not even the senior technician has ever seen sporozoites, other than in demonstration Giemsa-stained specimens, so rare are they in Trinidad.

3.6 in November and nil in December 1949. The animal husbandry of these Indian villagers seems, as at Glandon [(Section VI (ii)], to protect them completely from malaria.

(IV) DENSITY REQUIRED FOR MALARIA TRANSMISSION.

In Section VII (iii), data are given on 170 collections in which a survival rate from Abdomen Stage II to Abdomen Stage VII of 5.9 per cent was found. A further set of data on 378 collections in which no survival to Stage VII was found are also recorded. Taking all the collections tabulated in Table XXXIX survival rates of 10.5 to 1.8 per cent are shown. Therefore, an all-over survival rate to mature ovary of 5 per cent of fresh feeds is considered to be a fair approximation to general conditions on the Caroni Plain. The length of one gonotrophic cycle has not been more closely approximated (Section V) than an average of 12 days. Infection experiments in 1941-42 at the St. Ann's Mental Hospital (successful only with *P. falciparum*) showed a developmental cycle to sporozoites of 12 to 13 days.* It would, therefore, be safe to assume that, in Trinidad, *A. aquasalis*, when following exact gonotrophic concordance, is only likely to transmit malaria at its fourth feed. Assuming, therefore, a 5 per cent survival rate during one cycle, and making no allowance for the undoubtedly considerable mortality which must occur during the oviposition flight with mature ovaries and the subsequent return to the following feed, it may be calculated that for one specimen to survive to do its fourth (potentially infective) feed the following original population is necessary (round figures):

Day	1-3.	76,000 are reduced to	3,800
Day	4-6.	3,800 are reduced to	190
Day	7-9.	190 are reduced to	20
Day	10-12.	20 are reduced to	1

This calculation would directly apply only to transmission by a purely anthropophilic vector which ingested gametocytes at its first feed. But the anthropophilic index of *aquasalis* has nowhere and on no occasion in Trinidad been found to exceed 36, and in the cattle-protected Indian villages (Table XXXVIII) averages 3 to 6 only. Whilst again perforce making the improbable assumption that the first feed is (a) human, and (b) on an effective gametocyte carrier, the original population required for one successful malaria transmission in these villages may be calculated as under:

A.I. = 3 $\therefore$ 1 in 33 bites man: $\therefore$ 4th (potentially infective) contact is made by $(1 \text{ in } 33)^4 = 1 \text{ in } 1,185,921$ (Hackett, 1937).

But survival during a life of 4 bites = 1 in 76,000: $\therefore$ initial population required to produce one specimen making its first and fourth bites on man is: $1,185,921 \times 76,000 = 90,129,906,000 = 90,129 \text{ million} = 9 \times 10^{10}$. This is an astronomical figure.

* Floch and Abonnenc (1943, Tables III and IV) fed 840 specimens on *falciparum* and 741 specimens on *vivax* patients. Only 9 of those fed on *falciparum* remained alive (or were held) up to 12 days, and an unspecified number of these 9 showed sporozoites. No *vivax* fed specimens were dissected later than the 8th day, and no gland infections were found.

But when in semi-urban conditions, a rather different picture presents itself. On the Success-Laventille A.I. of 30 (Table XXXVIII) 1 in 3.3 bites man. $\therefore$ 4th contact is made by $(1 \text{ in } 3.3)^4 = 1 \text{ in } 118$.

Assuming that mortality remains at the survival rate of 1 in 76,000, then the initial population required to produce one specimen doing its first and fourth bites on man is: $118 \times 76,000 = 9,011,000 = 9 \times 10^6$.

But D.D.T. must enormously affect the survival rate. Its effect is not here considered. But it is possible to calculate the semi-urban pre-D.D.T. mortality figure in quite another fashion, i.e. from the oöcyst : sporozoite ratio of 1.47 : 0.03 (Table XLVI) = 49 : 1. On these data 1 in 50 of the human biters survives to do its fourth bite, that is, the survival rate was not 1 in 76,000, but 2 per cent. But this calculation would rest on a much surer basis if more than one gland infection had been found. It does not take into account mortality in the first three days of engorged life. The only survival rate figures other than the above that have been calculated from direct observational data are those of Russell and Rao (1942) on the Indian *A. culicifacies*. These authors showed that reduction by death is in a geometrical progression of 50 per cent every two days.

Now oöcysts are usually not apparent in dissections until the third day after an infective feed, thus on a gonotrophic cycle of three days a mosquito showing small oöcysts would normally be ready for its second feed. Mortality deduced from the oöcyst : sporozoite ratio will therefore apply to that occurring between second and fourth feeds, and must, on Russell and Rao's data, be doubled to represent the mortality between first and fourth feeds. Taking Hackett's calculation for proportion biting man at fourth bite, and survival rates based on Russell and Rao, the relative vectorial capacities of the three most dangerous species of the Ethiopian, Oriental and Neotropical regions in comparison with semi-urban *aquasalis* in Trinidad work out as in Table LV. In this connection the house anthropophilic index findings of Deane, Vernin and Damasceno (1949) in Brazil should also be considered. No calculation based on dissection data is possible for Indian village conditions in Trinidad, as sporozoites have not been, nor are they ever likely to be, found, owing to the numbers that would require dissection to give any real chance of finding a gland infection. The calculations are full of assumptions based on analogies with other species, but do serve to bring out the relative innocuousness of *aquasalis* in Trinidad, even under the most favourable vectorial conditions.

The dissection data in Table II from Brazil and the Southern Windward Islands, however, reveal *aquasalis* as a quite potent vector, and it is therefore considered essential that local investigations should be pursued into the causes underlying this difference in behaviour. These will be considered in a subsequent paper.

At first sight it might be considered that the calculations in Section IX (iv) on which are based the numbers required (a) for a single specimen to survive to its fourth gonotrophic cycle, and (b) for the likelihood of a single specimen making its first and fourth feeds on man, result in such enormous figures that it appears extremely improbable that *aquasalis* can exist in such numbers. But the author thinks that the argument adduced below refutes this.

(a) The maximum outside resting density so far measured for numbers and area occurred on July 28, 1949, when in a bush quadrat of 4,624 sq. ft. (0.1 acre), much of it short grass unsuitable for resting, 698 ♂ and 702 ♀ were captured, a density of 55.9 ♂ and 56.2 ♀ per man-hour. Of the 702 ♀, 49.1 per cent were in Abdomen Stage I. This density is therefore equivalent to 3,450 Stage I females per acre, and to a total per acre population of 14,000 of both sexes. If such a large continuous area of resting *cum* breeding ground exists, this would be equivalent to a total population of nearly 9 millions to the square mile.

(b) The highest single night's trap catch of *aquasalis* (one ox bait) on record is 14,257, taken in trap B1, situated less than a mile east of the area referred to in (a). The hungry female density of this catch represents no more than the population of about $4\frac{1}{2}$ acres, an area covered by a circle of no more than 40 yds. radius. Whilst not suggesting that the normal flight range of *aquasalis* is restricted to any such short distance, the trap catch does suggest that the effective flight range to blood is quite short. Zulueta (1950a, 1950b) measured the outside resting densities of *A. brasiliensis*, *parvus* and *pergandui* on the tall grass llanos of Eastern Colombia by 'tenting' and driving up resting mosquitoes by light applications of pyrethrum. The method is only usable in areas of uniformly low and thornless vegetation, such as grasses. Under these circumstances the method has been tried in Trinidad. As a method of general insect collecting it is excellent, but it was found that *aquasalis* is unwilling to fly up into bright sunlight, which, on the light values given in Section VII, is to be expected.

Zulueta found an average of 1 ♀ anopheline to every 4 square metres. He points out that this density means that an area with a radius of 100 metres contains more than 7,000 anopheline females, which is enough to fill an animal bait trap during the night with thousands of mosquitoes, his maximum catch in such on the llanos being 4,600 anophelines and culicines combined. He states 'it is sufficient to say here that the dispersal of mosquito females from the breeding places over vast areas of open country . . . does not mean that the mosquitoes searching for blood are attracted by an animal bait over such wide areas. There are in fact two different kinds of flights: a long-distance flight of dispersal from the breeding areas and a short flight towards the source of blood. Males take part as well as females in the long-distance flights of dispersal, though they have a shorter flight as judged by the results obtained from the tent captures'.

These results accord well with the data calculated from observations (a) and (b) above, though the species of *Anopheles* are different. It is no wonder, therefore, that traps placed 33 yds. apart, as in the individual species preference and hours of entrance experiments at Curepe set out in Sections VII and VIII, showed maxima on different days. Each trap was apparently drawing on separate resting populations of widely dispersed hungry insects, only attracted to the individual traps over a short range from each. The Nelson Bamboos catches [Section VI (vi)] are also apparently illustrative of this short range blood attraction phenomenon. The following argument is perhaps too full of assumptions to be really usefully pursued, but from the data in (a) it can be calculated in regard to Section IX (iv), that one specimen out of the original, unfed, female population of 23 acres survives to accomplish its fourth (normal-cycle) feed, and that specimens which have bitten man at their first and fourth feeds, in a closely populated area, exist at no greater

density than one in 4 square miles. But to apply such a calculation to intensity of malaria transmission in Trinidad is considered to be fallacious. Man is not, as has been shown [Section VIII (ii)], a very tolerant host, not more than 41 per cent of attacks resulting in full feeds. It is, therefore, likely that a specimen feeding within a house is likely to attack two or three inmates to obtain one full meal, and, if that specimen be infective, to distribute malaria accordingly. Only daily spot maps of attacks would serve for the experimental observation of such theoretical deductions.

SUMMARY AND CONCLUSIONS.

1. The geographical distribution of the *tarsimaculatus*-group of *Nyssorhynchus* and the position within it of *A. aquasalis* is discussed. Recent work on its bionomics in Brazil is summarized, and what is on record of its capacity as a malaria vector throughout its distribution area is tabulated. This shows that it is a fair vector from 23°S. to the Equator. North of the Equator it is either not at all, or, at best, a very feeble vector up to and including Trinidad in 10°N. In the Windward Islands it is a potent vector. Nothing is known of its vectorial capacity near its northern limit of 17°N. In Tobago it is assumed to be a vector on epidemiological grounds only.

2. The races of *aquasalis* in the various countries of its distribution are examined, using the maxillary index as the separatory character. The species is shown to consist of five statistical groups ('demes') which fall into two separate clines apparently worthy of sub-specific nomenclatorial distinction. It has only one deme in Trinidad, which it shares with British Guiana. This deme is replaced by a geographically narrowly restricted but quite separate one on the ancient land of Central Tobago. In Trinidad its density decreases with distance from the sea.

3. There are statistically significant differences between the number of eggs in the ovaries in certain months. Though the larva will tolerate salinity up to 23.50 per mille during the rains, almost all the breeding is in water not exceeding 3.5 per mille, though in the dry season about 14 per cent of this occurs in water of higher salinity. Pupation is favoured by rather higher concentrations than larval findings indicate as optimum. Shade and turbidity are unfavourable to breeding. Saline ammonia content appears to play no part in regulating either oviposition or larval life. The average larval *cum* pupal life is $8\frac{1}{4}$ days (though it may extend to 26 days), and the time for a complete generation is just over 12 days. Young larvæ in particular appear to be incapable of holding position in the face of the slightest water movement.

4. Five general types of breeding places were subjected to extended surveys. An account of breeding in each type is given. Second growth mangrove, particularly *Rhizophora*, is shown to be more productive than virgin areas in other countries. *Ruppia maritima* is shown to afford a good nidus for larvæ. Of three species of salt-tolerant reeds, *Cyperus articulatus* is shown to be the most productive. Rice-fields are shown to present very little hazard, most of their larval production being of *A. albitarsis*. *A. aquasalis* is only present to any appreciable extent during the first three months of the crop season. Water-cress cultivation is shown not to be

highly productive of *aquasalis*. Breeding in the submontane length of one of the streams descending from the side valleys of the Northern Range is shown to be dependent upon seasons of low rainfall.

5. Adult activity is shown to continue throughout the night, though it is greatest before 20.00 hours, and is affected by moonlight. Day-time resting places are described, and the discovery of these has furnished the material for all subsequent investigations into adult bionomics. Mortality rate during one gonotrophic cycle is shown to be about 95 per cent, and is a 'density-dependent' phenomenon. Predators are suspected as the cause of this mortality, but very little information has yet been gained concerning them. Certain plants are more preferred for day-time resting than others. Removal of scrub and keeping grass short are shown to be inimical to resting. The micro-climates affecting day-time resting have been studied, particularly light incidence values. Evening activation of day-time resting females is shown to be entirely a light effect. Activation is compounded of two movements, upwards from the lowest foot of shaded vegetation and then flight outwards from shade.

Swarming and mating studies have been commenced. These are yet not extended enough to dogmatize, but females entering, and the few caught mating, in the swarms appear all to have empty guts. Swarming here seems to occur on an average 20 minutes after sunset, at a light value not exceeding 5 foot-candles. *A. oswaldoi* and *A. albitarsis* have also been seen swarming, the former in swarms mixed with *aquasalis*. The male swarms do not appear to be the main site of fertilization, which is still undiscovered.

As regards fertilization, a long series of spermathecal and ovarian stage dissections is set forth. The import of relict eggs in the ovary is discussed. A subdivision of Christophers' Ovary Stage II was tried out, and found of value.

So far only data on short range movement have been obtainable.

Density fluctuations are shown to occur at a shorter interval than the length of one gonotrophic cycle. Data are presented indicating that in a large proportion of specimens more than one blood meal is required per ovulation. Long period density fluctuations related to breeding intensity are discussed. They are climatic in origin, and it is shown that the critical period of maximal densities, though not strictly fixed either locally or annually, is between the end of May and early September.

The former belief of infiltration into the northern valleys from the plain is shown to be ill-founded.

6. Blood preferences deduced from precipitin reactions on wild-caught specimens are examined at length. Bovines and equines are shown to be the preferred hosts and man is only seriously attacked in the lack of sufficient deviation by those animals. Other domestic animals are not favoured hosts. In the presence of adequate ox-horse deviation entrance into a trapped house is shown to be negligible. A large proportion of feeds are shown to be multiple on more than one species of host. Experiments to evaluate specific host preferences and multiple feeding are described. These tendencies are shown to fluctuate significantly in a single area over the year, fluctuations not being related to changes in density. These fluctuations are not understood.

Observations of flower feeding by both sexes at different common species of flowers are set forth. Results from the chromatography of flowers and of gut contents at various stages of pre-blood feeding existence are tabulated. Nectar meals seem to follow, and not succeed swarming and mating.

7. The dissection data from Trinidad are discussed in detail for transmission. Almost astronomical figures of density are required for a successful human infection. Such densities, however, are shown to exist.

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TABLE XLIX.*
Success Village—All elevations—1950: A. aquasalis: precipitation results.

1950.	M	O	G	H	P	D	$\frac{M}{O}$	$\frac{M}{G}$	$\frac{M}{D}$	$\frac{O}{G}$	$\frac{O}{D}$	$\frac{G}{D}$	$\frac{H}{P}$
Jan.	3	15	6	3	2	...	0	0	0	0	0	0	0
Feb.	3	21	11	3	3	...	2	0	0	1	0	0	0
Mar.	1	10	11	2	1	...	1	0	0	0	0	0	0
Apr.	2	3	2	0	0	...	0	0	0	0	0	0	0
May	1	3	4	0	0	...	0	0	0	0	0	0	0
Jun.	4	6	1	0	0	...	0	0	0	0	0	0	0
Jul.	22	28	18	8	8	3	0	0	0	1	0	0	0
Aug.	4	42	11	6	7	0	1	0	0	1	0	0	1
Sep.	10	20	4	4	2	0	0	1	1 ¹	0	1	0	0
Oct.	1	27	2	15	0	0	2	0	0	0	0	0	0
Nov.	3	22	6	4	1	0	6	0	0	0	0	0	0
Dec.	2	41	16	5	1	0	1	1	0	2	0	1	0
TOTALS AND AVERAGES.	56	244	92	50	25	3	7	2	1	5	1	1	1

M = man. O = ox. G = goat. H = horse. P = pig. D = dog.

* Table I to Table XXXVIII have appeared with Part I of this paper in the *Indian Journal of Malariology*, Vol. 5, No. 3, September 1951, p. 295; and Table XXXIX to Table XLVIII have appeared with Part II in the *Indian Journal of Malariology*, Vol. 5, No. 4, December 1951, p. 463.

TABLE XLIX--*conold.*

1956.	Σ	M	O	G	H	P	D	Negative.	δ percentage.	$\frac{\delta}{\rho}$ density per man-hour.	Anthropo- philic index.
Jan.	29	3	15	6	3	2	...	2	82.4	1.6	10.3
Feb.	47	5	24	12	3	3	...	4	88.2	2.7	10.4
Mar.	33	2	17	11	2	1	...	1	87.6	1.4	6.1
Apr.	7	2	3	2	0	0	...	0	88.4	0.2	28.6
May	8	1	3	4	0	0	...	0	70.0	0.2	12.5
Jun.	11	4	6	1	0	0	...	1	54.1	0.3	36.4
Jul.	89	22	29	19	8	8	3	5	67.1	4.3	24.7
Aug.	76	5	44	12	7	8	0	5	67.4	3.3	6.6
Sep.	46	12	21	5	4	2	2	2	69.2	2.4	26.1
Oct.	40	3	29	2	15	0	0	3	32.4	1.4	6.1
Nov.	42	3	24	9	5	1	0	0	61.3	2.1	6.5
Dec.	75	4	44	19	6	1	1	3	70.1	2.0	5.3
TOTALS AND AVERAGES.	512	66	239	102	53	26	6	26	76.2	1.8	12.0

M = man. O = ox. G = goat. H = horse. P = pig. D = dog.

TABLE L.
New la Paille, 1950: A. aquasalis precipitation results.

1950.	M	O	G	H	P	D	$\frac{M}{O}$	$\frac{M}{H}$	$\frac{M}{D}$	$\frac{O}{G}$	$\frac{O}{H}$	$\frac{O}{P}$	$\frac{O}{D}$	$\frac{P}{D}$	$\frac{M}{O \cdot G}$
Jan.	0	51	1	8	10	...	0	0	0	1	2	2	...	...	0
Feb.	2	54	2	1	17	...	1	0	0	1	1	3	...	...	0
Mar.	1	7	0	2	1	...	0	0	0	1	0	0	...	...	0
Apr.	1	11	1	0	1	...	0	0	0	0	0	0	...	...	0
May	1	29	2	0	3	...	0	0	0	2	1	0	...	...	0
Jun.	8	118	1	5	15	24	1	0	0	1	4	0	6	...	0
Jul.	33	161	3	15	1	19	10	1	1	0	0	2	2	1	0
Aug.	4	145	1	59	2	10	2	1	0	1	5	0	0	0	1
Sep.	2	105	0	5	0	6	2	1	0	1	1	0	0	0	0
Oct.	5	77	1	6	1	0	3	0	0	1	4	0	0	0	0
Nov.	2	26	0	5	0	0	1	0	0	2	0	0	0	0	0
Dec.	0	43	0	5	0	3	2	0	0	0	1	1	3	0	0
TOTALS AND AVERAGES.	59	817	12	111	51	62	22	3	1	11	19	8	11	1	1

M = man.

O = ox.

G = goat.

H = horse.

P = pig.

D = dog.

TABLE L—*concd.*

1950.	$\Sigma +$	M	O	G	H	P	D	Negative.	σ percentage.	$\frac{\sigma}{\rho}$ density per man-hour.	Anthropo- philic index.
Jan.	80	0	56	2	10	12	...	4	53.5	12.3	0.0
Feb.	88	3	60	3	2	20	...	3	40.1	14.7	3.4
Mar.	13	1	8	1	2	1	...	7	31.0	4.2	7.7
Apr.	17	1	11	2	1	2	...	1	41.9	4.1	3.0
May	41	1	32	4	1	3	...	2	16.2	7.3	2.4
Jun.	199	9	132	4	9	15	30	7	11.2	19.2	4.5
Jul.	232	33	161	3	15	1	19	13	40.2	53.6	14.2
Aug.	242	8	154	2	66	2	10	9	46.2	45.4	3.3
Sep.	127	5	109	1	6	0	6	5(3)	38.4	25.4	4.0
Oct.	106	8	85	2	10	1	0	4	41.9	18.8	7.5
Nov.	39	3	29	0	7	0	0	2	21.7	5.8	7.7
Dec.	65	2	50	0	6	1	6	0	37.2	9.1	3.1
TOTALS AND AVERAGES.	1,247	74	887	24	133	58	71	57(3)	40.0	18.3	5.9

M = man. O = ox. G = goat. H = horse. P = pig. D = dog.
 Numbers in brackets after numbers negative denote negatives found 'mammal' on the new polyvalent anti-serum.

TABLE LI.
Bamboo Village: outside resters in scrub.
A. aquasalis: precipitation results: fortnightly catches combined by months.

1949-50.	M	O	G	H	P	D	M	N	O	G	O	H	P	H	D	M	O	G	P
Nov.	1	33	1	15	0	...	1	0	2	0	0	0	0	0	0	0	0	0	0
Dec.	0	71	0	5	0	...	0	0	0	0	0	0	0	0	0	0	0	0	0
Jan.	0	13	0	9	0	...	0	0	0	0	0	0	0	0	0	0	0	0	0
Feb.	0	57	0	7	0	...	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar.	2	66	0	13	1	...	1	0	2	6	0	1	0	0	0	0	0	0	0
Apr.	0	7	1	5	0	...	0	0	1	0	0	0	0	0	0	0	0	0	0
May	2	25	0	2	0	...	0	0	0	1	0	0	0	0	0	0	0	0	0
Jun.	7	85	0	12	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0
Jul.	3	201	1	33	0	8	3	2	2	5	1	0	1	0	1	1	1	1	1
Aug.	5	184	1	30	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Sep.	0	77	0	17	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
Oct.	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nov.	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dec.	0	91	1	6	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0
TOTALS AND AVERAGES.	21	883	5	164	1	12	6	2	9	24	1	1	1	1	1	1	1	1	1

D = dog.

P = pig.

H = horse.

G = goat.

O = ox.

M = man.

TABLE LI--*concl.*

1949-50.	Σ +	M	O	G	H	P	D	Negative.	♂ percentage.	♀ density per man-hour.	Anthropophilic index.
Nov.	56	2	36	3	15	0	...	0	24.1	5.7	3.6
Dec.	76	0	71	0	5	0	...	3	8.6	0.3	0.0
Jan.	22	0	13	0	9	0	...	1	23.6	1.8	0.0
Feb.	64	0	57	0	7	0	...	1	10.0	5.7	0.0
Mar.	102	3	75	2	20	2	...	2	16.9	4.7	2.9
Apr.	15	0	8	2	5	0	...	0	8.0	0.5	0.0
May	31	2	26	0	3	0	...	3	13.6	1.3	6.5
Jun.	109	7	86	1	12	0	3	5	12.0	6.2	6.4
Jul.	340	9	274	4	42	2	9	3	23.0	16.8	2.6
Aug.	212	5	165	2	39	0	1	3	23.2	9.2	2.3
Sep.	114	0	87	0	27	0	0	4(2)	30.0	4.9	0.0
Oct.	2	0	1	0	1	0	0	0	64.1	0.7	0.0
Nov.	3	1	2	0	0	0	0	0	47.9	0.6	(33.0)
Dec.	34	1	24	1	8	0	0	0	29.9	1.2	2.9
TOTALS AND AVERAGES.	1,180	30	925	15	193	4	13	25(2)	25.1	4.5	2.6

or

M = man. O = ox. G = goat. H = horse. P = pig. D = dog.
 Numbers in brackets after numbers negative denote negatives found 'mammat +' on the new polyvalent anti-serum.

1, JM

TABLE

A. aquasalis density and animal ratio 1950

Locality population.	Average population—2 censuses 1950.					Average M : O + H.	Ratio M : all animals.	Last spleen rate pre-D.D.T.	Density	
	M	O	G	H	P				Dry.	
Predominantly Indian.										
Cacandee	1,082	244	32	5	36	4.3	3.4	0.6	5.2	8.8
Debo	1,100	79	89	37	30	10.3	5.6	2.8	1.3	0.9
Rousillac	608	59	82	8	36	7.9	3.3	18.7	2.8	3.7
Fishing Pond	441	28	36	8	21	12.2	4.7	70.6	0.3	0.2
TOTALS	3,231	401	209	58	123	7.0	4.2			
Predominantly Negro.										
Plaisance	908	38	76	14	96	17.3	4.1	7.3	0.7	1.7
Petit Bourg	529	28	44	23	37	10.4	4.0	?	0.6	0.5
Chatham	196	8	23	2	57	19.6	2.2	25.7	0.4	7.4
Erin	241	5	20	0	34	48.2	4.1	2.6	1.0	0.4
La Lane	340	16	20	24	94	10.0	2.3	9.3	0.1	0.3
Moruga	238	23	28	9	53	10.2	2.9	20.0	0.2	0.3
Guayaguayare	607	8	44	45	217	11.4	1.9	57.1	1.9	3.7
Biche	701	27	23	10	40	18.9	7.0	8.7	0.1	+
S. Manzanilla	430	12	25	9	53	24.8	4.3	16.1	1.4	0.5
Salybia	120	3	0	1	6	30.0	12.0	38.1	0.0	0.2
Matina	508	34	20	9	53	10.8	4.4	15.0	+	0.5
Guaymare	15	0	5	1	16	15.0	0.7	?	0.0	0.1
Curmana	303	2	27	16	56	16.8	3.0	35.0	+	0.0
Blanchisseuse	631	6	12	21	90	23.4	4.9	28.0	0.0	0.0
Pilletto	150	5	28	0	34	30.0	2.4	30.0	+	0.2
TOTALS	6,007	209	395	184	938	15.3	3.5			
Mixed.										
Chagoana	1,324	114	25	50	51	8.1	5.5	2.7	0.1	0.1
California	394	7	20	6	60	30.3	4.2	24.7	1.9	0.6
Claxton Bay	503	16	17	2	49	27.9	6.0	25.5	0.2	+
Leacos	470	97	100	7	94	4.5	1.1	17.9	8.4	13.6
Navel	496	51	25	6	85	8.3	3.0	10.6	0.1	0.2
Plan	104	4	14	9	3	8.0	3.5	16.0	0.1	0.0
Cocal	63	12	16	5	21	3.7	0.3	?	0.0	+
TOTALS	3,354	301	362	85	363	8.7	3.0			

M = man. O = ox. G = goat.

LII.

against pre-D.D.T. spleen rate.

per man-hour.		M bites.	Percentage bites—both seasons.						Anthropo- philic index.	REMARKS.
Wet.	Wet.		M	O	G	H	P	D		
♂	♀									
0.7	10.0	137	3	120	1	9	2	2		
0.8	0.3	7	1	6	0	0	0	0		
1.3	1.7	39	4	19	5	1	10	0		
0.5	0.8	6	1	3	0	2	0	0		
		189	9	148	6	12	12	2	4.2	
0.3	0.2	10	3	5	0	1	1	0		
0.2	0.4	11	0	4	0	1	5	1		
0.0	0.2	70	4	8	16	32	10	0		
1.3	1.1	8	0	1	5	0	2	0		Fishermen.
0.0	0.2	4	0	4	0	0	0	0		
0.1	0.1	8	0	1	2	1	4	0		
0.4	0.6	38	2	4	0	12	20	0		Oilfield area.
0.2	0.6	4	0	4	0	0	0	0		
0.7	0.7	12	0	3	3	5	1	0		
0.0	0.0	1	0	0	0	1	0	0		
0.1	0.3		No fresh bloods							
0.0	0.0		"							
0.0	0.0		"							
0.0	0.0		"							
0.0	0.0	4	1	0	2	0	1	0		Larval control.
		170	10	34	28	53	44	1	5.9	
0.3	0.4	2	0	2	0	0	0	0		Small town among cane.
0.3	1.0	9	0	8	0	0	2	1		" " "
+	0.4	5	1	4	0	3	0	0		
4.9	10.9	183	17	61	54	12	29	10		
0.0	0.1	2	0	2	0	0	0	0		
0.2	0.2		No fresh bloods							
0.2	0.2		"							Coconut Est. No village.
		201	18	72	54	16	31	11	8.1	

H = horse.

P = pig.

D = dog.

TABLE LIII.

		SUCCES-LAVENTILLE HOUSES.					BARATARIA HOUSES.				
		Gut.	+	Percentage.	Gl.	+	Gut.	+	Percentage.	Gl.	+
1941.											
Sep.	...	50	1	2.0	66	0	...	...	...	...	...
Oct.	...	66	1	1.6	72	0	...	...	...	...	...
Nov.	...	263	2	0.8	264	0	...	...	...	...	...
Dec.	...	105	1	1.0	107	0	...	...	...	...	...
1942.											
Jun.	...	68	1	1.5	57	0	A38	0	0.0	37	0
Jul.	...	121	1	0.8	115	0	89	1	1.1	85	0
Aug.	...	118	7	6.0	119	0	102	5	5.0	97	0
Sep.	...	2	0	0.0	1	0	214	22	10.3	209	0
TOTALS	...	793	14	1.8	801	0	443	28	6.3	428	0

A = one specimen from S. El Socorro.

TABLE LIII—*concd.*

		HOUSE TOTALS.						STABLE TOTALS.					
		Gut.	+	Percentage.	Gl.	+	Gut.	+	Percentage.	Gl.	+	M. CHRISTO—MCLEOD, CURLEE, GOVT. FARM.	
1941.													
Sep.	...	50	1	2.0	66	0	...	...	...	...	...	...	...
Oct.	...	66	1	1.6	72	0	...	...	...	...	...	...	...
Nov.	...	263	2	0.8	264	0	15	0	0.0	16	0	0	0
Dec.	...	105	1	1.0	107	0	...	...	...	...	...	...	...
1942.													
Jun.	...	106	1	1.0	94	0	95	0	0.0	84	0	0	0
Jul.	...	210	2	1.0	200	0	71	0	0.0	68	0	0	0
Aug.	...	220	12	5.4	216	0	115	0	0.0	108	0	0	0
Sep.	...	216	22	10.2	210	0	8	0	0.0	7	0	0	0
Totals		1,236	42	3.4	1,229	0	304	0	0.0	283	0	0	0

TABLE LIV.
A. aquasalis dissections, 1945-46.

Year	SUCCESS-LAVENTILLE-MORVANT HOUSES.						BARATARIA HOUSES.					
	Gut.	+	Percentage.	Gl.	+	Gut.	+	Percentage.	Gl.	+	Gut.	+
1945.												
Jul.	4	0	0.0	9	0							
Aug.	3	0	0.0	3	0							
Sep.	13	0	0.0	13	0	30						
Oct.	1	0	0.0	1	0							
Nov.	3											
Dec.	33											
1946.												
Jan.	42	0	0.0	42	0	140	0	0.0	142	0		0
Feb.	25	1	4.0	25	0	75	0	0.0	75	0		0
Mar.						12	0	0.0	12	0		0
Apr.												
May						11	0	0.0	11	0		0
Jun.	58	0	0.0	58	0	30	0	0.0	28	0		0
Jul.	21	0	0.0	21	0	42	0	0.0	42	0		0
Aug.						15	0	0.0	15	0		0
Sep.						6	0	0.0	6	0		0
Totals	167	1	0.6	172	0	331	0	0.0	331	0		0

TABLE LIV—*concl.*

EL SOCORRO AREA.						HOUSE TOTALS.					
	Ent.	+	Percentage.	Gl.	+	Ent.	+	Percentage.	Gl.	+	
1945.											
Jul.	25	0	0.0	25	0	29	0	0.0	34	0	
Aug.	66	0	0.0	66	0	69	0	0.0	69	0	
Sep.	84	0	0.0	84	0	97	0	0.0	97	0	
Oct.	44	0	0.0	44	0	45	0	0.0	45	0	
Nov.	...	...	...	...	...	...	...	...	...	...	
Dec.	...	...	...	...	...	...	...	...	...	...	
1946.											
Jan.	...	...	...	...	...	182	0	0.0	184	0	
Feb.	25	0	0.0	25	0	125	1	0.8	125	0	
Mar.	9	0	0.0	9	0	21	0	0.0	21	0	
Apr.	...	...	...	...	...	...	...	...	...	...	
May	...	...	...	...	...	11	0	0.0	11	0	
Jun.	33	0	0.0	33	0	121	0	0.0	119	0	
Jul.	24	0	0.0	24	0	87	0	0.0	87	0	
Aug.	2	0	0.0	2	0	17	0	0.0	17	0	
Sep.	6	0	0.0	6	0	12	0	0.0	12	0	
Totals	318	0	0.0	318	0	816	1	0.1	821	0	

R. A. Senior White.

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TABLE LV.
Comparison of vectorial capacities.

Species.	Dissected.	Gut +.	Gland +.	Anthropophilic index.	Biting man at 4th bite.	Mortality, 1st to 4th feeds percentage.*	Authority.
<i>gambiae</i>	16,245	2,037	1,138	90	(9 in 10) ⁴ = 1 in 1.5	75	Boyd (1949), Table CIV West African figures. Anthropophilic index deduced from East African data (Gibbins, 1933).
	Percentage	12.5	7.0				
<i>fastidius</i>	Ratio	2:1					
	1,871	132	63	83	(8 in 10) ⁴ = 1 in 2.4	75	Senior White, figures from papers on the Jeypore Hills.
	Percentage	7.1	3.4				
<i>darlingi</i>	Ratio	2:1					
	582	129	20	63	(6 in 10) ⁴ = 1 in 7.7	87	Brazil figures, Boyd (1949), Table CIV and Gabaldon in Boyd, pp. 766-768.
	Percentage	20.8	5.0				
<i>aquasalis</i>	Ratio	4:1					
	G. 3,792	56	1	30	(3 in 10) ⁴ = 1 in 123.5	$\frac{100}{9 \times 106^9}$	As calculated on pages 53-54.
	Gl. 3,302	1.47	0.03				
	Ratio	49:1					

* Oöcyst: sporozoite ratio doubled to allow for 1st G.T. cycle mortality at 50 per cent.

IRRIGATION AND MOSQUITOES IN THE UNITED STATES OF AMERICA.

BY

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FOREWORD.

DURING the summer of 1949, the author had the privilege of being detailed to the Malaria Institute of India while serving as Malaria Consultant, South-East Asia Regional Office, World Health Organization, United Nations. His principal assignment was on field surveys of irrigation projects in India, construction of which was proposed or underway at that time. Although he acquired some first-hand knowledge of irrigation developments in India, the projects visited were in the early stages of construction and his acquaintance with practices in the irrigated croplands themselves consequently is largely confined to a limited review of the literature.

More recently (1951), he has made a field study of irrigation design and practices in the United States in their relationship to mosquito problems, while serving as Consultant to the Communicable Disease Centre, U.S. Public Health Service, and has been invited to contribute an article to this journal. Although the problem would be more informative to readers in India if presented on a comparative step-by-step basis, he is obliged to confine the greater part of this article to a direct presentation of irrigation in the United States.

INTRODUCTION.

Much interest has been demonstrated in and many studies have been made of irrigation practices and problems in relation to disease, and in their correction. In the malarial aspects of irrigation alone, the names of Afridi, Chang, Christophers, Covell, Hackett, Harbhagwan, Hill, Knipe, Menon, Mulligan, Perry, Rao, Robertson, Russell, Sinton, Sitapathy, Sur and many others come to mind or are found in the literature.

The recent increased interest of the public health professions, sociologists, economists and others in food supply and disease control as they relate to human populations, emphasizes the need for a joining of forces by those concerned with programmes of public health, agriculture and irrigation. Such mergers already have been manifested in India by joint agricultural development-malaria control programmes engaged in by the Government of India and the World Health and the Food and Agriculture Organizations of the United Nations, and by the co-operative agreement between the Malaria Institute of India and the Central Board of Irrigation.

The development of knowledge and insight into each other's problems and in the combined ecology of these fields is deemed essential to further progress. Such development can be aided by the exchange of information on problems, practices and programmes in various parts of the world. This paper has been prepared as a minor contribution towards that objective.

IRRIGATION AND HEALTH

Irrigation obviously yields a net benefit to man's survival, mainly because famine would sweep over the arid and semi-arid regions of the world if irrigation were suspended. But it is also known that irrigation has introduced or added a heavy burden of death, disease and discomfort to the agricultural populations of irrigated areas. The principal disease and discomfort problems can be summarized as follows:—

- (1) Irrigation malaria—occurring in all continents.
- (2) Schistosomiasis due to irrigation—with Egypt offered as an outstanding example.
- (3) Other gastro-enteric infections arising from irrigation of certain truck crops with polluted water—which probably occur to a varying extent in all countries where irrigation is practised. These include the dysenteries, the typhoids and the lesser salmonelloses.
- (4) Human discomfort wherever nuisance mosquitoes are produced in large numbers in irrigated areas.

Related to these are important economic losses in livestock due to nuisance mosquitoes and to animal disease, such as mosquito-borne equine encephalomyelitis.

Irrigation in the U.S.A. imposes a minor toll on human health compared with many other countries and from a global viewpoint the problems here might even be 'charged off' as negligible. Full explanation would require lengthy epidemiological discussion. Mosquito nuisance arising from irrigation is extensive and reaches a critical level in some areas. The most significant irrigation—health-comfort problems in the U.S.A.—are considered to be: malaria, encephalitis and mosquito nuisance. These are summarized briefly below:

Malaria.—Hyperendemic malaria was a serious consequence of the wet cultivation of rice along the South Atlantic seaboard during the 17th and 18th centuries and occasioned one of the earliest known measures of conscious malaria control in history when the citizens of Savannah, Georgia, early in the 18th century

purchased easements for adjoining marshlands to perpetually prohibit wet rice cultivation for the stated purpose of malaria control. Needless to say, the rôle of the mosquito in malaria transmission was not known at that time, although the association between malaria and wet rice cultivation was clearly recognized.

Not all of this seaboard problem was irrigation-connected in the normal sense of the term, since some wet rice cultivation was carried on in fresh marshlands enclosed by dykes with water-control structures which were located below the high tides of river estuaries or could admit water during flood stages. The general malaria problem in these ricelands was solved when rice cultivation became unprofitable and was abandoned due to the establishment towards the close of the 19th century of prairie land rice cultivation in the States of Arkansas, Louisiana and Coastal Texas.

Thereafter, malaria related to irrigation never became a major problem in the United States, although irrigated acreages expanded rapidly in some malarious States (Arkansas, California, Louisiana, Texas and New Mexico) and the malaria vectors, *Anopheles quadrimaculatus* and *Anopheles freeborni*, were found in great numbers in irrigated areas of these states. This does not mean that there was no malaria in these irrigated areas. In arid New Mexico and West Texas, most of the infections were probably irrigation-connected, but the incidence of malaria was of a relatively low order (Barber and Forbrich, 1933).

At the same time, anophelism sin paludism made its appearance in the irrigated rice-growing areas of Arkansas, Louisiana and Coastal East Texas (Barber *et al.*, 1926: 1929). Although adjoining unirrigated areas of Louisiana and Arkansas which were topographically similar to the irrigated rice-growing areas experienced hyperendemic malaria, infections were few in the irrigated areas. At the same time, *A. quadrimaculatus* was notoriously abundant in these irrigated regions. For example, observers noted over 300 *A. quadrimaculatus* adults on approximately 1 sq. ft. of resting surface and over 2,700 resting in one barrel (Barber *et al.*, 1926).

Likewise in California, Herms (1919) stated that rice culture as practised in that state was responsible for vast hordes of *Anopheles freeborni*, but there had been little or no increase in malaria as a consequence. Lenert (1922) stated that the more malarious portions of California were not found near the ricefields, but that there were few dwellings in their immediate vicinity.

Various hypotheses have been advanced for this phenomenon in some of the above areas. In Arkansas particularly, great hordes of the vicious biting nuisance mosquitoes *Psorophora* spp. are produced in the ricefield areas. This occasioned an unusually high degree of screening of houses and porches with consequent anti-malarial benefit. However, the question has never been definitively answered.

The discussion has dealt so far with the history of malaria in the past. With the current near-eradication of malaria in the United States, there is at present no significant malaria transmission either in irrigated or unirrigated areas.

Mosquito-borne encephalitides.—There are 3 recognized types of mosquito-borne encephalitis which affect man in the U.S.A.—the eastern, western and St. Louis strains. Two other strains have been tentatively identified.

Occasional outbreaks have been responsible for as many as 1,000 to 3,000 reported human cases a year in the U.S.A.; there is no known treatment and very young children usually have severe permanent brain damage. The case-fatality rate of these virus infections in man varies from 5 to 60 per cent, depending principally on strain. As many as 66,000 cases of encephalomyelitis in horses have been reported from one State (Iowa) in a single year (1938). Inapparent infections in man are believed to be very common. However, clinical encephalitis in man does not appear to be a principal cause of illness.

As with malaria- and filth-borne diseases, encephalitis is not limited to irrigated areas, although the principal recognized vector, *Culex tarsalis*, breeds readily in many types of irrigation waters. In many of the arid regions where irrigation is practised, production of this species in other breeding places is minor or nil. Also, there are at least 2 localities where encephalitis is endemic. Both are irrigated areas where *Culex tarsalis* is unusually abundant due to inefficient irrigation. The adult of this species rests mainly outside of houses and is not effectively controlled by D.D.T. house spraying (Reeves *et al.*, 1948).

The mosquito-borne encephalitides are considered more important than is indicated by incidence records. Infections are difficult to diagnose, are confused with other illnesses and are believed to be materially under-reported. All of the indigenous strains probably have not yet been identified. Outbreaks of far greater extensiveness may well occur (as in Japan), since the infected avian 'reservoir', the mosquito vectors and favourable climatic conditions have all been noted over large areas and cases of human encephalitis and/or equine encephalomyelitis which presumably were mosquito-borne have been reported at one time or another from every one of the 48 States.

Nuisance mosquitoes.—In the author's opinion, nuisance mosquitoes constitute the greatest health-comfort handicap provided by irrigation in the U.S.A. at the present time. Apart from their direct effect on human comfort, nuisance mosquitoes may at times impose important economic losses by lowering milk and beef production by bothering cattle, by reducing patronage at pleasure resorts and from the forced sale of farms and other real estate.

Aedes spp. constitute the principal nuisance mosquitoes in irrigated areas of the western states, the more important species being *Aedes nigromaculis*, *Aedes dorsalis* and *Aedes vexans*. *Psorophora confinnis* and *Psorophora discolor* comprise the principal nuisance mosquito problem farther east in the Arkansas ricefields and are also important in some of the western irrigated states, although secondary in importance to *Aedes*. The *Culex pipiens-quinquefasciatus* complex and *Culex tarsalis* present the greatest irrigation-nuisance problem in the genus *Culex*. Both species are reported to be vectors of one or more types of encephalitis with *pipiens-quinquefasciatus* incriminated so far only in unirrigated areas. *A. freeborni* in California and *A. quadrimaculatus* in the ricefields of Arkansas, Louisiana and East Texas become so prevalent at times as to cause primary nuisance problems, apart from their malaria-transmission capabilities. Since approximately one hundred species of mosquitoes have been identified in irrigated areas of the United States, it follows that some unmentioned species may possess primary local importance as nuisance problems.

The chief importance of the principal aedines in the western states is due not only to their abundance and aggressive biting tendencies toward man, but also because they are strong fliers, ranging up to 5 to 10 miles in sufficient numbers to constitute a nuisance. In this respect they rival the salt marsh species *Aedes sollicitans* and *Aedes taeniorhynchus*. Although some *Aedes* mosquitoes have only one or two broods each year, *A. nigromaculis* in California has as many as 18 generations and is a major problem from April to October (Thurman *et al.*, 1951).

Nuisance mosquitoes are produced in great numbers in some irrigated areas due to inefficient practices. As many as 3 lakhs of mosquitoes were collected from a standard electric light trap in an irrigated locality in California in 3 nights. This was in a mosquito-control area. As many as 20 million aedine eggs per acre have been found in pastureland in an area where there were several hundred thousand acres of pasture. More than Rs. 1 crore annually are spent in this state by public agencies for organized mosquito control, chiefly larvicidal measures, and it is estimated that Rs. 5 crores would be required for fully effective results. More than 50 per cent of the mosquito production in California probably occurs in irrigation water and in some districts irrigation is considered responsible for 85 to 90 per cent of the mosquito problem.

No other irrigated state is spending a like sum for recurrent mosquito control because of lack of financial resources and lower densities of human population, but avoidable nuisance mosquito production occurs in virtually all irrigated areas and in some of them has reached levels that interfere seriously with land occupancy and development.

IRRIGATION BACKGROUND.

Climate and irrigation.—As is commonly known, quantity and frequency of precipitation and its seasonal distribution are the principal determinants of irrigation need. But other factors such as temperature, humidity and air movement (winds) are also important, since they affect the transpiration of plants and evaporation from the earth's surfaces. Where the annual precipitation is exactly the same as the potential loss through evaporation and plant transpiration, there is neither water deficiency nor water excess, and the climate is neither moist nor dry. The line of equilibrium (zero line), along which precipitation balances moisture requirements, passes from north to south across the United States, dividing the country into approximately equal halves. The area west of this line is included in the 17 western states, while the eastern half of the country contains the remaining 31 states and the easterly portions of several of the western states. Due to high elevation or nearness to the Pacific Ocean, there are a few western areas, principally in the Pacific Northwest and the Northern half of California, where the climate is humid or even superhumid, but the climate of the 17 western states area as a whole is moisture-deficient. Some portions are sheer desert with a moisture deficiency approaching 100 per cent, others are classed as arid (deficiency between 40 and 60 per cent), semi-arid (deficiency of 20 to 40 per cent), and dry subhumid (deficiency between 0 and 20 per cent). Compared with India, the semi-arid portions are similar to the dry plains of the Punjab. The dry subhumid zone is comparatively limited in size, the greater part of the irrigable lands in the west being arid or semi-arid.

Annual precipitation in the United States averages 30 inches, varying from practically zero in some western deserts to over 60 inches in Louisiana. According to the U.S. Geological Survey, approximately 200 thousand million U.S. gallons of water are used daily for irrigation projects, industries, municipalities, farms and homes. Fifteen per cent or 30 thousand million gallons are obtained from wells, the remainder from surface sources. Total irrigation consumption is estimated at ninety-five thousand million gallons daily (an Imperial gallon is 1.2 U.S. gallons, liquid measure).

Water obtained from surface sources for all purposes is equal to about one inch of annual precipitation for the nation, as compared with 8 inches which flow to the sea in streams. Nevertheless, water rather than land is the limiting factor in western state irrigation since precipitation and stream runoff are much lower in this region and only a portion of the stream flows can be utilized for irrigation due to physical and economic limitations.

The irrigated areas of the western states probably have a greater variation in seasonal and annual mean temperatures than those of India. In some south-western irrigated areas, the mean annual temperatures exceed 70°F., while those of the interior high plains of the north-west may be as low as 43°F. In the former mean monthly temperatures may vary from 50°F. in January to 90°F. in July. In the latter, a typical range is from 13°F. to 68°F. Extreme temperatures for the west are from 66°F. to 134°F. The average length of frost-free period in irrigated areas is from as short as 40 days to as long as 320 days.

Irrigation is practised in every one of the 48 states. In the humid and super-humid east, however, irrigated acreages tend to be limited in extent because crops can be grown without irrigation. Such irrigation is called supplemental irrigation as required in humid areas either to meet the needs of very wet crops (such as rice in Arkansas, Louisiana and East Texas and cranberries in New Jersey), or to supply optimum moisture to drier crops during 1 or 2 relatively dry months in the growing season or for longer periods during years of abnormal drought. But about 90 per cent of the irrigated lands are located in the 17 western states area where even dry crops generally cannot be grown successfully without irrigation.

Hence this article mainly discusses irrigation in the west, which is composed of the following states comprising three-fifths of the area of the Continental United States: Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, New Mexico, Nevada, North Dakota, Oklahoma, Oregon, South Dakota, Texas, Utah, Washington and Wyoming.

History of irrigation in the United States.—The history of irrigation in the United States dates back well over a thousand years, primitive systems having been established by the American Indians in the west at that time. Modern irrigation is considered to have been introduced in 1847 by newly arrived white settlers in the State of Utah, although these early developments were primitive by present-day standards. The early developments were undertaken both by individual farmers and by loosely organized groups who joined together to construct and operate small irrigation systems for mutual private benefit. The irrigated land was individually owned.

There are many mountain chains in the west with steep, humid slopes which rise abruptly from flat, arid and semi-arid plains. The primary flows of small streams provided by melting snow in the growing season were sufficient for the irrigation of limited acreages near the bases of the hills. Land, bordering the streams, could be irrigated by simple diversion works, without provision for storage or long supply channels. As lands bordering the streams became utilized, arriving settlers occupied tracts which were farther removed; this required longer supply channels, because of increasing distance from streams *per se* and to provide the added gravity-head required. At the same time, small storage works became necessary due to the utilization of primary flows by earlier developments. The resultant increasing complexity and cost of irrigation projects brought forth a new type of organization—the local irrigation district. Such districts typically were formed by popular vote in tracts of irrigable land varying in size from 5,000 to 50,000 acres and were public corporations managed by locally elected officers, with power to tax all lands within their boundaries and to develop and operate irrigation systems. To a limited extent private corporations were created to develop irrigation systems and to sell water commercially under franchise as public utilities.

Another manifestation of the growth period which carried up to and through the creation of public irrigation districts was the establishment of the common law doctrine of water rights in the western states. This represented an effort to avoid chaos. Under this doctrine (also called the Doctrine of Appropriation) water-users had a continuing right to appropriate water from a stream in undiminished quantity. This in effect was a rule of 'first come first served' and was necessary to provide security of investment in irrigation systems and irrigated land. Stream diverters in each state filed a water-use claim with offices of public record when the development was undertaken. Upstream land owners were subsequently denied diversion rights which might jeopardize existing developments and potential downstream users were denied legal right to claim more than the residual flow of the stream as it reached them. This doctrine is opposite to the riparian rights doctrine which prevails elsewhere in the United States and which considers the stream as a resource to be shared in common*. All persons in consequence must let the stream pass through their property substantially undiminished in quality and quantity.

Further increases in the cost and physico-hydraulic complexity of irrigation projects and wide acceptance of the water rights doctrine set the stage for a third phase of irrigation development—the introduction and expansion of irrigation source-water projects financed by state and federal governments. The increasing scarcity of nearby sources of water supply and the need to 'reach out' for water soon surpassed the financial capacity of the local irrigation district form of organization. Allied to this were four other factors. The bringing of water from longer distances necessitated projects which provided large quantities of water in order to justify the greater cost of project development. Such projects provided water which would meet the needs of several local irrigation districts, which in consequence indicated development by a higher echelon of government. Some planned developments crossed state boundary lines; others which did not, drew from inter-state

* In some western states the 2 doctrines are concurrently recognized; with the riparian rights doctrine applying to water which has not been assigned by the state to appropriators.

streams which were to yield irrigation water to several states at many different diversion points. The Colorado River, for example, has 9 existing reservoirs serving 7 states and 1 foreign country, and 14 more dams have been proposed.

These larger planned projects also tended to become multi-phasic in function and this too favoured increasing state and federal participation. The unit cost of water as well as total project cost tended to increase rapidly with the prior exploitation of cheaper sources and it became advisable to incorporate hydel power into project plans in order to keep the cost of irrigation water within economic limits and to meet expanding demands for electric energy. Flood control and navigation became important in the planning of larger projects, both for intrinsic public benefit and as an added means of offsetting costs which otherwise would be charged to irrigation. More recently other socio-economic values have entered into project planning, including those of municipal water supply, stream sanitation, wild life and recreation, ground water replenishment, mosquito production and mining and industrial development; the latter 2 being in connection with hydel, navigation and water supply factors.

On some projects there is also need for hydel power to pump irrigation water. In 1951, for example, pumping plants were placed in service on 3 federal projects in the States of California, Colorado and Washington, to lift stream water for the ultimate irrigation of 27.5 lakhs* of acres. Two are trans-watershed diversions; the third at Coulee Dam on the Columbia River in Washington will irrigate nearby elevated tablelands with 16,000 cusecs of water lifted 346 feet.

Although a few state governments have been active, by far the larger burden of financing, planning and designing these 'third phase' projects has been borne by the federal government. The principal federal agency in irrigation activities is the U.S. Bureau of Reclamation (established in 1902) which is responsible for the construction and operation of practically all federal irrigation systems†. The U.S. Corps of Engineers also constructs and operates many multiple-purpose dams which may provide water for irrigation, but the irrigation canals leading from the dams are under the dominion of the Bureau of Reclamation.

The process of organizational growth of irrigation was not one of metamorphosis of structure but rather one of synthesis, since individuals, private groups, local governments and the federal government to-day all play important rôles in the control of existing source-water developments and its delivery to the fields. As for the actual utilization of water in crop production, this is not only almost exclusively private in character but is dominated by individual landowners. Communal land developments are negligible in quantity and transient in duration; the irrigated acreage held by private agricultural corporations is larger but is minor compared with individually-owned holdings.

* U.S. equivalents for Indian terms are :—

Re. 1/- = U.S. \$0.21

Cusec = Cubic foot per second

1 lakh = 100,000

1 crore = 10 million.

† Excepting irrigation works operated by the U.S. Bureau of Indian Affairs, which are capable of irrigating 8 lakhs of acres.

There are two principal explanations for the co-existence of these 3 phases of organizational pattern : public policy and technological development.

In the face of growing shortage in nearby surface water sources, individuals and local districts turned increasingly to the exploitation of ground-water resources for irrigation. This was made possible by the existence of abundant water in underlying sand and gravel formations, and by the development of efficient-power-driven deep well pumps capable of delivering large volumes of water from drilled wells. Between 1909 and 1939, for example, the yield of pumped irrigation wells increased by 500 per cent to nearly 1 lakh of cusecs and the acreage of irrigated land in the 19 states area, served entirely or partly by pumped wells, rose to 38 lakhs. Largely for this reason, progress in new-lands developments by local action has continued. In recent years, however, ground-water resources in the west have been over-exploited and this type of development appears to have passed its zenith.

As for public policy, federal and state governments have confined themselves to a rôle of source-water developers, in a sense 'water manufacture', and characteristically and traditionally dispose of water to local districts as far upstream as possible in the irrigation system. This is in accord with the political climate of the western states and of the nation as a whole.

Many federal source-water developments are planned and designed to provide and sell supplemental water to existing local irrigation districts in order to 'firm-up' local sources of supply. On 'new-lands' projects the U.S. Bureau of Reclamation fosters the establishment of local irrigation districts as early as possible following project completion, sells the downstream irrigation system to the local district under a plan of long-term repayment and thenceforth limits itself to the delivery of 'wholesale' water from the upstream supply canal. This policy of decentralization has obviously served to maintain and even increase the rôle of local government in irrigation activities.

Tables I to III, derived mainly from the data collected for U.S. Censuses of Agriculture, illustrate some of the discussion in this section.

The cost of source-water developments by type of irrigation organization is shown by the figures in Table I which are for irrigation works constructed in the 19th and 20th centuries which were in use in 1939.

TABLE I.

Type of enterprise.	Average investment per acre primary enterprises.
Individual and partnership ...	17.69
Co-operatives ...	25.65
Local irrigation districts ...	52.46
U.S. Bureau of Reclamation ...	89.81

TABLE II.

Percentage of irrigation capacity by type of source-water enterprise—comparison between 1910 and 1940.

Type of enterprise.	YEAR.	
	1910.	1940.
Individual and partnership	39.9	34.3
Co-operatives	30.5	28.5
Sub-total private individuals and groups ...	70.4	62.8
Local irrigation districts	3.9	17.7
Sub-total local enterprises	74.3	80.5
U.S. Bureau of Reclamation	3.9	8.4
Commercial companies	14.6	7.0
All others	7.2	4.1
TOTAL	100	100

TABLE III.

Increase of irrigation capacity in million acres by type of enterprise—comparison between 1910 and 1940.

Type of enterprise.	INCREASE.	
	Million acres.	Per cent.
Individual and partnership	1.5	19
Co-operatives	1.8	29
Sub-total private individuals and groups ...	3.3	23
Local irrigation districts	4.2	521
Sub-total local enterprises	7.5	50
U.S. Bureau of Reclamation	1.6	200
Commercial companies	—1.0	—50
All others	—0.3	—28
TOTAL	7.8	38

Remarks—Tables II and III.—1. The term 'local enterprises' has been used to indicate irrigation works resulting directly from the activities and participation of farmers. Actually some commercial enterprises and some in the 'all others' category may be local in character, but they do not represent 'farmer' types of irrigation organizations.

2. The thirty-year period 1910-1940 includes the eras in which both local irrigation district and federal 'source-water' enterprises became prominent. During this period (Table III), new developments by private individuals and groups continued, but at a less rapid rate (23 per cent) than new developments of all types (28 per cent). Commercial companies were unable to compete with public enterprise and declined sharply (50 per cent). Local irrigation districts were quite rare in 1910, serving only about 800 thousand acres, but increased very rapidly during the period (over 500 per cent) to nearly 5 million acres. The increase in federal developments was also great (200 per cent). In spite of the rise in local irrigation district and federal activities, however, the older 'individual and group' forms of organization still controlled nearly two-thirds of the 'source-water' works in 1940 (Table II).

The current trend towards federal source-water projects is somewhat obscured by the figures presented. Within the 30-year period, the great expansion in source-water developments by local irrigation districts took place between 1910 and 1930 (from 800 thousand acres to 4 million 800 thousand acres, as compared with a gain of from 786 thousand acres to 1 million 900 thousand acres by the U.S. Bureau of Reclamation projects). From 1930 to 1940, however, the acreage capacity of new federal developments was $2\frac{2}{3}$ times that of new local irrigation district developments. At the time this page is being written, preliminary data for the decennial census of 1950 is not available, but the indications are that the trend established in 1930 to 1940 has continued. New irrigation districts are being formed but mainly to serve as 'middlemen' for the distribution to farmers of water purchased at wholesale from federal projects. Established local irrigation districts are still developing new sources of supply in some cases, but at a much slower rate than previously.

The rôle of the U.S. Bureau of Reclamation in source-water development also tends to be obscured by the large proportion of supplemental source-water developments in its programme. These are not reflected in many statistics (including Tables I to III), which credit irrigated acreages to the primary enterprise. Between 1910 and 1940, the Bureau constructed source-water projects which were capable of supplying supplemental water to 35 lakhs of acres of existing irrigated lands which were credited to other types of enterprise, and exceeded any other type of enterprise as a supplier of supplemental water.

Thus, with certain significant differences as to organization and with compression of time, the history of irrigation source-water development in the United States has followed the more lengthy history of India.

There is one difference that might be remarked on, namely, the relative absence of small local tanks for irrigation in the United States. Individual irrigated land holdings tend to be far larger in size than in India and such tanks in consequence are too small to serve an irrigation purpose. However, many farmers in both irrigated and unirrigated areas have small tanks for the watering of livestock and

for fish cultivation. These are formed by low earth dams built across small streams or dry valleys which catch seasonal runoff, or earth excavations which are filled with water from small wells.

Extent of irrigation.—The extent of irrigation in the United States is measured by censuses in three ways, the significance of which can be understood best by examining them in terms of a specific irrigation enterprise. A certain local irrigation district embraces tracts of land totalling one lakh of acres. Three-fourths of a lakh are deemed suitable for irrigation from the combined standpoints of soil characteristics, topography and other factors. This is termed 'irrigable acreage'. The irrigation works however are capable of supplying only half a lakh, due to limitations in the supply of source water and in the capacities of impounding structures and transmission channels. This figure is called 'acreage which irrigation works are capable of supplying'. During the census year, however, only two-fifths of a lakh actually were irrigated. This is termed 'irrigated acreage'. The quantitative relationship between these three values is shown by the following figures for the 17 western irrigated states, as reported in the 1940 Census of Irrigation:

Acreage irrigable in existing enterprises	...	...	3.02	crores.
Acreage existing works were capable of supplying with water	...	...	2.70	„
Acreage irrigated	...	...	2.04	„

'Irrigable acreage' is probably the least significant item. It does not properly reflect the potentialities of irrigation because it is limited to areas located within existing enterprises. This term was probably adopted by the census takers in an attempt to appraise the extent to which irrigation could be expanded readily, but the true measure of irrigation expandability in arid regions is water rather than land.

Of the remaining two terms, figures on acreage of land actually irrigated during the crop year immediately preceding the census-taking period are more commonly cited, but the capacities of existing works may provide at times a more reliable index of irrigation development. This is demonstrated by the following equation which indicates the variables which influence the land area under irrigation in *any specified year*.

$$A = (C - R) \pm (C^1 - R^1) - (I \pm E \pm W \pm P \pm L).$$

Definition of Terms:

A = Total acreage under irrigation.

C = Irrigation works capacities of enterprises in areas which never receive sufficient precipitation and must be irrigated each year.

R = Acreage equivalent of abnormal drought which may reduce the supply of source water in 'C' areas below irrigation requirements.

C¹ = Irrigation works capacities of all remaining areas, which in some years receive ample rainfall on the fields and in consequence do not practise irrigation every year.

R¹ = Acreage not irrigated in C¹ areas due to abundant precipitation.

I = Acreage of irrigable land which has not yet been connected to irrigation systems but for which water is available.

E = Acreage equivalent of the supply of water available in existing irrigation works, in excess of irrigated and irrigable lands in existing enterprises.

W = Previously irrigated land which is no longer irrigated due to water-logging, salinization, alkalization or other factors which have retired the land from irrigation agriculture. Most of this is wasteland.

P = Effect of prevailing crop prices on acreage of land placed under cultivation and irrigation.

L = Effect of prevailing labour supply along the same lines.

It will be seen from the above that irrigation agriculture in the United States is sensitive to many climatic and socio-economic forces and the general correlation between irrigation development and area irrigated is affected by many variables.

The Census of Irrigation, under the Bureau of Census, U.S. Department of Commerce, is performed in the first year of each decade, and is part of a more comprehensive census in which the human population is counted for the nation and data on industry, agriculture, commerce and many other conditions and activities are collected.

The Census of Irrigation was first undertaken in 1890 for the arid western states region. Subsequently, this specialized census was extended in scope to include three humid states: Arkansas, Louisiana and Florida; the last named having been added in 1940. Irrigation is practised in all of the 28 remaining humid states, but the total acreage in these states is only about half a lakh.

Because of changes which have occurred in the census-taking area, it is customary to segregate figures for the 17 western irrigated states area from the 3 humid states.

TABLE IV.
Irrigated acreage in lakhs.

Crop year.	17 STATES AREA.		TOTAL AREA.	
	Acreage.	Annual increase ¹ .	Acreage.	Annual increase ¹ .
1860 ⁴ ...	4	...	...	...
1880 ⁵ ...	36	3.2	37 ⁵	...
1890 ⁵ ...	75	3.0	77 ⁵	4.0
1900 ⁵ ...	140	6.5	144 ⁵	6.7
1910 ⁵ ...	186	4.6	192 ⁵	4.8
1920 ⁵ ...	189	0.3	195 ⁵	0.3
1930 ⁵ ...	204	1.5	211 ⁶	1.6
1940 ⁵ ...	249	4.5	262 ⁶	5.1

Footnotes.— ¹Annual increase for preceding 10-year period.

²Source: Israelsen (1950).

³Source: Census of irrigation for following year.

⁴Source: Preliminary figures for 1950 census.

⁵10 states area, including Arkansas and Louisiana.

⁶20 states area, including Florida.

TABLE V.

Acreage in lakhs which existing works were capable of supplying with water.

Census year.	17 STATES AREA.		19 STATES AREA.	
	Acreage.	Annual increase.	Acreage.	Annual increase.
1890 not reported	...	...	...	...
1900 " "	...	...	...	...
1910 " "	193	...	203	...
1920	251	5·8	260	5·7
1930	251	0·0	261	0·1
1940	270	1·0	281	2·0
1950 not yet available	...	...	...	...

Earlier in this article, under the heading 'History of Irrigation', it was indicated that irrigation in the dry western states was approaching maturity in rate of growth. This is unquestionably the long-term trend, as is indicated in the following section on the future of irrigation. In view of this it is surprising that the acreage irrigated increased by 45 lakhs between 1939 and 1949, as compared with only 15 lakhs in the preceding 10 years, and was exceeded only in the 20-year period 1899 to 1919. Statistics on all irrigation attributes are not yet available from the 1950 Census of Agriculture and it is too early to make a complete evaluation. In particular, information is not available on the changes in capacities of existing works (Table V). Nevertheless, some of the incomplete preliminary data are revealing.

Comparison of irrigated acreages by states is of interest. The great percentage increases between 1939 and 1949 occurred in the humid states, as indicated in Table VI.

TABLE VI.

	Acreage irrigated.		Per cent increase.
	1939.	1949.	
Arkansas	161,001	418,644	159
Florida	132,362	362,009	174
Louisiana	447,095	598,056	34
Sub-total	741,058	1,370,609	86
17 western states	20,395,043	24,868,899	22

The percentage increase for the '3 humid states' area was exceeded only by one western state of irrigation importance: Texas. For the 17 western states area there was a decrease in 5 states, a relatively small increase (under 1 lakh of acres each) in 6 states, substantial increase in 4 states (between 2 and 5 lakhs each), and a large increase in only 2 (California and Texas). In these 2 states irrigated acreage increased by a total of nearly 37 lakhs, as compared with a net increase of only 18 lakhs for the remaining 15 western states. Irrigation in California and Texas accordingly warrants special scrutiny.

California is the leading state in irrigated acreage and has been for some time. Irrigated acreage rose from 51 lakhs (1939) to 66 lakhs (1949), a gain of 15 lakhs. The position of Texas changed from eighth to second place in the 10-year period. Irrigated acreage there increased from 10 to 31 lakhs, a gain of 21 lakhs. According to the Bureau of the Census, these increases largely were realized by new source-water supplies in the form of pumped and drilled wells (tubes). This was also the case for the western state having the third greatest gain (Arizona).

The increase of irrigated area accordingly was realized largely by expansion of irrigation works capacities, encouraged by high crop prices. The area of greatest new development was the interior high plains of Texas. In general the irrigation development, which took place in all 3 states (Arizona, California and Texas), involved ground water resources which had been previously exploited in varying degree.

Since irrigation from pumped wells is characteristically undertaken in these states by individual farmers, it is significant to note that the 10-year gain was achieved largely by private individual initiative, although some ground water irrigation systems were developed by private groups and local irrigation districts. Under the impetus of favourable crop prices and increasing mechanization, farmers can develop new ground water supplies on their properties and place new lands under irrigation in a few months, but development of remaining surface water sources requires large-scale construction by National Government. (The largest post-war irrigation projects of the U.S. Bureau of Reclamation have been in California. Although some of these had been completed by 1951, this programme did not importantly affect census results for the 1949 crop year.)

Other statistics of interest in the preliminary census reports for the 17 western states are:—

The average cost of water to farms for the 1949 crop year approximated Rs. 25 per acre. The capital investment in irrigation works and water rights increased by 80 per cent to a total of Rs. 915 crores and by over 100 per cent in 5 of the 17 states. There were 1.12 lakhs of irrigation source-water enterprises, of which 91 per cent were owned and operated by individual farmers, each serving a single farm. The total number of irrigation enterprises of all types, however, served an average of 3 farms. This was due to the larger size of group and government enterprises. According to the Bureau of the Census, the 116 source-water projects constructed by the U.S. Bureau of Reclamation supplied all or part of the irrigation water, directly or indirectly, for about one-fifth of the irrigated land in the western states in 1949. There were approximately 3.3 lakhs of irrigated farms in the 17 western states, with an average of about 75 irrigated acres per farm.

The average area tended by each full-time farm worker has not been reported, but is estimated at about 50 acres.

The phenomenal increase of ground water development discussed in the preceding section, continued during the 10-year period in the 17 western states and the 3 humid states. In 1949, 68 lakhs of acres (26 per cent of total) were served exclusively by pumped wells and 25 lakhs (9 per cent) were served jointly by surface and ground water, a total of 93 lakhs (35 per cent). This represents an increase of 145 per cent over the 38 lakhs so irrigated in 1939, as compared with an increase of only 24 per cent in the irrigated acreage served by all types of water supplies.

Extent in relation to other countries.—The United States is believed to rank third among the nations of the world in extent of irrigation. Israelsen (1950) gives a list of countries and irrigated acreages, based mainly on estimates assembled by the U.S. Bureau of Reclamation as of 1948. He reports 5 crores of acres under irrigation in China, with the observation that 'some writers estimate the area for China as about (8 crores of acres)'. India and Pakistan together are credited with 6 crores by Israelsen. The Central Board of Irrigation of India reported elsewhere 4.8 crores for India in that year, which indicates the irrigated acreage in Pakistan totals about 1.2 crores, placing it in fourth position. According to the figures of Israelsen, fifth to eighth positions are occupied by Russia, Japan, Egypt and Mexico, which are credited with acreages of eight-tenths to six-tenths crores each.

Future of irrigation.—The physical criteria which determine the potentialities of new irrigation are: water supply, supply of irrigable land and distances and topography between the two. Social and economic criteria include food supply needs, changing costs of irrigation development relative to other variable items of income and outgo in irrigation agriculture, and subsidy by government. Here again we are concerned with an equation having many variables. For this reason few attempts to predict the future extent of irrigation in various areas have been accurate.

For the 17 western states area, as a whole, water supply rather than irrigable land is the limiting physical factor. In theory, it is possible to determine the upper limits of irrigation development by estimating the water and land resources of each major stream basin and accepting the lower of the 2 results as the irrigation ceiling for the basin. The sum of all river basins in an area determines its potentialities. But this is not so simple in practice. Trans-watershed diversions from a water-surplus basin to a water-deficit basin become feasible as economic needs for irrigation become more acute. The flows of streams can be determined by measurements at a few gauging stations but the suitability of extensive land areas for irrigation may require large-scale surveys. Thus, the 10 lakhs of acres 'blocked in' as irrigable during the early planning stages of a project may dwindle to 2 lakhs after the soil technologist has completed his detailed field studies. Moreover, surface water resources can be measured more accurately by limited advance studies than can ground water resources. The more important errors in estimates of the recent past have resulted from underestimating the future extent of ground water development (in many cases overdevelopment), of irrigation development in relatively new regions where irrigation had not been popular previously

with farmers, and in overestimating the area of irrigable land in other regions as a result of lack of adequate soil surveys. Many proposed developments also are alternative projects; if some are built, others cannot be built due to competition for water supply, and it is necessary to guard against such duplication.

According to the U.S. Department of Agriculture (1948): 'Perhaps the most optimistic statement of overall irrigation possibilities from the standpoint of irrigable soils (in the 17 western irrigated states) appears in the National Resources Board Report of 1936.—This report mentions an ultimate irrigable acreage of (5.15 crores)'. At the time this estimate was published, the irrigated acreage was about 2 crores, indicating a potential development of slightly over 3 crores. The estimate was subdivided by states.

The U.S. Department of Agriculture (1948) reported that subsequent plans of the U.S. Bureau of Reclamation for 'further development underway or proposed' as federal undertakings, greatly exceeded in some states the estimate made 12 years earlier by the National Resources Board. It also stated: 'Re-examinations indicate that the delineations of the National Resources Board were too optimistic in many areas. Under conditions likely to occur in the foreseeable future, not more than one-third to one-half of this further potential is likely to be developed'. These figures agreed closely with the list of projects underway and proposed by the Bureau of Reclamation, referred to previously, which totalled 1.3 crores of acres. Since the status of many of these 'proposed' projects was very nebulous, the Department of Agriculture stated: 'As irrigation is pushing closer and closer to the maximum limit of available water supply in many areas and because even now some areas are overdeveloped in terms of permanent water supply, and may eventually decline, it can reasonably be expected that within the next 25 years an additional ($\frac{1}{2}$ to 1 crore) acre will be newly irrigated'. The base line of existing area of irrigated land used in the report was an acreage of 1.95 crores, based on a 1945 Census of Agriculture. The Department of Agriculture accordingly estimated a total irrigated acreage of 2.5 to 3 crores by 1973. By comparison, the subsequent 1950 census of irrigation (based on the 1949 crop year) reported 2.49 crores of acres under irrigation one year after the aforementioned report was issued. This approximated the lower limit set for 1973.

The unexpectedly large rise in irrigated acreage reported in the 1950 Census of Irrigation, coupled with increasing rate of population growth in the United States, has given rise to a recent recasting of estimates by some irrigation authorities. This is indicated by Wood (1951), who states:

'At present rate of increase, U.S. population will be 190,000,000 by 1970. The 40,000,000 increase will probably mean the irrigation of every available acre for which water is available regardless of cost. There is a tendency to view an irrigated acre as a food-producing unit which may go on producing for hundreds of years.'

The ceiling imposed on the extent of arid area irrigation by soil and water conditions could be altered substantially in the United States by technologic developments for freshening sea water and conditioning soils. According to Laurence (1952), the American Research and Development Corporation of Boston, Mass., has developed a continuous-flow electrolytic method of treating sea water,

employed in conjunction with a new synthetic membrane. Two-thirds of the water treated is economically desalted, the salt content being concentrated in the remaining one-third, which may be wasted or used for by-product recovery. Southern California is the principal irrigated area which would be benefited by the economical bonification of sea water.

According to Laurence (1951), the Monsanto Chemical Co. has developed a synthetic soil-conditioner called Krilium, which has been tested for 3 years by a large number of soil scientists and is expected to be in mass production by 1953. One pound of Krilium is reported to have essentially the same effect on soil structure as 200 lbs. of peat moss or 500 lbs. of commercial compost. By restoring the flocculation of soil particles it shows promise in the reclaiming of saline and alkali soils; available as virgin, uncultivable land or resulting from past improper irrigation.

Irrigation by sprinklers also has become increasingly popular in some areas, due to introduction of light weight portable pipe lines, increasing scarcity of water and higher crop prices. This method can be practised on some lands which are not irrigable by other methods, thus increasing the irrigation potential.

At the present time, however, the overall picture in the 17 western states area is one of acceleration in growth of irrigation towards a relatively fixed ceiling. This upper limit appears to be in the range of 4 to 5 crores of acres of land under actual irrigation in a given year, as compared with at least 8 crores for India (and possibly far more). The higher figure cited for the United States, however, is twice the irrigated acreage in 1949 (Table IV).

The potentialities of supplemental irrigation in the 31 humid states are not subject to the physical limitations that exist in the arid states, and cannot be estimated on a quantitative basis. The supply of source water is ample for irrigation development of large proportions. The total reported acreage of farmland in the United States in 1949 was 113 crores; most of this was in the humid area. Only about 2 per cent of all farmlands in the nation were irrigated in that year. Irrigation potentialities in the humid region are contingent mainly on socio-economic factors and on local patterns of seasonal rainfall in the crop-growing season. Since irrigation is not essential to crop production in humid areas, it tends to be practised in the occasional farm rather than in a majority of the farm in any locality except in rice-growing areas. Ground water supplies developed as private individual enterprises generally are more suitable in such situations.

Supplemental irrigation in the humid region will undoubtedly expand, but whether it will ever be practised on a large scale outside of the present 3 humid irrigated states (Arkansas, Louisiana and Florida) is indeterminable.

Principles of water application.—'Soil water may be classified as hygroscopic, capillary and gravitational. Hygroscopic water is in the surface of the soil grains and is not capable of movement through the action of gravity or capillary forces. Capillary water is that part in excess of the hygroscopic water which exists in the pore space of the soil and which is retained against the force of gravity in a soil that permits unobstructed drainage. Gravitational water is that part in excess of the hygroscopic and capillary water which will move out of the soil if favourable drainage is provided' (Israelsen, 1950).

Rice and other crops which grow in fields that are flooded a large part of the time, utilize both capillary and gravitational water, but over 90 per cent of the irrigated acreage in the United States is devoted to crops which require air in the soil-root zone most of the time, as grown under what is known as 'dry crop' irrigation in India. Such crops utilize neither gravitational water nor the unavailable hygroscopic water, depending on capillary moisture in the soil. Furthermore, not all of the capillary moisture can be utilized: when the concentration becomes too low, plants reach the permanent wilting point before they can extract the remainder; when it approaches the concentration level of gravitational water, the soil air supply becomes inadequate for the growth of many types of plants.

The objective of soil irrigation practice is to maintain a suitable supply of capillary moisture in the soil reservoir during the growing season by applying water as infrequently as possible. Excessive frequency of water application is considered undesirable since this is harmful to plant root development and to soil characteristics and results in excessive evaporation and labour costs.

To attain this objective the capacity of the soil-root zone is determined and a quantity of water equivalent to the 'field capacity' is applied during each irrigation period. Under this procedure the soil reservoir is considered empty at the permanent wilting point and full at the point where gravitational water is formed. A slight over-supply of water may result where the soil air supply is unduly reduced but the effect is very temporary during the growing season and is not harmful. The 'field capacities' of different croplands vary over a considerable range, being influenced principally by depth of root zone (determined by type of crop and depth of topsoil), soil texture and soil structure.

It will be seen from the above that the principles of water application are in close agreement with mosquito-prevention interests wherever 'dry crop' irrigation is practised. Mosquito breeding largely occurs only where good agricultural practices are not followed, i.e. where inefficient irrigation is practised.

The following agricultural objectives are listed because of their relationship to mosquito-prevention principles:—

- (1) Flooding of the fields should be temporary—usually a few hours—since flooding for a longer period will exceed 'field capacity'. Water standing on the ground for longer periods is detrimental to soils and crops in other ways as well.
- (2) Water should be applied as infrequently as possible.
- (3) Application of water only to the point of 'field capacity' results in minimum deep percolation loss with resultant absence of distant seepages and waterlogged areas.
- (4) Applications of water which do not exceed 'field capacity' tend to avoid a high ground watertable within and near the fields, with resultant absence of local seepage and waterlogged areas, alkali and saline soils, etc.
- (5) Efficient application of water demands adequate levelling of the fields. Resultant elimination of low spots prevents residual ground pools in the fields after the irrigating period.

Kinds of irrigated crops.—The list of irrigated crops grown in the west is very lengthy. The more important ones listed in the 1940 U.S. Census of Irrigation for the year 1939 are given below :—

Crop type.	Acreage in lakhs.	Acreage in lakhs.
All small grains	...	33·8
Barley	9·8	...
Oats	4·9	...
Rice	8·5	...
Wheat	9·0	...
Other small grains	1·6	...
Cotton	...	7·6
Sugar beets	...	6·3
Maize	...	6·4
Sorghums	...	3·1
Potatoes	...	3·7
Alfalfa seed	...	2·2
Beans and peas	...	7·2
Orchards and vineyards	...	13·7
All hay crops	...	64·5
Alfalfa	36·5	...
Wild hay	16·5	...
Clover and timothy	5·4	...
All other hay	6·1	...
GRAND TOTAL	...	148·5

The economic importance of different crops does not correspond to the number of acres devoted to their production, since yield and market value per acre vary greatly. The most valuable crops listed are probably alfalfa seed and some types of orchard-grown fruits and nuts, the least valuable is wild hay.

Some crops can be grown in parts of the west without irrigation, such as wheat and hay; and the unirrigated acreage may greatly exceed that irrigated. With others, the benefits of irrigation are so great that practically all of the production in the western states is from irrigated lands. These include cotton, rice and sugar beets.

The only crop listed which requires prolonged flooding is rice, a wet crop. Among the intermittently irrigated crops however are some which might be classed as moist, such as alfalfa and cotton; while others, such as wheat, have a very low moisture requirement. (In other parts of the United States, cotton is not considered a moist crop, but a special strain of seed was introduced in 1948 in the south-west.) The average yield of irrigated cotton was 580 lbs. per acre (1-1/6 bales) in 1939, and is considerably higher at present; up to 3 bales per acre in some tracts.

Rice production in the United States.—Although irrigated rice production in the United States is not as important as in Asia, it deserves special attention. As the only wet crop grows in this country to any extent it possesses a special significance in mosquito ecology. Rice production also has world-wide importance as an irrigated crop and possesses special malariogenic characteristics. Rice

culture will be discussed principally from a standpoint of contrast with practices elsewhere.

California and Texas are the only western irrigated states in which rice is grown in commercial quantities. Rice is produced in Texas however only along the humid east coast, which is actually located in the eastern half of the United States and which climatically and topographically is similar to the nearby ricelands of Arkansas and Louisiana.

Rice production in the 4 important rice-growing states in 1950 totalled 19 lakhs of short tons. Unirrigated domestic rice is not commercially grown in the United States. According to the U.S. Department of Agriculture the irrigated acreage under rice cultivation in 1950 was as given below :—

			Lakhs of acres.
<i>Western states area :</i>			
California	...	...	2.3
<i>Eastern states area :</i>			
Arkansas	...	...	3.4
East Texas	...	...	4.8
Louisiana	...	...	5.5
TOTAL	...	...	16.0

This is an increase of nearly 100 per cent over the acreage reported for 1939 in the Census of Irrigation. The acreage actually used for rice culture over a 3-year span is considerably greater than the above since crop rotation is customarily practised. Land used for rice growing one year is commonly utilized for the unirrigated culture of legumes for 2 succeeding years in order to replenish the soil. Wet fallow is not practised either in the rotation years or in the non-growing season of the rice year.

There are no nursery or transplanting beds. Rice seed is sown directly on the field from which it is harvested. The yield may not be as high with this practice but labour costs are too high to permit rice to be transplanted. In fact rice culture is a highly mechanized operation. Due to the level land in which it is grown, all steps in land preparation, sowing, fertilizing and harvesting are carried out by motorized equipment. Sowing practices are illustrative. Some seed is sown by mechanical drills on dry land but principally it is sown on flooded fields by low-flying airplanes, which at the same time apply commercial fertilizer, and D.D.T. wettable powder to control rice water-weevils and for the incidental control of mosquitoes.

The varieties grown are short, medium and long-grain rice. The average yield per acre of bagged, milled rice in 1950 was 2,360 lbs. The yield in 1939 in terms of paddy was 51.5 bushels per acre.

Rice is sown between April 10 and June 1, maturing in September or October after a growing season of 125 to 170 days, depending on variety. It germinates in 7 to 20 days and the mature plants reach a height of 39 to 50 inches. The growing season does not permit more than 1 crop annually.

In Arkansas, the first irrigation is carried out about 4 weeks after sowing, when the rice plant is 4 to 6 inches high or just before sowing when aircraft are used. The water is maintained at a depth of about 2 inches for 3 to 4 weeks by continuous flow varying from 1 to $1\frac{1}{2}$ cubic feet per minute per acre.

The second application of water takes place from 10 to 14 days after surface water from the first application disappears. This second application is held on the field at a depth of about 5 inches until 2 weeks before harvesting when it becomes necessary to dry the ground for mechanical harvesting. The average duration of the second application is $2\frac{1}{2}$ months.

In California the fields are generally flooded in late April or early May, just before sowing the wet germinating seed by airplane. Some California farmers use 2 irrigation cycles as in Arkansas; others keep the field continuously flooded until shortly before harvesting.

Intermittent irrigation with many cycles, as carried on to some extent in India, Portugal, Java and elsewhere, has never been utilized to any extent by farmers in this country. Farmers tend to keep the fields flooded all or most of the time not only because they feel rice needs this much water but also to prevent terrestrial weed growth.

The rice-growing season conforms roughly to the breeding season of the ricefield anophelines *A. quadrimaculatus* and *A. freeborni*, and to the malaria season, and water is in the fields during the greater part of these seasons.

Unlike some anophelines elsewhere, most of the production of these 2 species which is associated with rice cultivation, takes place in the flooded fields rather than in ditches, structures and minor pools and seepages. Also, breeding in the fields is not inhibited by tall stands of rice to the extent that occurs elsewhere with some other anophelines, as with *Anopheles culicifacies* in India. For example according to Sperbeck (1951) there were 3 peaks in the larval density of *A. freeborni* in ricefields under study in California in 1950. The third peak was during the week of September 9 in ricefields which were planted in late April and the first week of May and apparently harvested in October. *A. quadrimaculatus* in the eastern states is also somewhat shade-tolerant and is found breeding in fields where there is mixed sunlight and shade.

To a considerable extent the greater importance of breeding in the fields may reflect differences in irrigation systems. In Arkansas for example a single field of 100 acres may be served by a deep well pump located at the high end of the field. Supply and drainage channels are negligible in length compared with systems where water is brought from a distance and delivered to very small individual fields which are also interspersed by other land on which seepages and small pools may be found.

Although field-production is the greater, it does not follow that production outside of the fields is unimportant. This is especially true of California where the size of field is often small, with consequent greater occurrence of stagnant ditches,

supply channels and ground pools. According to Markos (1951) 'Aside from the ricefields proper, the problem is further complicated by mosquito-breeding in drainage ditches, roadside pools and seepage areas'. Even in the larger eastern ricefields similar problems exist, although to a lesser extent.

Culex breeding in ricefields in California follows the same general pattern described for the anophelines. As for *Aedes* and *Psorophora*, where continuous flooding is practised, there is only one brood each season from any single ricefield since the female does not oviposit on water. But where there are 2 irrigation cycles they are a problem during the greater part of the growing season. As in Arkansas there may be a 6-week period during the spring between the time the first and last field in the flight-range area is first flooded. The elapsed time is nearly as long for the second flooding. Most of the more important species are many-brooded.

According to Chambliss (1920), irrigated rice in Arkansas prairie lands requires 48 inches of water during the growing season. The mean annual temperature in this area is 61.5°F., with hot summer months, and the soil is a compact silt loam with an extremely impermeable subsoil which lies at an average depth of 16 inches. The average annual precipitation is 52 inches, of which 20 inches falls during the rice-growing season. The deficiency of 28 inches is supplied by irrigation.

In California, it is reported that up to 11 ft. of water is applied on irrigated riceland during the growing season, but there is no good evidence that this much is needed. According to Blaney (1951), rice has a consumptive use factor of 1.0, indicating that in very hot climates the water duty of this crop as supplied by irrigation and precipitation during the growing season is in the order of 4 ft.

Irrigation methods in the United States.—Irrigation water is applied to land in the United States by four general methods, namely :—

(a) Flooding the surface, (b) in furrows, (c) sub-irrigation, and (d) sprinkling. These methods are subdivided as follows (after Israelsen, 1950) :—

(a) Flooding :

1. From field ditches.	3. Check.
2. Border.	4. Basin.

(b) Furrow :

5. Large deep furrows for such cultivated row crops as potatoes, corn and asparagus, and for orchards.
6. Corrugations or small shallow furrows for small grains, alfalfa and sugar beets.

(c) Sub-irrigation :

7. Controlled by lateral supply ditches.
8. Uncontrolled by excess application of water to higher lands.

(d) Sprinkling :

9. Pipe distribution system.
10. Circular system.

The more common methods are those listed under flooding and furrow. The 10 methods are largely self-explanatory except for those listed under flooding. These latter are described briefly below :

1. *Ordinary flooding from field ditches.*—This is the application of water from field ditches without any levees to guide its flow or otherwise restrict its movement. The water is brought to the field edge in permanent supply ditches and distributed within the field from temporary ditches built across the field which are spaced from 50 to 150 ft. apart, depending on the slope of the land, soil texture and depth, size of stream and type of crop. Except for wild-flooding, this type of uncontrolled flooding is the most primitive and inefficient method of irrigation and is indicated mainly on land with irregular surfaces that has not been properly prepared for irrigation. It is used for dry crops, principally pasture, hay and alfalfa grasses. Submergence of the land should be very temporary.

Wild-flooding consists of dumping water from higher lands on to natural pasture lands. It is practised to a considerable extent but is not a recognized method.

2. *Border-flooding.*—Dividing the farm into a number of strips, preferably not over 30 to 60 ft. wide and 300 to 1,300 ft. long, separated by low dykes (levees, checks or borders), is designated the border-strip method. Water is turned from the supply ditch into these strips along which it flows slowly toward the lower end, wetting the soil as it advances. The surface is essentially level between borders, so that the advancing sheet of water covers the entire width of land strip; but parallel to them the surface slopes somewhat according to the natural slope of the land.

3. *Check-flooding.*—With border-flooding the dykes cross the contours of the land and the water flows with the slope of the land. The narrow ends of the fields are not dyked. With check-flooding the long dykes (in a roughly rectangular field) tend to follow the contours and water fed from the supply ditches which border the narrow ends of the field, advances over the field due to head of water rather than because of land slope. This method requires level ground along the long dimension of the field and nearly level ground along the transverse dimension. The size of individual field in western dry crop areas varies from $\frac{1}{2}$ to 5 acres ($\frac{1}{2}$ to 2 acres recommended), and in the Arkansas ricefields from 10 to 100 acres, averaging 50 acres. The dykes are 10 to 12 inches high and have flat slopes so that farm machinery can cross. Crops are planted on the dykes themselves as well as in the fields.

The border-strip method is adapted to so-called 'normal' soils in the west. The check method is adapted to either extreme—very porous soils that require quick flooding at high rates in order to wet all portions of the field, or heavy soils into which water percolates so slowly that they are not sufficiently moistened during the time a sheet of water flows over them, making it necessary to hold the water temporarily on the surface to assure adequate penetration. The check method is also indicated for a very wet crop, such as rice, where water is to be held on the land for prolonged periods. Its main use however is with dry crops grown in abnormal soils.

4. *Basin flooding.*—The basin method of flooding is essentially the check method especially adapted to the irrigation of orchards. On some farms a basin is made for each tree, but under favourable conditions of soil and surface slope from 2 to 5 or more trees are included in one basin.

5. *Furrows*.—Referring back to the 4 general categories of methods, irrigation by flooding and furrows are the most widely practised methods. Although less efficient than sprinkling and sub-irrigation, a much lower investment is required. The furrow method is adapted to row crops, such as maize, and to orchards.

Large deep furrows vary in depth from 3 to 12 inches and are spaced from 3 to 12 ft. apart, depending on soil and crop characteristics. The slopes of furrows vary from 10 to 150 ft. per 1,000 ft., with 10 to 30 ft. being preferable. The recommended length of furrows is from 300 to 500 ft., but in practice they vary from 100 ft. in some gardens to as long as 2,500 ft. Water is admitted to the high end of each furrow, commonly by making a temporary break in the bank of the supply ditch or by use of a portable inverted siphon tube.

Any intermittent irrigation method which employs an advancing sheet of water must limit the length and rate of water travel if it is to be efficient. This applies to both the flooding and furrow methods. If the rate is too rapid (due to steep slope), water will run off from the fields, or if checked at the field edge, adequate irrigation of the near end of the field requires over-irrigation of the far end. If the slope is too flat or if it is satisfactory but the furrow is too long, excessive quantities of water may be absorbed by the near end of the field before the advancing water can adequately irrigate the far end. The common water-waste and mosquito-breeding consequences of the first condition are ponded water in the far end of the field or in adjoining areas, unless adequate drainage is provided; that of the second is excessive deep percolation, often resulting in seeped or waterlogged lands some distance from the causative fields.

Quantity of water applied and number and frequency of applications.—These vary over a wide range, depending on type of crop, length of growing season, precipitation and other climatic factors, soils and irrigation practices. With some perennial forage crops, such as alfalfa for example, the growing season coincides with the frost-free season. This may be as long as 320 days in the irrigated areas of South-eastern California to as short as 40 days in the high plains of Montana. On the other hand, some annual crops mature in 3 months, such as beans and potatoes. Irrigation efficiencies vary greatly, depending on type of soil, land preparation and farm management practices.

According to Houk (1951), the annual water requirements by type of crop are as follows:—

Alfalfa and other forage crops, including pastures	1.0 to 6.0 ft.
Potatoes, sugar beets and cotton ...	1.5 to 3.5 ft.
Cereals, except rice ...	1.0 to 2.5 ft.
Rice ...	4.5 to 9.5 ft.
Deciduous fruits ...	2.0 to 3.5 ft.
Small fruits and grapes ...	1.5 to 3.0 ft.
Citrus fruits ...	2.0 to 4.0 ft.
Walnuts and almonds ...	2.0 to 3.5 ft.
Vegetables, garden and truck crops ...	1.0 to 4.0 ft.

The figures above do not apply to unusually hot and arid interior valleys. Seasonal depths of irrigation water required for profitable production equal the

above depths minus such quantities of soil moisture as may become available as a result of precipitation.

Some crops require the most water early in the growing season, others late and still others have rather uniform requirements. This affects the spacing of irrigation applications. The usual practice is to apply from 2 to 6 inches of water at a time. In one area visited in Nebraska, water was applied in 3 cycles at 5 inches per cycle for a total of 15 inches for the crop-year. The first application was in May and the other two followed at 4-week intervals. On the other hand, in the California perennial pasture studied by Thurman *et al.* (1951), water was applied 18 times between April 1 and October 20, at a minimum interval of 6 days and a maximum of 16 days, depending on temperature. The quantity of water applied was not reported.

MOSQUITO-BREEDING PLACES AND DIRECTLY RELATED IRRIGATION PRACTICES.

This section is concerned with mosquito-breeding places found where rice and other crops which require sustained flooding are *not* grown, i.e. what is known as dry crop irrigation in India and may be termed 'typical irrigation' in the United States. Ecological factors of an irrigation-agricultural character are also presented.

Irrigation and precipitation in combination contribute to many of the breeding places under discussion; it should not be assumed that irrigation is wholly responsible in every case. In the western irrigated states, however, water from irrigation tends to be the more important. This is true even where precipitation exceeds irrigation water in quantity. For example, in an area with an annual rainfall of 25 inches, 15 inches of irrigation water are applied. But all of the irrigation water is applied within the mosquito-breeding season, while the seasonal rainfall totals only 10 inches. Moreover, the irrigation water is applied to the individual field on 3 spaced days during the growing season at 5 inches per application; a rate rarely if ever approached by precipitation in this area during the warm dry months. Surface runoff accordingly is far greater. In consequence, man-made water applications not only create ground pools which would not otherwise occur, but convert 'wet-weather' pools into semi-permanent or permanent bodies of water. In other cases, such as extensive waterlogged areas created by major water losses, the effect of rainfall may be imperceptible.

Breeding places within fields.—It is difficult to generalize about the relative importance of different types of breeding places since one type may be a minor problem in one region and a major one in another. Moreover, few if any mosquito ecologists have had opportunity to make observations over large multi-state areas and their individual judgments lack perspective.

It is considered by many however that breeding places in pasture lands constitute the most extensive problem for the western states irrigated area as a whole. The basic reasons are numerous: because of lower value of crop, water application practices are more careless and investment in land preparation is far lower; at least some grass will grow under soil, water and terrain conditions where cultivated crops would completely fail; pasture lands may be used as dumping grounds for random flows of surplus irrigation water from upslope fields

or from the open terminii of irrigation channels and field ditches to obtain a 'free' dividend from land and water that otherwise would not be utilized.

There are two commonly recognized types of pasture: wild and permanent. Natural grass grows in wild pasture, while permanent pasture has been planted to selected perennial grasses such as clover, alfalfa and timothy. Either type of pasture may be used for grazing or harvested for hay or forage crops. The *per capita* acreage devoted to grasslands of all types in the United States is of course greater than in many other countries because of the high consumption of meat.

The terms 'wild' and 'permanent' do not refer to the status of land preparation practices such as levelling and drainage. Land utilized as permanent pasture however tends to be in better condition in these respects than wild pasture. But in many areas there is little difference and mosquito breeding may be heavy in both.

Water is usually applied to both types of pastures by wild-flooding or by ordinary flooding from field ditches. It accumulates mainly as ground pools due to irregularity of land surface, excessive quantity of water applied and lack of drainage, soil permeability and depth of topsoil. Alkali, saline and waterlogged soil conditions at times may also be present.

Such residual pools may be generally distributed over the pasture or the water may be concentrated in a low corner or low end of the field due to terrain and lack of drainage outlets. They represent 'unwanted' water since they reduce the yields of the terrestrial grasses involved and degrade the land.

The following table (Thurman, 1951) of a study made in a one-acre permanent pasture in Central California illustrates clearly the persistency of ground pools during hot weather (July) and their relation to mosquito development.

Days after irrigation.	Per cent of area covered by water.	Aedes record.
0	97	Hatch.
1	73	
2	70	
3	60	
4	50	
5	No record	
6	29	Emergence.
7	14	Emergence completed.
8	10	
9	5	
10	3	

Residual ground pools within the crop areas of individual fields which persist long enough for mosquito emergence are relatively minor with most other types of irrigated crops. They may be found sporadically however in depressions of furrows, in field ditches used for water supply or drainage, where leakage occurs in underground supply pipes or supply structures within fields, in orchards where basin irrigation is employed, and in areas of low ground surrounded by growing plants.

Other mosquito-breeding places within fields may develop in supply structures themselves. These include uncovered small concrete 'turnout' boxes at the field edges which serve as valves and measuring devices. 'Standpipe' irrigation is used quite extensively on the Pacific Coast, especially in orchards. Water is supplied through underground pipe lines under low gravity pressure head. These supply concrete riser pipes of large diameter which extend above the ground surface. Each riser is provided with several radially spaced holes near the top out of which the supply water flows, generally into furrows. The risers (standpipes) and their outlet openings are located on a common hydraulic gradient and in this manner water is uniformly applied. The standpipes are usually uncovered and since the irrigation is intermittent, mosquito breeding takes place in the standpipes. When the tops are sealed with flat covers, mosquitoes still enter through the side port-holes which cannot be screened because of trash in the outflow. While minor in relation to the total irrigation-mosquito problem, prolific mosquito production takes place in the thousand or more standpipes which are sometimes found in a large orchard that may be free from other irrigation-mosquito problems. The indicated engineering solution is the provision of a valved drain at the low end of each underground supply line.

Mosquito breeding outside of crop areas.—Except for pasturelands, and orchards with standpipe irrigation, the great majority of the irrigation-mosquito production takes place outside of the area devoted to crops. This may occur at the untilled edges of the field but usually is outside of the field proper. Breeding places may be classified as follows:—

I. *Ground pools.*

- A. Seeped or waterlogged areas caused by leakage from irrigation channels or from deep percolation as a result of inefficient irrigation of the fields.
- B. Rainwater pools on the alkali soils of abandoned fields as a result of improper past irrigation.
- C. Undrained sumps, borrowpits and roadside ditches which receive water from adjoining fields due to excessive rate of water application, carelessness, unsuitable soils and terrain, lack of levelling and agricultural drainage and poor field layouts.
- D. Wastelands which receive spillage from adjoining fields as in C above or from terminating supply channels.
- E. Pools made by supply and drainage channel embankments which obstruct surface drainage, including improperly located cross-drains.

- F. Undrained borrowpits created incident to irrigation construction.
- II. *Still water in structures, channels and reservoirs.*
 - G. Supply canals and other channels of all sizes which may be held full between irrigation cycles, or before or after the irrigating season where crops are irrigated during only a portion of the growing season.
 - H. Improperly constructed or maintained drainage ditches of all types.
 - I. Idle 'turnout' and other outlet boxes located outside of fields—when not in use.
 - J. Idle stilling basins and inlet and outlet basins appurtenant to water control structures which form a part of the supply or drainage systems, such as ditch checks, inverted siphons and diversion chambers.
 - K. Reservoirs (generally large in size), with shallow grassy margins. This problem is limited because of substantial drawdown during the mosquito-irrigation season, steep shorelines, or both.

In most areas, ground pools (Category I) are far more important than the conditions listed under Category II. The most extensive conditions in Category I are judged to be items A and C.

Channel lining.—According to the 1940 Census of Irrigation, the average quantity of surface water entering irrigation canals in the 19 state census area was 6.2 acre ft. annually per irrigated acre and the quantity delivered to farmers was 4.1 acre ft., a conveyance loss of 34 per cent. This does not include losses from farm channels. The actual loss from extra-farm channels almost certainly exceeds the reported loss, since this data was not obtained for every irrigation enterprise but for a 'select segment' which could furnish these statistics. Irrigation engineering authorities state that combined conveyance and water-application efficiencies seldom are higher than about 33 per cent, a loss of 67 per cent. Conveyance losses include those from evapo-transpiration, waste and seepage. It is considered that on large irrigation projects where only small percentages of the canal lengths are lined, seepage losses vary from about 15 to 45 per cent. These can be reduced to less than 5 per cent by the more expensive types of lining.

From the standpoint of water conservation, much of the seepage loss from conveyance is not objectionable, this portion finds its way back into streams and is used by downstream irrigation works. There are also definite limits to the amount which can be economically justified for canal lining in terms of value of water saved, or even after considering all socio-economic benefits. Nevertheless, less than 4 per cent of the 127,500 miles of canals and laterals which existed in the 19 states area in 1940, were lined. The lining of a far greater amount is clearly justified and would greatly alleviate mosquito production in many areas of the western states, as is the case in India. The mosquito-production problem arising from conveyance seepage in the United States consists primarily of seeped or water-logged lands, whereas in India the seepage of water from channels into continuous chains of adjoining borrowpits is an added factor of great importance.

Lining is indicated not only to reduce seepage, but to increase canal capacity, provide safety against breaks, prevent weed growth and consumptive use of water by vegetation, and to prevent erosion.

The simplest type of channel lining is mud lining. In fact, many silt-laden irrigation waters provide this by natural process. Seepage losses from new canals may be reduced in this manner by as much as 80 per cent during the first year of life, although this is exceptional. Further siltation may increase seepage by constricting the channel section and increasing the 'head' of water. Mud lining is also artificially installed either by placing a blanket of clay on the sides and bottom of a dewatered canal or by pumping mud-laden water through 'well-points' into the sub-strata.

Other methods of channel lining practised include soil stabilization with portland cement and asphaltic compounds, precast concrete slabs, prefabricated asphaltic and rubber membranes, rock and brick masonry, poured-in-place portland cement concrete and asphaltic pavements, and bentonite-earth mixtures. Non-portland cements, such as the surkhi cement of India, have not been used to any extent.

One of the most promising developments is the use of buried asphaltic lining. The earth cross-section is excavated 1 ft. beyond 'grade', the asphalt lining is sprayed or laid in place and the earth is replaced. A prefabricated type of membrane is made in rolls 3 ft. wide and 36 ft. long and consists of an asphalt coating three-sixteenths of an inch thick on heavy kraft paper backing. The approximate cost of the material is Rs. 1.9 per square yard and the cost in place is Rs. 4. Burial permits use of a much thinner lining without loss of watertightness and provides protection against damage from the hoofs of cattle and other factors. A gravel topping may be placed over the earth backfill to resist erosion where necessary. This type of lining is applicable where the velocity of flow is not excessive and the growth of aquatic vegetation in the earth backfill is not an important problem.

Land preparation and project drainage.—By land preparation is meant preparation of the fields for irrigation by land levelling, construction of water-application systems, and field drainage. Project drainage refers to principal outlets for a general area containing many fields.

Very little land preparation or project drainage was carried out in the early days of irrigation in the west. The farmers' energies were concentrated on developing the irrigation supply system and, according to Wood (1950): 'The topography of the field was accepted as it existed in its natural state and the irrigation system was designed to fit it. This type of planning resulted in many small fields with a variety of row directions, ditches which followed ridges and irrigation water being diverted down steep slopes. The ultimate result was serious erosion problems and the use of an undue amount of labour in water distribution'.

'The present tendency in western land preparation is to modify the land surface to fit a pre-determined irrigation system wherever the depth of soil will permit. The various modifications now being employed consist in producing uniform slopes, reducing existing slopes, reducing to a minimum the number of fields and consequent row directions, and planning for a reduction in labour when water is applied to the land.'

The quotation above refers only to land levelling and water-application systems, but field drainage and project drainage are also essential for efficient irrigation in most cases. Some may consider that drainage offers a complete solution to the problem of mosquito production in ground pools where 'dry crop' irrigation is practised, since with perfect drainage there would be no residual water. Actually, however, land preparation methods in combination are required, since it is impractical to reach every ground pool with field ditches, and it is efficient irrigation in general rather than any specific component which provides the solution to mosquito production. However, drainage is the final step in the water supply-distribution-removal sequence and for this reason is apt to be the most neglected item.

The 1940 Census of Irrigation reported that drains had been installed for 39 lakhs of irrigated acres representing about 19 per cent of the land irrigated in 1939. This refers to project drainage in all degrees of adequacy and condition. The acreage served by proper field drainage is not ascertainable but is obviously also inadequate.

Further mention of land preparation is made later under the topic Conservation Irrigation.

INDIRECT INFLUENCES ON MOSQUITO PRODUCTION.

Those engaged in public health and mosquito control activities are prone to think mainly of measures directed specifically against mosquitoes as the solution to any mosquito problem. This is especially true since the development of the chlorinated hydrocarbon insecticides, particularly D.D.T. But there are a number of reasons why such measures do not provide a general solution to the irrigation-mosquito problem, even though they may be found useful as principal or supplementary measures in certain irrigated localities or regions.

In the first place, the great majority of the individual mosquitoes in irrigated areas which provide nuisance or encephalitis hazards do not enter, and remain in houses and so are not amenable to control by economical residual house-spraying techniques. Some represent species which do not enter houses to any appreciable extent, but also with many so-called 'house-entering' species, the bulk of the population either does not enter houses or does not rest indoors long enough for insecticidal effect. In this connection it should be realized that the problem of combined control is concerned with the total population of the biting species, rather than with a small infected percentage.

For these reasons mosquito control methods must be against the immature stages, thus greatly increasing costs of control due to the extensive breeding areas involved. Moreover, in California where the attack is heavily concentrated on larviciding, pronounced resistance and even cross-resistance to chlorinated hydrocarbon insecticides have developed, and this phenomenon may be expected to occur in other places where the species populations are drastically suppressed by these chemicals.

The extensiveness of irrigation and irrigation-mosquito problems and the low densities of human population that prevail under the highly mechanized, productive

agriculture of many irrigated areas, all tend to make direct antilarval measures economically and organizationally infeasible in the greater part of the irrigated areas of the United States. For this reason attention is directed to broad influences which, by virtue of their impact on irrigation-agriculture, either improve mosquito production conditions or make them worse.

Carelessness and inattention.—In a recent symposium held in California (Henderson, 1951), it was stated by managers of mosquito control districts that the over-application of water as a result of carelessness and inattention was responsible for a large part of the irrigation-mosquito problem in certain areas of that state. (This, of course, is only one phase of water over-application, since it is also practised deliberately.) The following excerpts from the discussions sum up this problem: 'It is water wastage rather than what we might term untidy irrigation practices. You are just putting too much water on the land'.....'One interesting thing we all have is the farmer who has too much to do. He lines up a lot of work, he sets his pump going and comes back an hour or so too late, and we have water coming out by hundreds of gallons per minute; you get too much water too soon'.....'The farmer either has too much to do or hires incompetent help in his irrigation'.

It is difficult to evaluate the relative importance of carelessness and inattention, or even to distinguish between them at times. Carelessness may be considered avoidable but this is only partly true of inattention resulting from 'too much to do', and it is unlikely that this problem can be fully corrected as long as agriculturists in the United States pursue the objective of high productivity per farmer.

Unlike many other countries, man-power is at a premium in the United States in terms of both wage level and its availability for employment, and the prevailing high standard of living can be raised or even maintained only by constant impetus towards increased production per man. Agriculture is no exception. Although production per acre is high, it is not as high as in some other countries where farms are smaller and more intensively cultivated. It is rather in agricultural productivity per man that the United States is supreme. According to the 1950 U.S. Census, there were only 53·8 lakhs of farms in the entire nation. The total acreage of land in farms was about 113 crores; 34 crores being in harvested cropland. The total farm labour supply was slightly over 88 lakhs of workers at the beginning of the crop season, or one worker for each 129 acres of farmland and for each 33 acres of harvested cropland. The above farm labour supply included farm operators, family labour and hired labour and much labour was expended outside of the harvested acreage. Yet in spite of this small labour supply, the diet of the 150 million people in the United States is exceeded by few other countries in quantity and by none in diversity, and the nation has enough to spare to be the world's largest exporter of cotton and a major exporter of wheat and some other foodstuffs. The dynamic improvement in productivity of agricultural labour is indicated by the continued long-time decline of farm population in the face of a steadily increasing total population, and by the increased use of tractors and electric power. In 1950, the working population on farms was 8·7 lakhs less than in 1940, although the total population increased by 1·8 crores. The number of farm tractors increased from 15 lakhs in 1940 to 35 lakhs in 1950. The number of farms with electricity increased by 14 lakhs in the 6-year period 1945 to 1950 alone.

According to the Food Protection Committee, National Research Council (1951): 'When this country was settled, 9 farm workers produced enough food for themselves and one city dweller. By 1940, one farm worker could produce enough for himself and 9 others. To-day the ratio has further increased; 14 urban dwellers now depend on each farm labourer. This efficiency has been achieved even though there have been increasing difficulties from disease and insect pests and the depletion of three-quarter million acres of fertile land by soil erosion'.

These trends are probably even more pronounced in the dynamic irrigation-agriculture segment of the total agricultural society and it must be expected that mosquito production arising from inattention arising from 'too much to do' will continue for many years.

Conservation irrigation.—Within the past 5 years, scattered, individual measures to improve irrigation practices have been brought together and given the name 'conservation irrigation'. Conservation irrigation is simply using irrigated soils and irrigation water in a way that will ensure high crop production without the waste of either soil or water and represents a fusion of soil erosion control, agricultural soil science and irrigation engineering interests. A number of agricultural-irrigation agencies are engaged in a programme of promoting conservation irrigation in irrigated areas—State agricultural colleges, State agricultural extension services, soil conservation district associations, the Production and Marketing Administration of the U.S. Department of Agriculture and the U.S. Bureau of Reclamation. But the chief exponent of conservation irrigation is the Soil Conservation Service (S.C.S.) of the U.S. Department of Agriculture, a federal service established in 1933 for the nation-wide control of soil erosion in agricultural and forest areas. More recently (*circa* 1947), this service also adopted the more comprehensive programme of conservation irrigation in the irrigated areas served.

The Soil Conservation Service (S.C.S.) is composed principally of a group of about 10 thousand local agents and staffs who serve as technical advisers to farmers in local soil conservation districts established by popular election. At the end of 1949, there were 2,194 local districts which contained about 80 per cent of the farms and farmlands in the nation, including over 120 lakhs of irrigated acres in the western irrigated states. The programme is still expanding in area served at a relatively rapid rate. The annual appropriation made to the service is about \$5 to 6 crores, including \$15 lakhs for research. Technical advisory services, including the making of engineering surveys and preparation of 'farm improvement' plans, are rendered at the request of farmers, who thereupon carry out the work themselves or make arrangements with private contractors. They may be reimbursed for a portion of the cost by the Production and Marketing Administration, which in the 5-year period, 1944 to 1948 for example, partially subsidized the levelling of 38 lakhs of acres for irrigation within and outside of organized soil conservation districts.

The objectives of avoiding waste of soil and water, along with the maintenance and improvement of crop yield, entail the following basic measures, all of which are consistent with the prevention or abatement of mosquito production: (1) land levelling for more efficient and more uniform distribution of water in the fields; (2) elimination of gross water leakage from channels; (3) improved drainage;

(4) improved layouts of irrigated fields; (5) control of soil erosion, which among other things silts drainage ditches; and (6) restriction of irrigation to suitable soils and terrain.

The principles of conservation irrigation and mosquito-avoidance are almost completely harmonious. Conservation irrigation at optimum level would eliminate virtually all mosquito production outside of artificial structures and reservoirs—a probable overall reduction of 90 per cent. It is antagonistic in only one minor respect—the provision of stilling basins in erosion control structures such as ditch ‘checks’, but this can be avoided by improved engineering design.

It is rather in differences of emphasis by the S.C.S. farm advisers and by the irrigation farmers themselves, and in the incomplete adoption of conservation irrigation principles and practices, that deficiencies appear in mosquito-prevention accomplishments. Good land-levelling practice for example calls for eliminating both ridges and hollows, but the farmer’s attention is apt to be concentrated on the ridges since they directly prevent flooding of the field. In this connection, a current objective of the Communicable Disease Centre of the U.S. Public Health Service is the assignment of a small number of mosquito-prevention specialists to the health departments of irrigated states who will serve as technical advisers to all agricultural agencies serving farmers on conservation irrigation programmes.

A complete record of conservation irrigation accomplishments attained under the S.C.S. programme is not available but at the end of 1949, work plans entailing the following improvements for 1950 had been formulated at the request of farmers: land levelling—10 lakhs of irrigated acres; improved water application (mainly field layouts)—25 lakhs of irrigated acres; general farm drainage (not listed separately for irrigated farms)—46 lakhs of acres; and improved acreage (all farms)—11 crores of acres.

The research programme of the S.C.S. is concerned with the technical development of conservation irrigation *via* research, demonstration, in-service training and preparation of technical literature.

A third element of the S.C.S. programme is the rendering of irrigation-engineering service to local water-users groups in charge of irrigation works and systems, on the rehabilitation, operation and maintenance of such systems.

A fourth element is concerned with new-lands developments of the U.S. Bureau of Reclamation (B.R.). The B.R. now makes soil surveys of all proposed new-lands projects which are to be federally financed to determine the water-duty of the land and to classify soils and terrain as to suitability for irrigation. The S.C.S. reviews these survey results and makes recommendations to the B.R. A number of extensive irrigated tracts exist in the west which were developed before soil technology became recognized, and which it is generally agreed never should have been placed under irrigation. It is reported that one tract recently proposed for irrigation has been reduced in acreage from over 100 lakhs to 20 lakhs due to adverse soil-terrain findings, and the importance of these studies hardly can be over-emphasized.

Like the S.C.S., the B.R. now has within its organization a group of conservation-irrigationists with basic training in water-soil sciences. While this organization as a constructing agency exercises more direct control over irrigation

projects than does the advisory S.C.S., its main sphere of interest in conservation irrigation is limited to tracts for which new, federally financed primary or supplemental source-water projects have been proposed. S.C.S. on the other hand potentially serves every irrigated acre.

The state and federal governments finance a nation-wide system of State Agricultural Experiment Stations which also include field extension services. A total of 5,042 local agricultural agents and assistants, 1,933 specialists and 767 administrators and supervisors were employed in 1949. Both the experiment stations proper and the local agricultural agents who advise the farmers have a comprehensive interest in agricultural matters, including conservation irrigation. Their interests are so diverse, however, that in most irrigated areas their effectiveness in the advancement of conservation irrigation is secondary to that of the S.C.S. and B.R.

Irrigation economics.--Modern irrigation economics is a very complex subject that involves many controversial elements. Only a brief summary is possible in this paper. As an indirect influence on mosquito production, economic factors influence or determine not only the acreage of land under irrigation but also the quantity of water used, extent of canal lining, efficiency of water application, including investments in land levelling, field layouts and drainage systems, and many other conditions.

With irrigated tracts which are served by source-water developments that are locally financed and operated, and which serve only an irrigation purpose, the economic equation from a local point of view is that increase in crop value from irrigation must exceed the combined costs of water, field development, and of increased farm labour requirements and taxes. It is important to realize that each of the 'outgo' items may be significant and that increase in farm income cannot be balanced against cost of water alone. On a given project the increased crop value may be Rs. 500 per acre and the annual cost of water may be only Rs. 20, yielding a superficial profit of Rs. 475 per year. But the cost of properly preparing the tract for irrigation where cultivated crops are raised will vary from about Rs. 200 to Rs. 1,500 an acre for land levelling, drainage and field ditches (or supply pipes and sprinklers). This cost is amortizable over a number of years but yet often costs more than the water. The annual cost of extra labour required for irrigation and for harvesting the increased yield generally is greater. According to Houk (1951) for example, 'one man can seldom irrigate more than 3 or 4 acres a day', where flooding from field ditches is employed. At a wage level in cash or equivalent of Rs. 750 per month for a farm worker, added labour costs frequently exceed any other item chargeable to irrigation-agriculture, even though with intermittent application one man in the course of a month can irrigate many such field units. A consolidation of all of these costs in one representative study of 5 farms by the U.S. Bureau of Reclamation in 1949, reported average annual costs per acre totalling Rs. 200 per acre for surface irrigation and Rs. 245 for sprinkler irrigation (Houk, 1951). Even with pasture land, which required no cultivation and minimum attention, the total cost of production in a study made in California in 1948 was Rs. 163 per acre (Jones and Brown, 1949).

In spite of these sizable costs, most irrigation-agriculture up to the present has been very profitable, both to the farmers and to the national economy. For

example, according to the California Agricultural Experiment Station (1944): 'California's irrigated crop area is reported to be about one-half of the total acreage for all crops in the state and yet the value of irrigated crops was 83 per cent of the total for all crops'. This is in a state with much humid agricultural land where irrigation is not needed.

When irrigation works are planned and financed by other than farmer groups, such as the federal government, it is customary to ignore the costs of irrigation to the farmer beyond the actual price he is to pay for water, but in spite of this the economic considerations become greatly more complicated.

From the standpoint of national planning to improve agricultural resources, agricultural productivity can be improved by a variety of measures, of which irrigation is one. This is true both in India and in the United States. These measures include many types of improved farm management in unirrigated areas, such as use of better seeds, more or better fertilizers and soil conservation practices, and the reclamation of wet land. According to the Engineers Joint Council (1950): 'Drainage of 20 million acres of wet, swampy and overflow land in the United States could be accomplished at reasonable cost'.....'Another 29 million acres in organized districts need improved drainage'. This compares with an irrigation potential of about 13 million acres in new lands and 8 million acres in new supplemental water developments. This source also states: 'There is an opinion that worn out and eroded lands and other lands of low fertility can be put into satisfactory production at a capital investment of under \$50 (Rs. 250) per acre; that wet lands can be cleared, drained and made ready for cultivation at from \$50 to \$100 per acre; but that the cost of supplying water to dry lands by means of irrigation will be in the order of \$200 to \$500 per acre on the better remaining possible projects. Irrigation projects are now under consideration where the cost may exceed \$1,000 (Rs. 5,000) per acre'.

The preceding comparison is incomplete since it is necessary also to consider differences in land productivity, which are generally favourable to irrigated land, but does point up the high investment per acre required by new irrigation source-water developments. The cost of new irrigation is not only high relative to some alternative methods of agricultural improvement but also to past irrigation developments which utilize more accessible water sources and were constructed in pre-inflation periods. According to the U.S. Census of Agriculture (1940), the average investment in primary irrigation works constructed up to 1939 was only Rs. 144 per acre, based on works capacities.

The high cost of so many of the remaining 'marginal' irrigation opportunities not only serves to restrict the rate of new development and to encourage multi-phasic developments in which a large part of the project costs can be borne by other than irrigation interests, but has also resulted in a degree of direct subsidization by the federal government. This has prompted the following statement by the Engineers Joint Council: 'Irrigation projects should be self-liquidating; the progressive trend toward shifting the entire burden on to those who bear the expense of government through taxation should be stopped'.

A contrary view is expressed by the Water Resources Policy Commission to the President of the United States (1950) which stated: '.....increasingly as the government has undertaken large investments for public purposes rather than

simply to serve private purposes not fulfilled by private capital, the principle of full reimbursement has ceased to be useful or necessary'. Conforming to this concept, the U.S. Bureau of Reclamation (1948) has tended in recent years to justify the federal irrigation programme, not as a self-liquidating activity, but on water revenues plus enhancement of the national income and increased general taxes accruing to the federal government. In the case of one project it has cited an annual federal income-tax revenue of Rs. 33 crores extracted from an irrigated urban-rural area supported by irrigation works costing Rs. 22 crores, of which the gross federal investment was Rs. 10.5 crores. Most of the taxes were not derived from the farmers but it is reasoned that the water supplied for urban domestic purposes and for agricultural irrigation in this arid area is fundamental to the existence of the city.

Speculative farming.—This term refers to the cultivation of waste lands for temporary periods when crop prices are abnormally high. In irrigated areas such farming may be practised by landowners but more often is carried on by leasing an uncultivated tract at a small fee for the duration of the speculative venture.

Such irrigated farming probably is practised more in California than in any other state and in recent years cotton has been a chief speculative crop. The extensive use of machinery for all stages of cotton production in that state has made possible sudden sharp expansion of acreages that could not have been achieved when ploughing, seeding, fertilizing, cultivating, insecticiding and harvesting were performed by manual labour.

In recent years, the price of cotton has varied from about Re. 1-6 to Rs. 2 per lb., depending on supply and grade of staple. Yields of 1,000 to 1,500 lbs. per acre have been realized on good irrigated land amounting to Rs. 1,500 to Rs. 3,000 per acre. A gross income of Rs. 750 to Rs. 1,000 per acre can be obtained even on poor irrigated land where the yield may be only 500 lbs. an acre, about twice the average for unirrigated land in humid areas of the United States. A farmer leasing 100 acres of marginal land can realize a tidy sum after all expenses are paid, provided his labour and land costs are held to a minimum. Since occupancy and use of the land are temporary and wastelands are involved, land and water management practices followed represent the antitheses of conservation irrigation. Mosquito production occurs in proportion and the land may be degraded from a semi-usable to an unusable state by the operation.

Farm tenancy and farm ownership.—Tillage of the soil by farm operators who lease, rather than own, the land under more established land tenure, generally does not result in the gross abuse experienced under some types of speculative farming. Farm operators who own the land they cultivate, however, are more interested in undertaking land preparation measures of a conservation-irrigation character and in all types of land improvement than are either farm tenants or absentee owners. Other things being equal, efficient irrigation and minimum mosquito production are favoured where the owner operates the farm. This condition prevails in a majority of the western irrigated farms and is fostered by national legislation which prohibits the U.S. Bureau of Reclamation from developing projects for water-users groups where the maximum size of any irrigated tract under one ownership exceeds 160 acres.

Old versus new irrigated areas.—In some of the older irrigated areas where irrigation was introduced under primitive conditions, a heritage of inefficient irrigation has been passed down from father to son. Although conservation-irrigation practices are making headway in these areas, it is said that progress is more gradual than in others where extensive irrigation is a new venture and past malpractices do not exist.

Water rights.—The common law 'Doctrine of Appropriation' which mainly determines 'Water Rights' in the irrigated western states was mentioned under 'History of Irrigation'. This doctrine actually preceded irrigation, having been established during the pioneering days of 'placer' mining for gold. The development of irrigation, however, entrenched its position in the body of unwritten law.

The Doctrine of Appropriation asserts that the ownership of all waters is vested in the state rather than in the owners of adjoining or overlying lands. Unlike privately-owned property, rights to water-use can be assigned, confirmed or withdrawn by the state. The doctrine declares that use establishes the right, and that disuse destroys or forfeits it. Public policy further limits usage by water-rights holders to 'beneficial use', which is declared to 'the basis, the measure, and the limit of the right'.

In reality, the underlying concept of the doctrine is the recognition of vested private interest in water, based on continuing use, independent of land ownership. The concept of state ownership of water is not applied to enhance the power of the state, but rather to separate private water usage from private land ownership, attainable only by establishing the state as the nominal owner of water resources. The application of this concept extends beyond the source-water development which obtains its water-use franchise from the state, and governs the pro-ration of water among individual water-users, such as the members of a local irrigation district.

The Doctrine of Appropriation reflects the history of the west. A little over a century ago the west was composed of vast tracts of land with ill-defined boundaries, which were nominally owned by government and others, but unoccupied and undeveloped. Settlers were attracted by the discovery of gold in the beds of streams. Their main interest was in recovering gold by hydraulic methods. In some areas, settlers arrived to till small tracts of soil in arid areas to the extent water was available. Water was primary in both cases and in neither case was water need or availability proportional to land ownership. The criterion of beneficial use was whether the water was to be put to sufficient private advantage for a new society to prosper and grow. This tradition that the welfare of the state as a servant of the people is best served by the welfare of the people in their own private enterprises has been maintained and preserved in the west up to the present.

Although the limiting factor of 'beneficial use' in legal theory provides protection from practices contrary to the public interest, and the Doctrine of Appropriation is essential in an irrigation society where water is taken by many from the same source of supply, the over-application of water to the land by farmers due to malfunctioning in execution of the doctrine is common.

This is partly because in actual practice the criterion of what constitutes 'beneficial use' largely is determined by whether the farmer is actually 'using' the water on the land, rather than the manner in which he uses it. If applied

to the land, it is being beneficially used, but if discharged into a wasteway, it is not, and the water right is subject to forfeit. The fact that a farmer could obtain equal or better results by applying 4 acre-feet instead of 8 is a refinement that largely extends beyond the present status of public opinion and enforcement mechanisms. To some extent this is because determination of what constitutes an 'optimum quantity of water' over widely variable conditions of crop, soil, terrain and climate, lies in the realm of professional judgment and cannot be converted into arbitrary standards.

As a result, the Doctrine of Appropriation and its related principles on numerous occasions have tended to encourage the deliberate over-application of water. In arid areas it is the 'right to use water' rather than ownership of the land which is valuable, for there is more land than water, and the land without the water is worthless. In the face of increasing appropriation of water resources, those who possess water guard it carefully lest a portion be taken away. The local irrigation district which has more water than it needs, may claim the whole quantity to protect its 'water rights' in full, and because who knows what the future holds in store? Each farmer in the water-users group may take more water than he needs so that in drought years his reduced ration will meet his full requirement. The state which seeks to establish its right to obtain more water from inter-state streams may not be interested in reducing the quantity in present use within its borders.

Under the free enterprise system which prevails and which is essential to a dynamic, productive American economy, the main solution lies in more costly water, rather than on stringent laws and an army of enforcement agents to 'police' each farmer.

Cost of water.—The cost of water to the farmer has an important influence on irrigation practices and mosquito production. When water is cheap, over-application to the fields is more apt to be practised and, in general, less attention is paid to the development of efficient irrigation methods, including land levelling and well-designed field layouts.

Where water is purchased by the farmer, as from a local irrigation district, two common practices are employed: he may buy it by the acre-foot, or pay a fixed price annually for each irrigated acre. Under the latter arrangement (flat rate per acre), he may either take as much water as he wishes on demand, or be limited to a maximum amount at scheduled intervals, according to a plan of rotation whereby each farmer is served in turn. In some private, co-operative enterprises known as mutual irrigation companies, a farmer may purchase shares of stock in an irrigation enterprise. These shares allow him a certain percentage of the total available supply for use on any lands under his control.

The trend in cost of irrigation water has been rising over the past 50 years due to many factors, but in spite of this the average cost is still relatively low, amounting to Rs. 11-6 per acre per year for all irrigated acreage in the 19 states area in 1939. This is due largely to extensive irrigation developments constructed in earlier years when labour and materials were cheap and surface water was near at hand, and is not representative of water costs for recent developments. The variation in cost accordingly is great. In one local irrigation district where project costs are largely met by the sale of hydel power, the cost to the farmer is only Rs. 5-0 per acre per

year, with some farmers applying as much as 10 acre-feet annually to rice, or Re. 0.6 per acre-foot. At the other extreme the U.S. Bureau of Reclamation has recently agreed to sell irrigation water to a municipal water supply at a price of Rs. 100 per acre-foot, and it is reported that on one new project the price to be charged from the farmer is Rs. 75 per acre-foot.

Some authorities feel that costly water (within economic limits) offers the basic solution to inefficient irrigation and irrigation abuses of all types, including the correction of conveyance losses as well as inefficient field practices.

One plan being used for new developments by the U.S. Bureau of Reclamation is to limit the sale of water to a local irrigation district at a favourable base price, to the quantity required by the tract under conservation-irrigation standards, as determined by soil-terrain-crop surveys. If more water is demanded, it is furnished at a premium or 'penalty' price.

Ground water recharge.—Overdraft of ground water is a critical problem in a number of western irrigated States, especially in Arizona, California and New Mexico. The annual pumpage of ground water from the alluvial valleys of California alone has been reported as about one crore of acre-feet. This heavy pumpage has created problems of overdraft and ocean water encroachment in many valleys.

This has created a need for state regulation of the output from wells and for recharging the ground water supply by use of 'spreading beds' on which the secondary flow of streams, and treated sewage and industrial wastes are applied for percolation into the soil. Seventy municipalities and sanitary districts in California alone dispose of their treated sewage in this manner. In the case of secondary stream flows which might otherwise be used for surface irrigation, recharging saves investment in surface storage reservoirs and conveyance system since underground reservoirs both store and convey water.

Spreading beds themselves sometimes give rise to mosquito production. In addition, the deliberate over-application of irrigation water to croplands in ground-water catchment areas has been proposed as an economic method of recharging which would eliminate the cost of 'spreading bed' systems. As far as is known, this is the only irrigation engineering concept which is antagonistic in principle to conservation irrigation.

Under carefully regulated condition, recharging through croplands might be carried on outside of the crop growing-mosquito production season without ill effect, but it has been advocated as a supplement to water applied during irrigation cycles as well. Up to the present time this is a plan rather than a practice.

CONCLUSION.

1. During the author's visit to India in 1949, he noted that extensive 'dry-crop irrigation' was being contemplated for some of the major irrigation projects under construction at that time. The stated purposes were to irrigate more acres than would be realized with rice irrigation (presumably obtaining more pounds of grain per acre-foot of water), and to reduce malaria hazards. Whether this type of irrigation has been extensively practised in the past in India is not known, since the available literature is concerned only with malaria and rice cultivation.

Since more than 90 per cent of the irrigated lands in the United States are 'dry-crop' irrigated, or at least the crops have a lower water requirement than rice, the main significance of this article is in relation to this type of irrigation.

The main irrigation-mosquito problem in India is concerned with the anophelines, whereas culicines constitute the chief problem in the United States. From an entomological standpoint the principal distinction between 'dry-crop' irrigation, which inherently is intermittent, and rice irrigation, which usually is not, is in differences in the length of time quiescent water remains in the fields or is present in adjacent areas and channels.

It is apparent that the production of anopheline species which might take place in other parts of the world under 'dry-crop' irrigation cannot be evaluated adequately in terms of culicine production occurring in the United States with this type of irrigation. Nevertheless, it is believed on the one hand that anopheline production can be greatly reduced by 'dry-crop' irrigation (even after considering the greater acreage of 'dry-crop' over 'wet-crop' irrigation), and on the other hand that 'dry-crop' irrigation without conservation irrigation does not provide protection against malaria hazards.

A large part of the mosquito problem in the 'dry-crop' irrigated areas of the United States comprises *Aedes* and *Psorophora* production and much of this occurs in ground pools which disappear before anopheline emergence would take place, as indicated by the table on page 107 for an irrigated pasture in California. Nevertheless, 3 per cent of the field held water 10 days after irrigation, or about 1,200 square feet per acre. Moreover, another large part of the mosquito problem in irrigated 'dry-crop' areas of the United States is composed of *Culex* species having developmental periods comparable with anophelines, and most of the larval habitats appear comparable in character to those in which one or more of the important malaria vectors of India are found, especially *Anopheles culicifacies*.

2. The most significant factor in the prevention of irrigation-mosquito production in 'dry-crop' areas is that the practices which prevent mosquito production largely are also good agricultural and irrigation practices from which farmers receive benefit in the form of improved crop yields, lowered water costs, or both.

The intermittent irrigation of ricefields in India (Russell *et al.*, 1942) saved water without reducing crop yield and thus provided potential opportunities for the irrigation of more land and reduction of the per acre irrigation tax. Efficient irrigation in the United States variously saves water (a precious commodity in arid areas), reduces water costs, increases crop production per acre and conserves the land. However, if the farmer is to be encouraged to conserve water, the profits must accrue to him rather than to the seller of water and to make this feasible it is important that water be delivered and sold by quantity rather than at a flat rate per acre irrigated.

According to Russell and Menon (1942), 'There is urgent need as regards malaria control for more effective co-operation between agriculture, public works, and health departments'. The programmes of agricultural agencies in the United

States which foster conservation irrigation among farmers possess much significance in this respect. When initiated they were not motivated by public health considerations, but were conceived to accomplish direct benefit to agriculture and for the conservation of land and water resources, yet they present the main solution to the irrigation-mosquito problem. Recently, the California State Health Department and the Communicable Disease Centre of the U.S. Public Health Service have become aware of this and are joining forces with agricultural interests.

At the present time the use of chlorinated hydrocarbons as residual house sprays is acknowledged to be the primary method of malaria control throughout the world, because of relative economy, effectiveness and the rapid benefit obtained. Yet the extent of malaria in India and some other countries is so great that it has not been possible so far to protect all persons at malaria risk, and may never be possible. For this and other reasons these insecticides at the present time constitute a chief tool, but not the only tool. The most economical mosquito-control methods are those which yield a profit through other direct benefits and provide mosquito-prevention as a free dividend, although the social obstacles to widespread adoption may often be great. Some types of drainage in urban and rural areas, some urban land filling and the conservation irrigation of both 'wet' and 'dry' crops come under this category. As stated by Russell (1936): '.....it would appear that for rural India, real malaria control will come only through cheap, automatic, naturalistic methods, *especially such methods as primarily will improve agricultural needs of the land*'. Moreover, the extent of 'man-made' malaria in India is already so great that its expansion through the poor planning or execution of new irrigation and transportation projects cannot be excused by relying on a perpetual protective blanket of D.D.T.

Although some steps in conservation irrigation, such as land levelling, are carried out in the United States by use of heavy machinery due to the large size of irrigated farms, all elements of land preparation and water application can be carried out in small holdings by the farmer's own efforts and do not require financial expenditure. The situation in this respect is analogous to the programme of rural drainage for malaria control in unirrigated farms which was carried on in low income areas of the humid South-eastern United States in previous years, and which functioned under the slogan: 'Malaria control costs labour, not money'.

That farmers can and will co-operate in India on programmes for direct agricultural betterment is being proved in demonstration areas under the 'Point IV' programme where American and Indian farm advisers are working with Indian farmers.

ACKNOWLEDGMENT.

The author gratefully acknowledges the help of the following persons who have reviewed this article and furnished helpful criticism: Paul F. Russell, M.D., Rockefeller Foundation; Richard F. Peters, Chief, Bureau of Vector Control, California State Health Department; Leslie C. Beadle, Entomologist, Communicable Disease Centre, U.S. Public Health Service; and Ivan D. Wood, Irrigation Engineer, U.S. Soil Conservation Service.

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A NOTE ON THE NOCTURNAL BEHAVIOUR OF *A. MINIMUS*
THEOBALD, 1961, IN DDT-SPRAYED HUTS
IN NORTH BENGAL.

BY

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[March 20, 1952.]

WHILE investigating the efficacy of DDT indoor residual spray in 1945 in the control of malaria in a tea garden area in the foothill region of the Darjeeling District, data collected regarding the time of entry of *A. minimus* at night in human dwellings of employees of the local tea factory and time of feeding of *A. minimus* have been analysed and presented in this note.

A. minimus had been incriminated as the sole vector of malaria in that area by Iyengar (1940) and confirmed by Puri and Krishnaswami (1947). This species was found to be mainly anthropophilic and an indoor rester by Thomson (1941). DDT indoor residual spray at dosages of 60 and 120 mg. per sq. ft. was found efficacious for a minimum period of 8 weeks in keeping down the density of *A. minimus* to intercept transmission of malaria (Puri and Krishnaswami, *loc. cit.*).

PROCEDURE.

Mosquito collections at night were made by two trained insect collectors under supervision of the author at two-hourly intervals for half an hour each time on a total of 12 nights. The collections were made commencing at odd and even hours on alternate nights from 19.00 and 20.00 hours and ending at 07.00 and 06.00 hours respectively. Eight labourers' huts with mud walls and zinc sheet roofs where the inmates slept without any mosquito nets were selected for observations. Indoor surfaces of huts had been sprayed with DDT prior to the start of observations and no other control measures were adopted.

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All mosquitoes resting inside the huts were collected in test tubes, identified* and their abdominal conditions recorded. They were then released in the same hut at every collection except the last. The abdominal condition of specimens of *A. minimus* were noted under the following categories :—

1. Flat abdomen with no trace of blood, obviously entering in search of blood meal ;
2. Abdomen half-filled with fresh blood, probably disturbed during the feed ;
3. Abdomen full of fresh blood ; and
4. Abdomen with blood in different degrees of digestion.

Analysis of data pertaining to specimens of *A. minimus* collected from June to August 1945 are given in Tables I and II and Charts 1 and 2. Accurate meteorological data during the period of observation were not available, but conditions in general remained uniform during the period.

TABLE I.
Collections of A. minimus at odd hours.

Time of collection, hours.	Number of <i>A. minimus</i> collected.	CONDITION OF ABDOMEN.							
		FLAT.		HALF FED.		FULL FED.		WITH DIGESTED BLOOD.	
		Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
19.00	43	20	46.5	1	2.3	2	4.7	20	46.5
21.00	40	7	17.5	0	0.0	2	5.0	31	77.5
23.00	41	24	58.5	2	5.0	8	19.8	7	16.7
01.00	64	14	21.8	6	9.5	33	51.6	11	17.2
03.00	66	8	11.7	5	7.8	48	72.7	5	7.3
05.00	53	4	7.6	5	9.5	41	77.4	3	5.5
07.00	33	0	0.0	4	12.0	24	73.0	5	15.0

* The following species of mosquitoes were found in the collections :—

- A. annularis* van der Wulp, 1884.
- A. culicifacies* Giles, 1901.
- A. fluviatilis* James, 1902.
- A. maculatus* Theobald, 1901.
- A. minimus* Theobald, 1901.
- A. vagus* Dönitz, 1901.
- Culex* sp.

TABLE II.

Collections of A. minimus at even hours.

Time of collection, hours.	Number of <i>A. minimus</i> collected.	CONDITION OF ABDOMEN.							
		FLAT.		HALF FED.		FULL FED.		WITH DIGESTED BLOOD.	
		Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
20.00	32	14	44.0	0	0.0	0	0.0	18	56.0
22.00	70	47	67.1	4	5.8	5	7.1	14	20.0
24.00	50	28	56.0	4	8.0	6	12.0	12	24.0
02.00	42	23	55.0	5	11.9	12	28.7	2	4.4
04.00	57	3	5.3	5	8.8	45	79.0	4	6.9
06.00	34	1	3.0	7	20.6	24	70.6	2	5.8

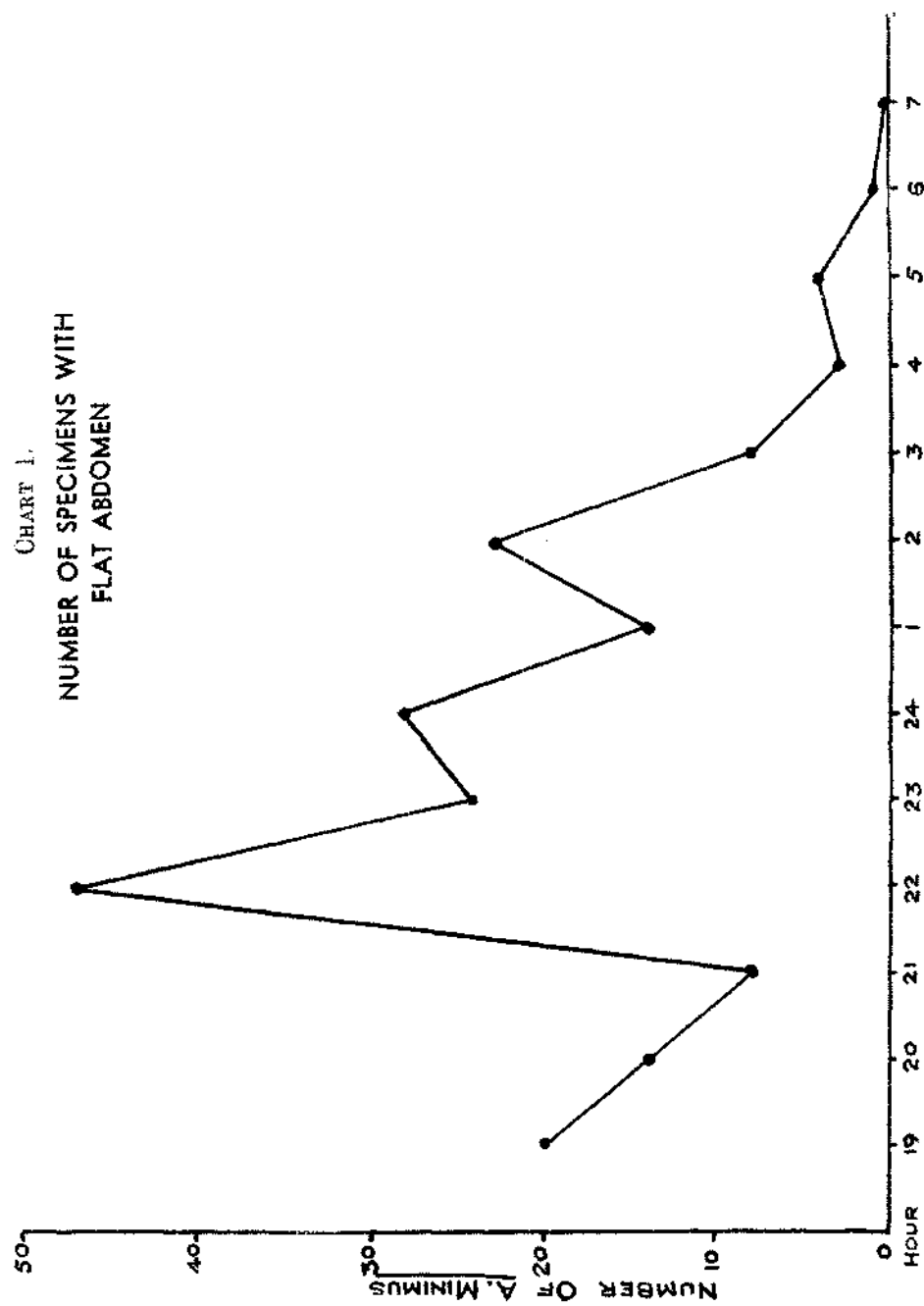
FINDINGS.

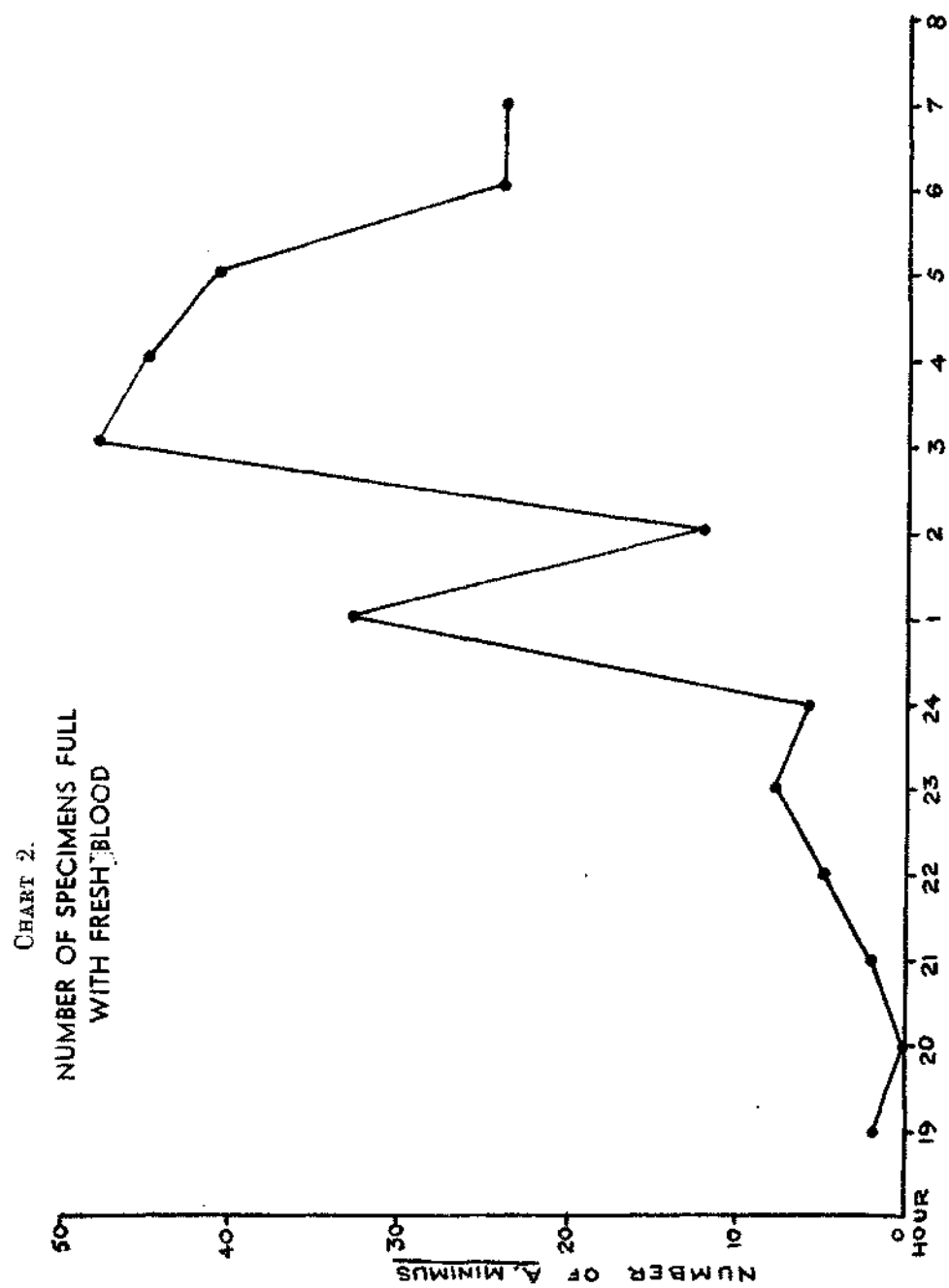
A. minimus, both unfed and freshly fed, were found among the collections. Specimens with flat abdomen entering huts for blood included both freshly hatched ones coming for their first meal, and those returning after oviposition for a subsequent feed, and were collected through the night. The maximum prevalence of such specimens, however, was among the collections made at 22.00 to 24.00 hours, indicating that majority of *A. minimus* enter human dwellings for feeding purposes before midnight.

In sharp contrast to this, majority of the specimens with stomach full of fresh blood were found in collections after midnight. It would appear from the data presented above that feeding activity of *A. minimus* during the earlier hours is negligible and majority of the specimens take their blood feeds mainly in the second half of the night.

There was a marked reduction in sporozoite rate among *A. minimus* collected in the mornings from the sprayed huts as compared with those from unsprayed ones (Puri and Krishnaswami, *loc. cit.*). Hospital records also showed a definite fall in the malaria morbidity and reduction in absenteeism due to fevers in the factory employees during the year.

Movements of *A. minimus* inside a DDT-sprayed house must have a very important bearing on the problem of controlling *minimus* malaria with residual applications of DDT.





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Bertram (1950) recorded a survival rate of 86 per cent with 45 mg./sq. ft. DDT in Assam. His finding suggests the possibility of *A. minimus* completely escaping the toxic effect of DDT deposit due to its excite-repellent action. But Macdonald (1950), recording observations of Gilroy in Assam, reports 100 per cent mortality among the specimens of *A. minimus* trapped from houses treated with DDT (45 and 90 mg./sq. ft.). Unfortunately the present studies do not include observations on the survival rate among trapped mosquitoes.

In view of the great importance of *A. minimus* as one of the principal vectors in extensive parts of India, detailed observations on a large scale are necessary to determine the true rôle of residual insecticides in the control of *A. minimus* malaria in the foothill regions of the Himalayas.

SUMMARY.

Time of entry of *A. minimus* into human dwellings in the Darjeeling Terai occurs mainly before midnight and maximum feeding after midnight.

A. minimus rests inside huts sprayed with DDT both before and after taking blood meals sufficiently long to pick up a lethal dose.

There was a definite fall in the malaria incidence as a result of residual application of DDT indoors.

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PROPHYLACTIC TRIALS AGAINST *P. GALLINACEUM* IN FOWLS.

BY

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JAMES and his colleagues recorded their observation on prophylactic trials in sporozoite-induced infection in human volunteers (James *et al.*, 1931). Quinine or pamaquin was administered before and after bites of infective mosquitoes, which had previously fed on a gametocyte carrier (*P. vivax*). Kikuth and Giovannola (1933) studied the action of quinine, pamaquin and mepacrine against *P. cathe-merium* and *P. praecox* (*P. relictum*) in canaries. Instead of mosquito bites they dissected out the salivary glands and injected the sporozoites. Later, Mudrow (1940) reported her observations on prophylactic action of pamaquin and other antimalarials on sporozoite-induced (by mosquito bites) *P. gallinaceum* infection in fowls.

Different techniques for assessing prophylactic and suppressive activities of various antimalarials were evolved during the last world war. Standard procedures for testing potential antimalarials against *P. gallinaceum* in 7 to 10 days old chicks were elaborated by Coatney *et al.* (1945), and later by Davey (1946a) and Tonkin and Hawking (1947). Methods employed for infecting the chicks consisted of bites of mosquitoes or subcutaneous or intravenous injection of either infected salivary glands or suspension of ground up mosquitoes. Wiselogle (1946) has described the different techniques in use in the various institutions and laboratories undertaking similar trials. Though the principle was essentially the same, the mode and route of introduction of the sporozoites differed.

In India, until recently, laboratory trials on antimalarials were restricted mainly to the assessment of therapeutic activity against blood-induced infections of avian or simian malaria.

The present report records observations on the prophylactic trials of the well-known synthetic antimalarials against sporozoite-induced infection of *P. gallinaceum* in fowls conducted at the Malaria Institute of India since 1949. The drugs

included mepacrine, chloroquine, amodiaquin (camoquin), sulphadiazine, proguanil, bromoguanide (Bami and Guha, 1948), pamaquin, primaquine, proguanil-pamaquin compound and pyrimethamine (50-63). For comparison, quinine was also used.

MATERIALS AND METHODS.

Fowls.—One hundred and twenty-four 6 to 12 months old white leghorn fowls weighing 0.3 to 1.7 kg. were used for these investigations. Prior to inoculations, blood smears from all the birds were examined on 3 consecutive days, as a part of routine procedure to exclude any protozoal or other infections like *Treponema anserinum*.

Plasmodium.—The strain of *P. gallinaceum* used in these studies has been maintained in these laboratories for a number of years. The course of infection after inoculation of infected blood or sporozoites is usually acute and in untreated cases mortality rates vary from 25 to 60 per cent.

Blood examination.—Blood and tissue smears were stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944). At least 200 fields of thin smears were examined before a negative result was recorded.

Aedes aegypti.—These mosquitoes were obtained from the colonies maintained in the laboratories and were fed on infected fowls. Only those mosquitoes which had fully fed were used.

Infective feed.—Attempts to feed mosquitoes through glass chimneys did not prove satisfactory. A convenient method consisted of introducing the fowls into large Barrand cages after their legs and the wings were tied up. Mosquitoes which had starved overnight were then introduced into the cages usually covered with a piece of black cloth during the feeding process.

Rearing.—Cages containing fully fed *Aedes* were placed over balls of moist cotton-wool wrapped in soaked lint in the insectarium where temperature and humidity ranges were controlled. Moist raisins were kept on top of the cages and changed frequently to avoid fungal growth.

Temperature and humidity.—Temperature in the insectarium was maintained between 68 and 78°F. by means of electric heaters in winter months and blocks of ice on the floor in summer. The relative humidity ranged from 66 to 76 per cent.

Dissection and inoculum.—Sample dissections of mosquitoes were commenced from the 4th day after the infective feed. Invariably small oöcysts were detectable at about this period and infection of the salivary glands was not observed till after the 7th day. Fowl serum with equal quantity of normal saline was used during dissections.

Only those glands which showed heavy infection were used for inoculation purposes. Each fowl received sporozoites from two pairs of glands, crushed and suspended in 2-3 c.c. of serum-saline mixture. In all cases inoculation was given intravenously through the wing veins. In this respect the technique employed was similar to that evolved by Tonkin and Hawking (*loc. cit.*).

Drug administration.—The drugs, either in solution or in suspension, were administered through the oral route by means of a rubber catheter with one end attached to a syringe. The first dose was administered soon after infective

inoculation and the drug was continued for 4 days in two daily doses. Dosage schedules were calculated in terms of mg. of base content per kilogram of the body weight.

Reinoculation.—The fowls which after infective inoculations (sporozoites) did not show any patent parasitaemia for at least a month on account of the effect of the drugs were inoculated with 30 million erythrocytic parasites of *P. gallinaceum* per kg. of the body weight. The course of infection was then followed up in each case.

Post-mortem studies were undertaken in all instances where the fowls died during the observation period either due to heavy infection or due to unknown causes. Special search was made for tissue phase of the plasmodium in the smears taken from the brain and the liver.

Uncontrolled infection.—Alongside the prophylactic trials, 12 fowls were inoculated, each with two pairs of heavily infected glands and the course of infection was observed. The average intrinsic incubation period was observed to be 7.5 days with a range of 7–10 days. The course of infection usually ran high in majority of cases with an average of 45–50 per cent erythrocytic cell infection.

RESULTS OF INVESTIGATIONS.

Quinine, mepacrine, chloroquine and amodiaquin.—For reasons that the known antimalarials are not effective causal prophylactic drugs, 20 fowls were placed under different regimes for comparison purposes as shown in Table I.

TABLE I.

Quinine sulphate, mepacrine, chloroquine and amodiaquin in sporozoite-induced infection of P. gallinaceum in fowls.

Antimalarial.	Schedule.	Dosage, mg./kg.	Number of fowls.	Patent parasitaemia in days.	RESULT OF REINOCULATION ONLY IF NEGATIVE FOR ONE MONTH.		REMARKS.
					Transient parasitaemia.	Acute infection.	
Quinine sulph.	I	5.0	2	8 (both)	...	...	...
	II	10.0	2	7 and 8	...	...	...
	III	20.0	2	7 and 10	...	...	...
Mepacrine ...	I	10.0	2	7 and 12	...	...	...
	II	20.0	2	7 and 10	...	...	Dose appeared to show toxic symptoms.
Chloroquine	I	2.5	2	8 (both)	...	...	...
	II	5.0	3	...	3	...	...
Amodiaquin	I	2.5	2	7 and 8	...	...	...
	II	5.0	3	8, 8 and 28	...	...	...

From Table I, it is apparent that quinine and mepacrine in different doses did not prevent the development of infection as patent parasitaemia was established in 7-12 days. The same results were observed in respect of chloroquine and amodiaquin under 2.5 mg./kg. dosage schedule. In 5 mg. dosage, 2 out of 3 under amodiaquin regime developed infection after the usual incubation period of one week, though in one case the period was prolonged by 3 weeks. With chloroquine in the same dosage, none of the 3 birds showed any patent parasitaemia within a period of one month. But after reinoculation in all the 3 cases parasitaemia was of a transient nature and the intensity was extremely low. There was complete disappearance of parasites from the peripheral circulation within a period of 3-5 days.

Pamaquin and primaquine.—Thirty-three fowls were similarly treated (see Discussion). The dosage schedules and number of fowls placed under each and the results have been shown in Table II.

TABLE II.

Pamaquin and primaquine in sporozoite-induced infection of P. gallinaceum in fowls.

Antimalarial.	Schedule.	Dosage, mg./kg.	Number of fowls.	Patent parasitaemia in days.	RESULT OF REINOCULATION IF NEGATIVE FOR ONE MONTH.		REMARKS.
					Transient parasitaemia.	Acute infection.	
Pamaquin ...	I	0.5	3	8 and 9	...	...	None effective.
	II	1.0	3	11	...	2	Effective in two.
	III	1.25	3	...	...	3	All effective.
	IV	2.5	3	...	...	3	"
	V	5.0	2	...	...	2	"
Primaquine	I	0.25	2	8 and 10	...	...	Ineffective.
	II	0.5	5	...	...	5	Effective.
	III	1.0	5	...	...	5	"
	IV	2.5	5	...	...	5	"
	V	5.0	2	...	...	2	"

From Table II, it may be noted that pamaquin in 0.5 mg. and primaquine in 0.25 mg. doses were ineffective. While primaquine showed prophylactic action in 0.5 mg. and higher doses, similar results were seen with $2\frac{1}{2}$ times the dose of pamaquin.

Sulphadiazine, proguanil, proguanil-pamaquin co., and bromoguanide.—Forty-seven fowls were utilized for evaluating the prophylactic action of these antimalarials (proguanil-13, bromoguanide-12, proguanil-pamaquin co.-12, and sulphadiazine-10). The results have been shown in Table III.

TABLE III.

Sulphadiazine, proguanil, bromoguanide and proguanil-pamaquin co. in sporozoite-induced infection of P. gallinaceum in fowls.

Antimalarial.	Schedule.	Dosage, mg./kg.	Number of fowls.	Patent parasitemia in days.	RESULT OF REINOCULATION IF NEGATIVE FOR ONE MONTH.		REMARKS.
					Transient parasitemia.	Acute infection.	
Sulphadiazine	I	30.0	2	8 and 10	...	...	Both ineffective.
	II	60.0	2	10	...	1	Effective in one.
	III	100.0	2	...	...	2	Both effective.
	IV	300.0	2	...	...	2	"
	V	600.0	2	...	...	2	"
Proguanil	I	2.5	3	10 and 13	...	1	Only one effective.
	II	5.0	5	9	...	4	80 per cent effective.
	III	7.5	3	...	...	3	All effective.
	IV	10.0	2	...	...	2	"
Bromoguanide	I	2.5	3	15	...	2	Two effective.
	II	5.0	3	17	...	2	Two effective.
	III	10.0	3	30	...	2	"
	IV	15.0	3	...	...	3	All effective.
*Proguanil-pamaquin co. (proguanil base).	I	2.5	6	1-8th day 3-10th day 1-12th day	...	1	Effective in one.
	II	5.0	6	...	...		All effective.

* The proportion of pamaquin base is one-tenth of the base content of proguanil.

In Table III, all the four compounds showed prophylactic action. Sulphadiazine proved effective in all cases in doses of 100 mg. and above. It is interesting to note that in 2.5 mg. dosage of proguanil and of bromoguanide, the results achieved were partial. But with a dose of 7.5 mg. and above of proguanil, all fowls were protected. With bromoguanide though protection was afforded to most of the cases under 5 and 10 mg. dosage, comparable results were obtained with a dose of 15 mg.

With proguanil-pamaquin co., only one out of 6 fowls was protected in Schedule I, and all the 6 in Schedule II.

Pyrimethamine.—A dose of 0.3 mg. per kg. or above of pyrimethamine protected all the 12 fowls. The results have been shown in Table IV.

TABLE IV.

Pyrimethamine (daraprim) in sporozoite-induced infection of P. gallinaceum in fowls.

Antimalarial.	Schedule.	Dosage, mg./kg.	Number of fowls.	Patent parasitaemia in days.	RESULT OF REINOCULATION IF NEGATIVE FOR ONE MONTH.		REMARKS.
					Transient parasitaemia.	Acute infection.	
Pyrimethamine	I	0.3	2	...	...	2	Both protected.
	II	0.625	2	...	...	2	"
	III	1.25	2	...	...	2	"
	IV	2.5	2	...	...	2	"
	V	5.0	2	...	...	2	"
	VI	7.5	2	...	...	2	"

DISCUSSION.

In the past, the term 'prophylactic' drug has been expressed rather loosely, so much so that many workers considered that quinine in daily doses possessed prophylactic action. However, Yorke and Macfie (1924) conclusively proved that quinine had no action against sporozoite-induced infections in human malaria. Since then ineffectiveness of quinine and mepacrine as prophylactics against human malaria have been reported by many workers including Coatney *et al.* (1948) and Cooper *et al.* (1949). Working with avian malaria, Davey (1946b) had observed that neither quinine, mepacrine nor sontochin possessed any action against pre-erythrocytic forms.

In the present series it has also been demonstrated that quinine and mepacrine in as high a dose as 20 mg./kg. body weight completely failed to protect the fowls.

Similar results were observed with chloroquine and amodiaquin in 2.5 mg. doses. In 5 mg. doses no parasites were detectable for a month in all the 3 cases under chloroquine.

The course of infection in fowls which received reinoculation of heavy doses of erythrocytic parasites was either (a) similar to acute primary infection or (b) was of a mild or transient character. Resulting course of type (a) was taken to mean that the drug administered soon after sporozoite inoculation and for 4 days thereafter had acted as a true prophylactic.

In the case of the latter type in which the course of infection was mild or transient in spite of the abnormally high dose inoculum, it was interpreted to mean that the drug acted as a suppressant, keeping the parasitaemia at a sub-patent level and thus allowed the development of immune bodies to counteract the effect of reinoculation.

The superinoculation test, in the fowls which received 5 mg./kg. dose of chloroquine, showed that in these cases the drug acted purely as a suppressant. Similar observations have been made in human malaria by Coatney *et al.* (1949), Coatney *et al.* (1950a, 1950b) and Lints *et al.* (1950).

On account of greater persistence of the drug in the tissues than both quinine and mepacrine, Findlay (1951) observed that 'plasma concentration of chloroquine on a given dosage are 10-20 times higher than in the case of mepacrine but concentrations of chloroquine tend to be higher than those of the other 4 amino-quinolines'. This then may be the explanation of the slightly better results obtained with chloroquine than with amodiaquin, though truly speaking none of them are causal prophylactic drugs.

During their studies with pamaquin both in blood and sporozoite-induced infections in human malaria in neurosyphilitics, James *et al.* (*loc. cit.*) had observed that the drug afforded complete protection against sporozoite-induced *P. falciparum* and astoundingly lengthened the incubation period of *P. vivax*. In similar investigations carried out in U.S.A. and Australia, it has been amply confirmed that 'pamaquin possesses properties not possessed by quinine, mepacrine, sontochin or S.N. 7618' (Davey, 1946b). Against certain avian malaria, pamaquin has been shown to be effective (Davey, 1946a), but Marshall (1946) has observed that although pamaquin is effective against *P. lophurae* and *P. cathemerium*, it has no action in *P. gallinaceum* infection in chicks. Findlay (*loc. cit.*) also considers that pamaquin is ineffective against the pre-erythrocytic form of *P. gallinaceum* in chicks.

During these investigations it was observed that pamaquin in doses of 1.25 mg./kg. and above afforded complete protection to fowls inoculated with heavily infected salivary glands. The explanation may be that the dose of one mosquito-equivalent used in chicks is extremely heavy. Greenberg *et al.* (1950), commenting on the investigations carried out by Coatney and his colleagues, observed that 'with this method of inoculation, it has been impossible to demonstrate that the 8-aminoquinolines have any effect on tissue forms'. Freire (1946) reported that

in *P. gallinaceum* infection the prophylactic dose of sulphadiazine varied directly with the number of sporozoites inoculated.

Secondly, it is likely that the metabolic breakdown process of pamaquin in chicks is not essentially the same as in fowls. Marshall (*loc. cit.*) considers that reactions of the same species of plasmodium to the same drug but in different hosts may depend on degradation of the antimalarial in addition to the absorption, distribution and tissue concentration factors.

Comparing the minimum effective prophylactic dose of pamaquin and primaquine (Table II), it will be noted that the latter is $2\frac{1}{2}$ times more effective than the former. Recently, Alving (1951) has observed that in 15 mg. weekly dosage the drug affords protection in human malaria.

Coatney and Cooper (1944) were the first to demonstrate the prophylactic action of sulphadiazine in *P. gallinaceum* infection. Similar results were also observed by Coggeshall *et al.* (1944). Davey (1946a) in a comparative study with various sulphonamides had observed that in 25 mg. dosage sulphadiazine protected all chicks against sporozoite-induced *P. gallinaceum*. Compared to this, four times as much of the drug was required in the same series for the same effect in fowls. Thus, it is again obvious that reaction of the same species of plasmodium to the sulphadiazine is not the same in chicks and fowls.

The classical investigations by Fairley (1946) conclusively proved that proguanil is a true causal prophylactic against *P. falciparum*. Against avian malaria like *P. gallinaceum*, similar observations were reported by Curd *et al.* (1945), Davey (1946b), and a host of others. In the present series, 80 per cent of the fowls were protected with a dose of 5 mg. and 100 per cent with a dose of 7.5 mg./kg. and above. The results obtained with bromoguanide were not altogether dissimilar though double the dose was required for 100 per cent protection.

When proguanil was administered along with pamaquin, protection rate was higher. A dose of 2.5 mg. of proguanil and 0.25 mg. of pamaquin proved ineffective in 83 per cent of cases while double the dose protected all the 6 fowls. In this connection it may be noted that in 0.5 mg. dosage, pamaquin alone was totally ineffective. Similarly, proguanil in 5 mg. dosage by itself protected only 80 per cent of the fowls but when both were administered together in 0.5 and 5 mg. doses respectively it became obvious that better results were achieved than either of the drugs administered separately.

Falco *et al.* (1951) observed that against sporozoite-induced *P. gallinaceum* infection, pyrimethamine was able to protect 29.4 per cent of the chicks for a period of 28 days. Working with the same species in fowls in the present series of investigations, the causal prophylactic action of pyrimethamine has been clearly demonstrated. Further it has been observed that dose for dose pyrimethamine (daraprim) is almost as effective as primaquine and slightly more so than pamaquin. It is found to be many times more effective as compared to the results attained with proguanil.

It would thus appear that in some ways pyrimethamine has shown quite encouraging results though final evaluation can only be done after it has been tried out against human malaria.

SUMMARY.

1. Prophylactic trials on quinine, mepacrine, chloroquine, amodiaquin, sulphadiazine, proguanil, bromoguanide, pyrimethamine (daraprim), pamaquin, primaquine, and proguanil-pamaquin combined, were carried out against sporozoite-induced *P. gallinaceum* infection in fowls.

2. Quinine, mepacrine and amodiaquin showed no protective action, though in one case with 5 mg. of amodiaquin, patent infection was considerably delayed.

3. In 2.5 mg. dosage, chloroquine proved ineffective, whereas in 5 mg. dosage patent parasitaemia was not established within a month due to its suppressive action as determined by superinoculation test.

4. Both pamaquin and primaquine seemed to have prophylactic action against *P. gallinaceum* infection in fowls. The latter appeared to be $2\frac{1}{2}$ times more potent than the former.

5. Sulphadiazine afforded full protection in 100 mg. dosage and the same results were obtained in 7.5 and 15 mg. with proguanil and bromoguanide respectively. But the last two compounds gave protection to majority of the cases in 5 mg./kg. dosage.

6. A dose of 5 mg. of proguanil combined with 0.5 mg. base of pamaquin showed better results than either of these administered separately.

7. Pyrimethamine (daraprim) seemed to be effective in small doses.

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A NOTE ON THE EFFECT OF PROGUANIL SUPPRESSION IN
ADDITION TO OTHER CONTROL MEASURES ON BENGAL
NAGPUR RAILWAY STATIONS.

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[September 22, 1951.]

SYSTEMATIC adult control measures with D.D.T. were commenced at various stations of Bengal-Nagpur Railway. Initially, only a few areas were involved but later by 1950 most of the malarious areas under the Bengal-Nagpur Railway, like the coastal areas of Orissa, Hazaribagh Ranges, Ranchi Plateau and Singhbhum Hills of Chota Nagur, were included under the control programme. In large colonies like Khargpur, Tatanagar, etc., antilarval control was the method of choice. However, selective anti-adult measures were also undertaken in certain places.

In view of the very nature of their duties, certain staff like the drivers, guards, khalasies, etc., who had to be away very often from their usual stations, it was realized as early as 1949 that additional measure like suppressive treatment would ward off overt malaria in these personnel. Different malarious areas where proguanil prophylaxis was tried are shown in the Map.

A weekly dose of 100 mg. proguanil was considered to be the most practical and economic method and, to begin with, essential staff of a few stations were placed under the protective regime. By 1950, this measure was extended to 54 stations. Some of the personnel refused the suppressive treatment for one reason or other, and they served as the comparison group.

The overall picture as analysed in 1951 is that malaria morbidity rate was considerably low in areas where control measures were operative but it was slightly lower still amongst the staff who received proguanil treatment as against the group who did not (Charts 1 to 5).

It may be stated here that, as it is, the average *per capita* cost is Rs. 3.7 (average 1947-50) mainly for mosquito control measures. Even then, it is usually not possible to protect the running staff in view of the nature of their duties. As such, additional expenditure on suppressive antimalarials for protection of these personnel, is considered as essential.

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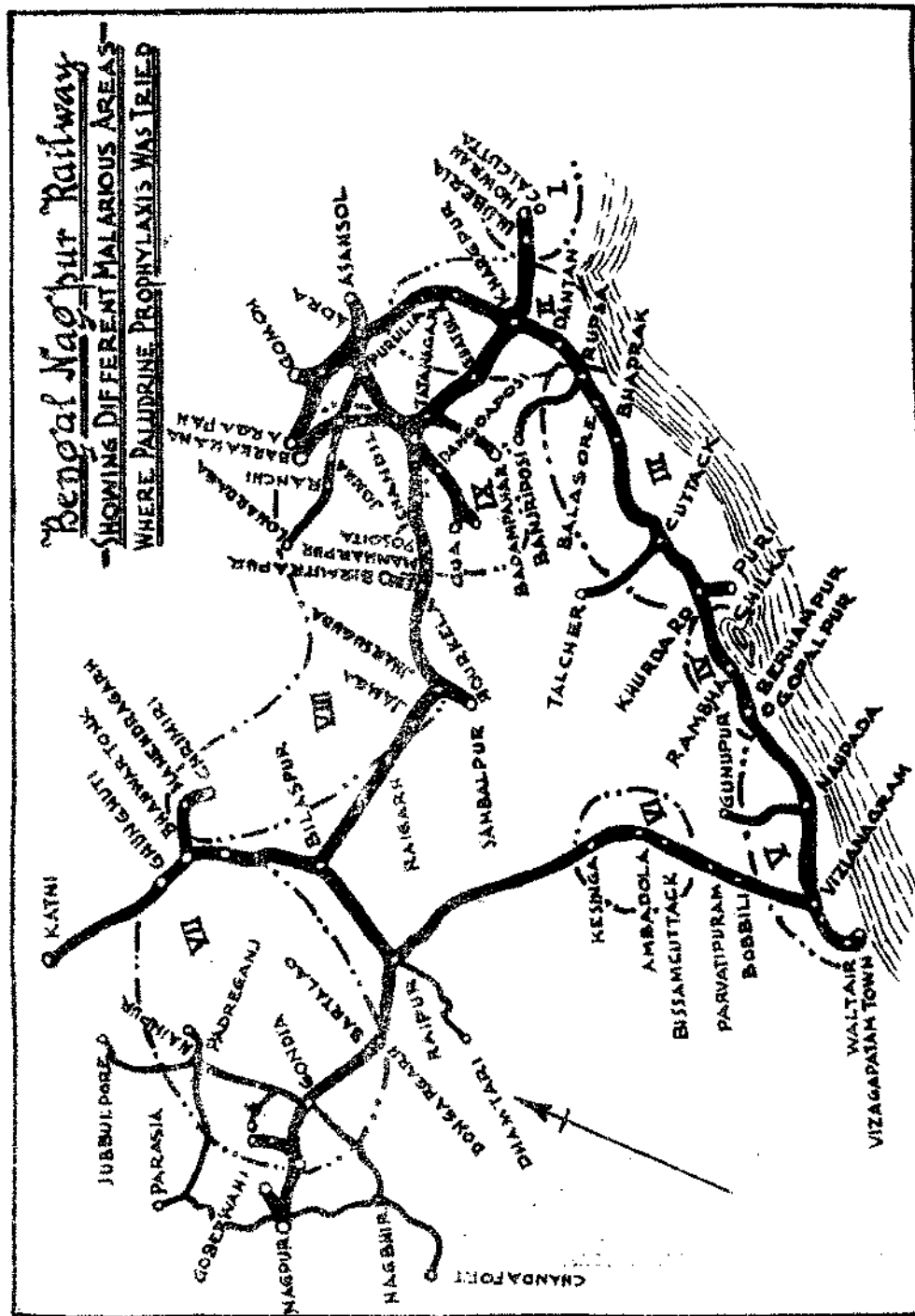


CHART 1.
Coastal plains (Area III of Map).
Malaria morbidity rates before and during prophylaxis.

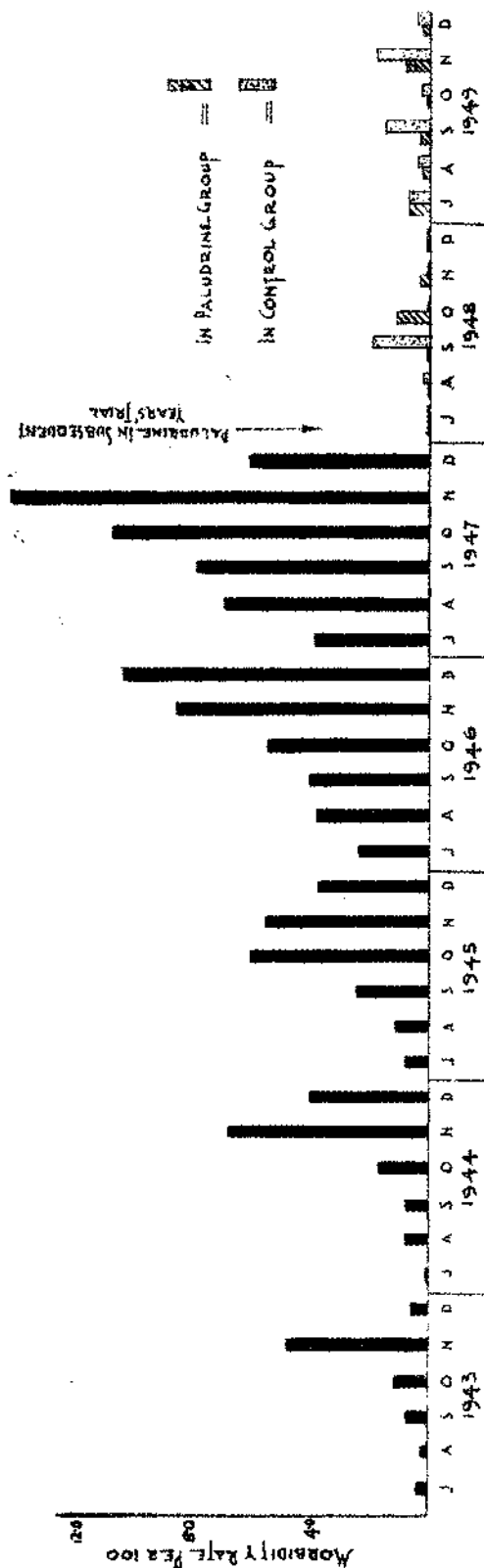


CHART 2.
Juppore Hill Tracts (Area VI of Map).
Malaria morbidity rates before and during prophylaxis.

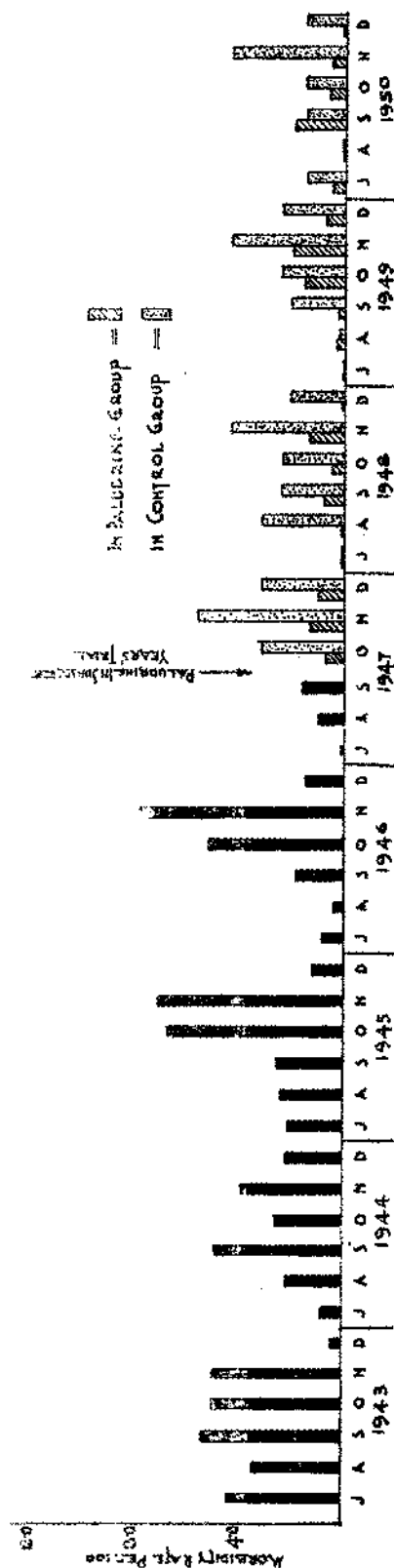


CHART 3.
Eastern Satpura Ranges (Area VII of Map).
Malaria morbidity rates.



CHART 4.
Hazaribagh Ranges (Area VIII of Map).
Malaria morbidity rates.

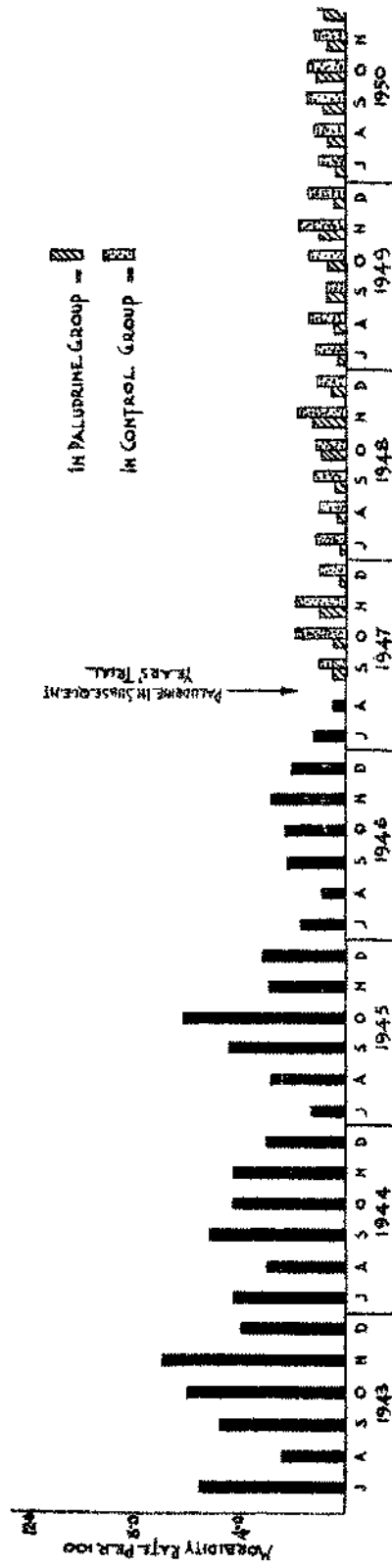
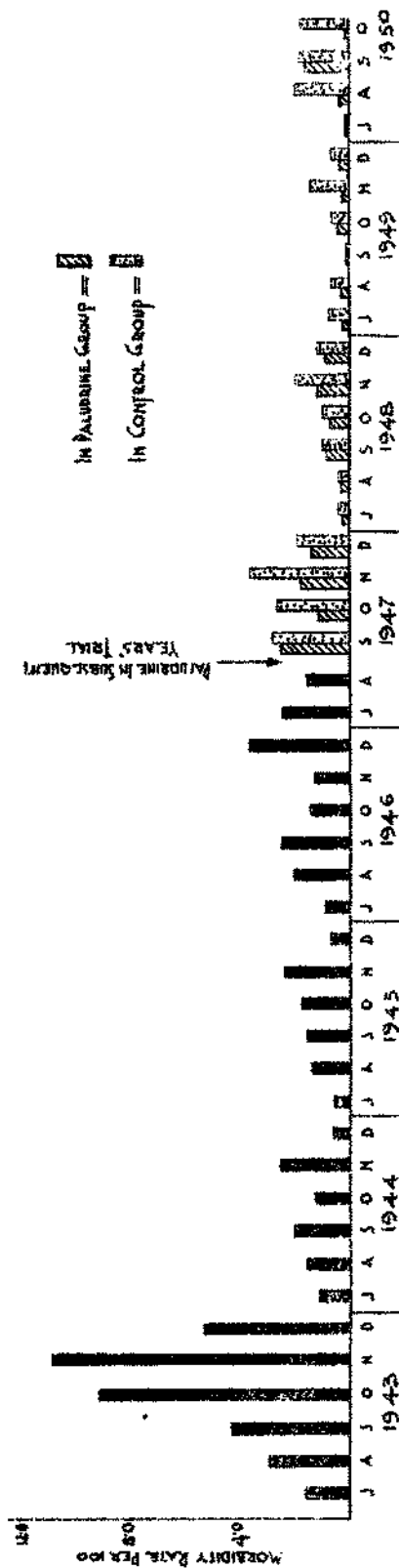


CHART 5.
 Singhbhum Hills (Area IX of Map).
 Malaria morbidity rates.



AN EXPOSURE CELL FOR USE IN RESIDUAL INSECTICIDAL STUDIES ON MOSQUITOES.

BY

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[February 14, 1952.]

SEVERAL CONTRIVANCES and techniques have been described in literature for exposing live mosquitoes for any specified period of time to surfaces sprayed with residual insecticides. Simmons and staff (1945) used a chamber $3 \times 3 \times 12$ inches consisting of 4 panels each measuring 3×12 inches. The plunger method used by these authors for the transfer of insects to treated panels has been further improved by Fay and Sheppard (1949) so as to bring the insect within an inch of the treated surface. Lindquist and McDuffie (1945) used treated cages as contact chambers and Roth and Lindquist (1949) treated jars. Ranganathan *et al.* (1949) and Hadaway and Barlow (1951) have inverted a funnel over treated surfaces and contrived contact. All these chambers have one serious drawback in that the exact period of contact of the mosquitoes with the treated surfaces could neither be controlled nor determined as the mosquitoes had enough space to fly about. Kartman and DaSilveira (1946) and Newman *et al.* (1949) have attempted to regulate the time of contact of the mosquitoes with the treated surfaces, the former workers by exposing mosquitoes in glass tubes 8 inches long and 0.4 inch in diameter, the inside of which was coated with insecticide and the latter by clipping off the wings of the mosquitoes. In the former case it was difficult to ensure uniform distribution of insecticide inside the tube while, in the latter case, the amputation of wings may interfere with the normal behaviour of mosquitoes sufficiently to detract the validity of the conclusions. Likewise, the use of anaesthetics like CO₂, chilling insects, etc., resorted to facilitate introduction and removal of mosquitoes may exert a biological interference and upset conclusions. It is, therefore, desirable to adopt a method of liberating mosquitoes with the least biological interference. Reference may also be made to the apparatus which Rajindar Pal (1951) has very recently described.

The problem in such experimental studies is, therefore, to devise a simple apparatus in which (1) the exposure of mosquitoes to treated surface is secured by physical contact through tarsi only for periods which could be precisely controlled, (2) no unknown effects as may be due to amputation, anaesthetization, chilling, etc., would be involved, and (3) many replications could be made easily and quickly.

A cell devised specially for the purpose is herein described.

The exposure cell (Figs. 1 and 2) essentially consists of three parts :

(1) A bottom panel consisting of a plate of 9 cm. square and made of any suitable material such as glass, wood, plaster of paris, clay, etc. Size chosen is such that it could be conveniently sprayed in a Potter's tower (Potter, 1941).

(2) A middle square frame, made of glass, metal or any other suitable material having exactly a thickness of 3 mm. and with outside and inside dimensions of 10.5 cm. square and 7.5 cm. square respectively.

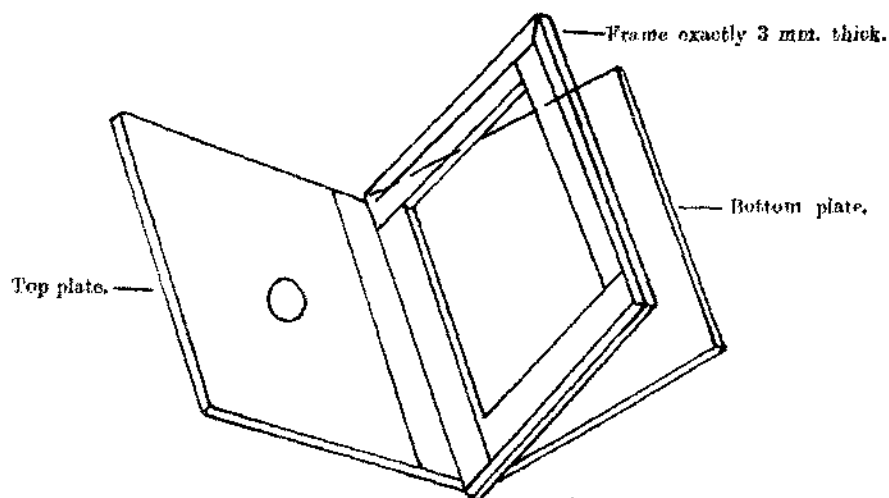


Fig. 1.—Exposure cell, opened

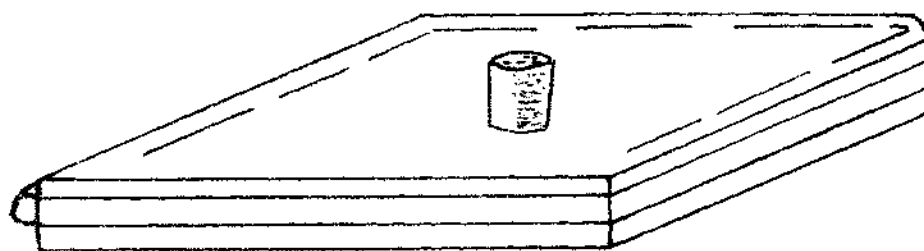


Fig. 2.—Exposure cell, closed, with cork-plug in position.

(3) A top panel made of glass or plastic of 9 cm. square having at its centre an entry hole of 1.5 cm. diameter. The three parts are fixed to each other along one edge only and they open out in the form of a book. This apparatus can be easily made in any laboratory. The three parts could be attached to each other by a specially made metal hinge or adhesive tape. The hole in the top glass plate may either be bored in the centre of a single plate or two plates of half the size, each with a semi-circular cut along one edge may be joined together to give a circular hole.

Only the bottom panel is treated with the insecticide leaving a central circular area untreated. Uniform distribution of the insecticide on the plate is ensured by using a Potter's tower. The properly treated plate is fitted to the apparatus and live mosquitoes are introduced through the hole in the top plate with a specially designed suction tube (Fig. 3) consisting of an outer and an inner glass tube. The inner glass tube has its distal end covered with wire gauze and functions as a plunger. Mosquitoes are gently sucked into the outer tube and allowed to rest for about 2 minutes. Then the outer tube is inserted into the hole on the top plate and pushed fully down to touch the bottom panel. The inner plunger tube is then gently pressed down and the mosquitoes pushed towards the bottom plate. When the end of the inner tube is just at the level of the top plate, the outer tube is gently raised up so that the mosquitoes are free to move into the cell. Often they do so immediately of their own accord but

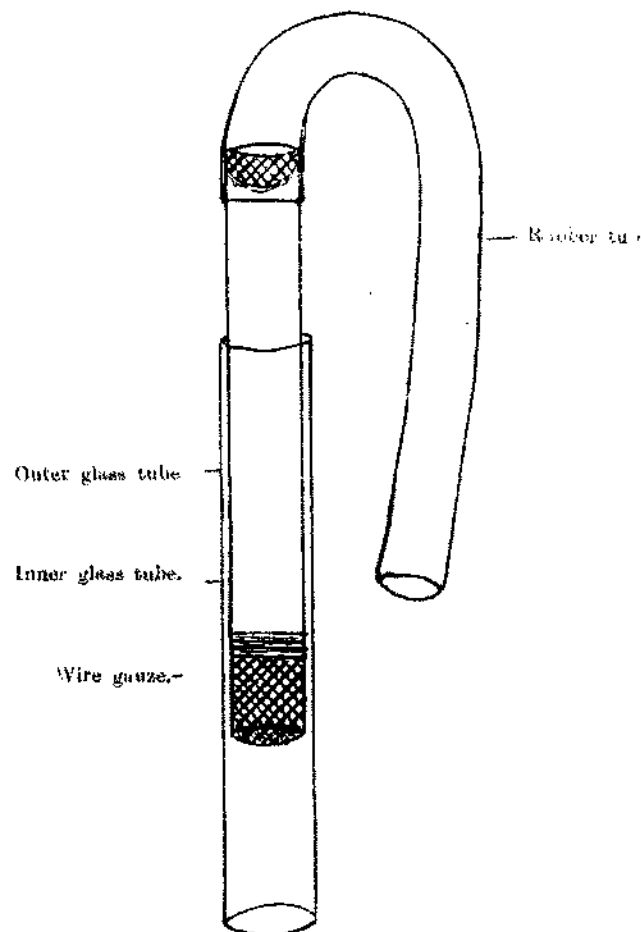


Fig. 3.—Suction tube.

sometimes a gentle blow of air is necessary. As there is no space for them to fly they walk in the cell. As soon as all the mosquitoes move in, the suction tube is withdrawn and a cork-plug pushed into the hole to rest on the bottom plate. During the whole process of introduction the mosquitoes do not come into contact with the insecticide till they actually crawl into the chamber, for the centre area of the bottom plate is secured free from insecticide by keeping that portion covered when the plate is sprayed in the Potter's tower. Once they have gone into the chamber they cannot come back to the untreated area as it is now fully covered up by the cork-plug.

By this method the exact time of the commencement of contact with the insecticide can be determined with an error of about 3 to 5 seconds at the most. As there is only a space of 3 mm. between the two panels of the cell, the mosquitoes cannot fly and can therefore only walk about on the treated panel and are continuously in contact with the treated surface. Rarely some culicines assume an upside-down position and such specimens are removed quickly from the cell with the aid of wire loop and discarded*. If this precaution is not taken it is not possible in the case of insects whose body gets contact with the sprayed surface to determine what area of the insect has come in contact. Results will then not be comparable from specimen to specimen. Five mosquitoes can be easily introduced into the cell at any one time*. When the end of the desired period of exposure is reached the whole cell is taken into a large gauze net fitted on frames (12 × 12 × 12 inches) and the cell opened like a book inside the sleeve of the net. The mosquitoes immediately fly out into the net which later acts as a recovery cage. Mortality is noted at the end of 24 hours, 48 hours, etc.

This simple apparatus has eliminated most of the disadvantages of the exposure apparatuses used previously in the studies with residual insecticides on mosquitoes.

The author is highly grateful to Dr. D. K. Viswanathan and Dr. T. R. Rao for their most valuable suggestions and criticism in the designing and use of this apparatus.

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* Removal of test insect from the cell with the aid of a wire loop may be a difficult operation. Moreover, the number of test insects exposed at one time is limited, and besides fumigant effect of the insecticide is more likely.—Editor.

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SCREENING OF ANTIMALARIALS AGAINST *P. GALLINACEUM* IN CHICKS.

Part I.

Preliminary Studies.

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SYSTEMATIC search for an effective antimalarial during the last war resulted in the synthesis of a very large number of chemicals. Necessity for correct appraisal of these compounds led to elaboration of various methods for biological assay. Although the essential principles of 'chick tests' evolved in U.S.A. and U.K. (Curd *et al.*, 1945; Wiselogle, 1946; Davey, 1946a) are not fundamentally different from Roehl test, they differed in certain techniques. Firstly, the species of plasmodium used was *P. gallinaceum* in chicks instead of *P. relictum* in canaries as used by Roehl (1926) and secondly, the infected blood was injected intravenously and not intramuscularly. Thirdly, the index of activity of a compound was determined in terms of its quinine equivalent, an expression used by Buttle *et al.* (1938). For reasons to be discussed later, the 'chick test' was considered to be superior to Roehl test and served as an essential feature for rapid evaluation of an amazingly large number of synthetics of diverse chemical structures.

In recent years, synthesis of antimalarials in India has been in progress in several institutes and laboratories, but biological assay was being conducted mainly against simian malaria in *rhesus* monkeys and to a lesser extent against *P. gallinaceum* in fowls. Cost of fowls and large animals like monkeys, and their maintenance, are considerably high and even then sufficiently large numbers are not always available. Furthermore, the quantity of chemicals required in trials with chicks or mice is much less than that necessary for trials in larger animals. Although some workers in India have tested compounds against *P. berghei* in white mice (Ramakrishnan *et al.*, 1951), certain degree of natural refractoriness to infection in some of the rodents was encountered (Ramakrishnan and Satya Prakash, 1950), unlike *P. gallinaceum* in chicks in which the infection is extremely severe in all cases, followed by death in over 99 per cent of cases.

Thus it would appear that screening of chemicals against *P. gallinaceum* in chicks, which are readily available, is not only economical but an extremely sensitive and rapid method.

Accordingly, a screening centre financed by the Council of Scientific and Industrial Research has been established at the Malaria Institute of India, Delhi.

The present paper records observations on such preliminary studies like the course and intensity of infection, their correlation with the dose of inoculum, mortality rates in uncontrolled infection, effects of known antimalarials and their relative value expressed in terms of quinine equivalent and similar other observations.

MATERIALS AND METHODS.

Avian host.—Observations were carried out in over 300 white leg horn chicks, hatched in the Institute laboratories or obtained from the Government Poultry Farm. The age of the laboratory-bred chicks was 7 days at the time they were taken up for experiments and the weight was more or less uniform between 50 and 60 gm. But ages and weights of those obtained from the poultry farm varied from 7 to 12 days and 40 to 90 gm. respectively. The birds were kept on standard diet consisting of mash and grain in the proportion of 70 and 30 per cent. Mash is made up of bran 22 per cent, ground wheat $12\frac{1}{2}$ per cent, ground barley $12\frac{1}{2}$ per cent, mixed ground cereals 4 per cent, milk powder 12 per cent and salt 1 per cent with 36 per cent of water. The scale of diet for 100 chicks was 10 lb. during the first week and 20 lb. during the second.

Parasite.—The strain of *P. gallinaceum* was the same as used by the authors during their studies on prophylactic effects of antimalarials in fowls (Jaswant Singh *et al.*, 1952).

Parasite count.—Thin blood smears were stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944) and the number of parasitized cells per 100 red blood cells per field of the oil immersion objective were recorded. For rapidity, the technique evolved by Gingrich (1932) was adopted. When infection was extremely low, estimation was based on number of parasitized cells per field of the oil immersion objective as regulated by Ehrlich's eye-piece adjusted to count 100 R.B.C. and until 30 such fields were counted. For infection of higher grade, estimation was based on parasites being counted by fields until 50 parasites were encountered. When infection was fairly high, parasite count was based on enumeration of individual red cells. For details the reader is referred to the original publication.

Inoculation.—For study of the course and intensity of infection, observation was recorded on three different doses of inoculum 1×10^6 , 0.5×10^6 and 0.1×10^6 per gm. of the body weight of the chick when the dose was injected intravenously through the right jugular vein*. Only one dose 1×10^6 was used when the inoculum was introduced subcutaneously. A small quantity of heparin was taken in the syringe while drawing blood from donor fowls and diluted in normal saline.

For assay of the suppressive action (A-1 test) of antimalarial drugs both in the experimental and in the comparison groups, an intravenous dose of 0.5×10^6 per gm. of parasites was also used.

* Initially it may not appear easy to inject into the vein but, if the neck is properly stretched on a wooden block with convex rounded top, the operation should present no difficulties.

For prophylactic trials, suspension of infected glands from *Aedes aegypti* were introduced intravenously as recommended by Davey (1946b) through the neck vein. The dose of infective inoculum consisted of sporozoites contained in the glands of one mosquito and expressed as one 'mosquito equivalent' (M.E.) dose. For comparison, doses of 0.1 and 0.01 M.E. were also used.

Insect host.—Freshly hatched *Aedes aegypti* from colony maintained in the laboratory were starved overnight and fed on infected fowls showing heavy gametocyte count. The mosquitoes were reared under conditions described previously by the authors (Jaswant Singh *et al.*, *loc. cit.*).

*Antimalarial drugs**.—Besides quinine, the well-known synthetic preparations like mepacrine, proguanil, chloroquine and amodiaquin (Camoquin) were used for A-1 test. The M.E.D. of each was determined and compared to that of quinine for determination of its quinine equivalent. For A-2 test (prophylactic trial) sulphadiazine, proguanil and pamaquin were used.

The dose was calculated in milligramme per 50 grammes of the body weight of the chicks, and on the first day drug administration commenced between 2 and 4 hours after infective inoculum (infected blood or sporozoites). Solutions or suspension of the compounds were introduced orally through a catheter attached to a syringe. Subsequently the dose was repeated twice on each of 3 consecutive days. Along with each series of investigation, a comparison group was included for assessing the intensity of uncontrolled infection. As a rule, 5 birds were kept under each dosage schedule. The time table adopted was somewhat similar to that observed by Curd *et al.* (*loc. cit.*) and consisted of the following procedure :

Monday	8 a.m.	..	The chicks were weighed and numbered.
	10 - 12 noon	..	Infected blood or gland was inoculated and the chicks were arranged in groups with different group signs marked with colouring agents.
	2 - 4 p.m.	..	The first dose of drug was administered.
Tuesday to	8 a.m.	..	Drug was administered and at the same time blood smears were taken from each bird.
Thursday	4 p.m.	..	
Friday	8 a.m.	..	Blood smears taken.

While observation was discontinued and the chicks destroyed in A-1 test, blood smears were continued to be taken daily for 26 days more in A-2 test.

Criterion of activity and determination of M.E.D.—The criterion of activity was determined by comparison of average parasite count on the 4th day after the day of inoculation (0-day)—that is the day following the last dose of drug to that of the untreated group. A schedule was considered to be effective if the average 4th day count of the treated group did not exceed 25 per cent of that of the comparison group (Wiselogle, *loc. cit.*).

The minimum dose which effected such deceleration in the course of parasitaemia is termed M.E.D.

* M.I.S. (Malaria Institute Survey) numbers 1 to 7 have been allotted to quinine, mepacrine, proguanil, chloroquine, amodiaquin, sulphadiazine and pamaquin respectively.

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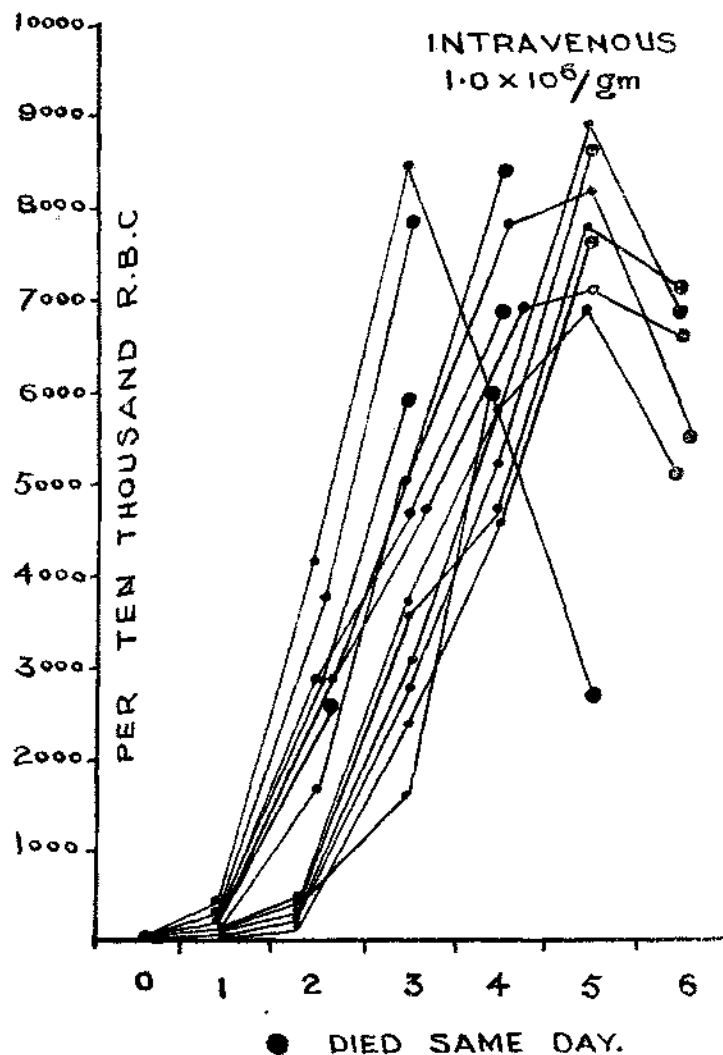
Quinine equivalent.—Quantitative evaluation of the effect of an antimalarial was made from its quinine equivalent (Q.E.) which is arrived at by dividing the M.E.D. of quinine by the M.E.D. of the test sample, i.e.

$$Q.E. = \frac{\text{M.E.D. of quinine}}{\text{M.E.D. of X}}$$

OBSERVATIONS AND RESULTS.

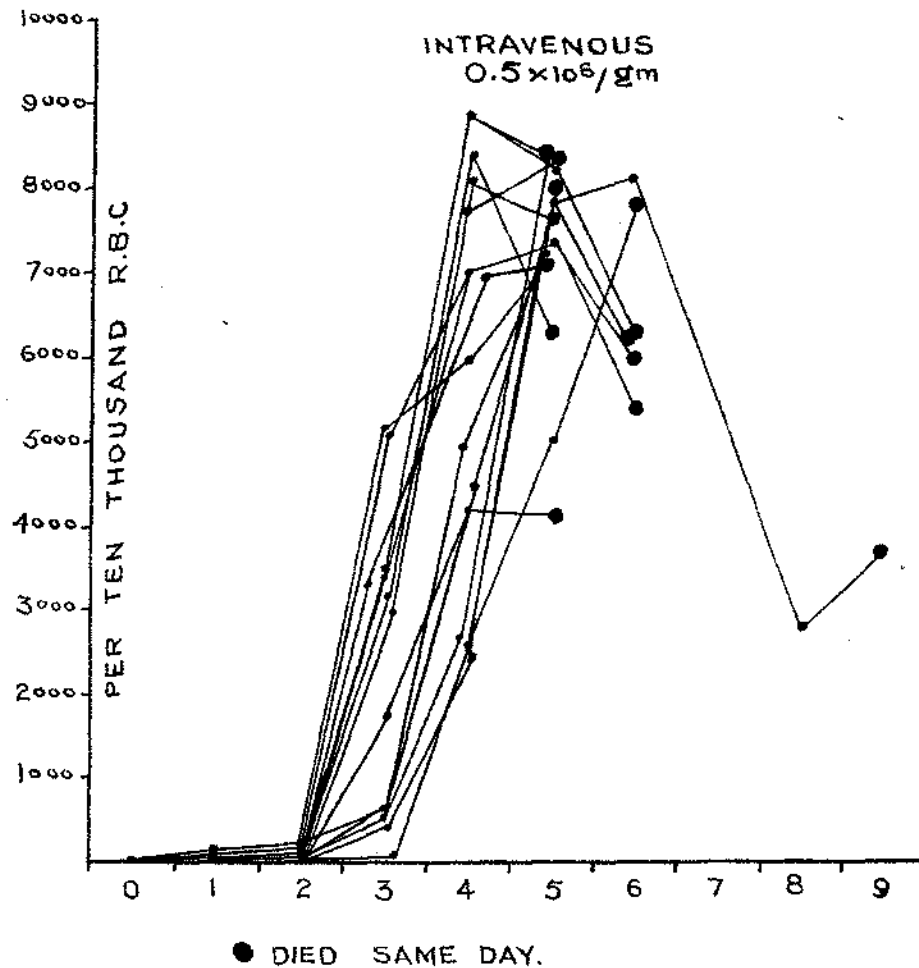
Uncontrolled infection.—Blood-induced infection, dose of inoculum, route prepatent period, intensity of infection, etc., are shown in Charts 1 to 4.

CHART 1.



There was no appreciable difference in the *prepatent period* in the groups of chicks which received inoculum of 1×10^6 or 0.5×10^6 gm. parasites intravenously. Patent parasitaemia was well established in both groups by the following day, whereas in the group which received the lowest dose of inoculum (0.1×10^6 gm.) there was delay by one day. But when an inoculum of 1×10^6 gm. was injected subcutaneously, the prepatent period varied between 4 and 7 days.

CHART 2.

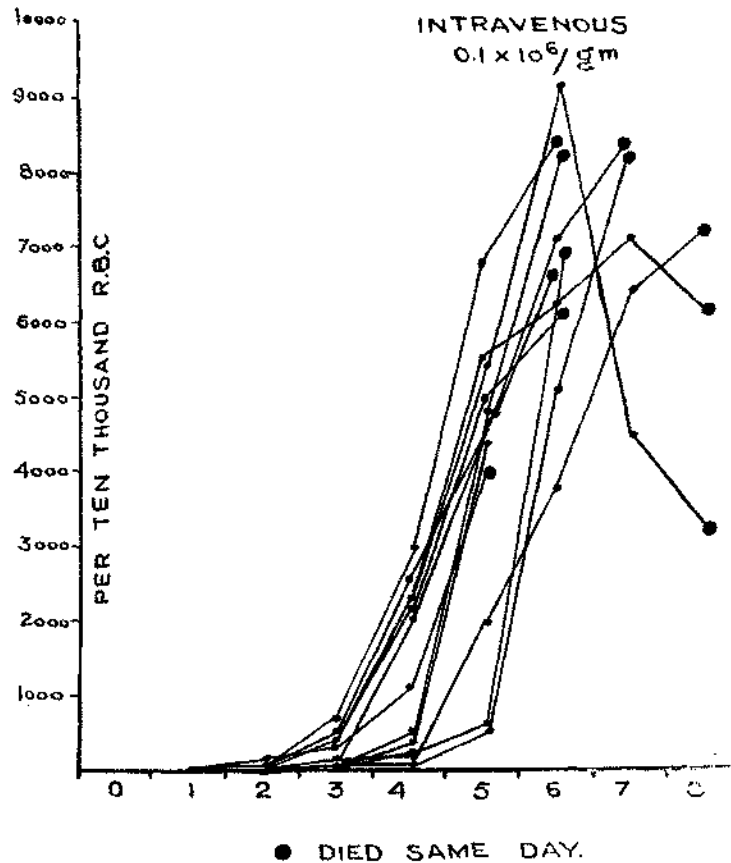


Course of infection.—Once patency was established the course was extremely rapid in those chicks which received intravenous injection of parasites. In most cases the increase appeared to be tenfold each succeeding day for 3 days till the infection attained a level of about 1,000 to 2,000 parasitized cells per

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10,000 erythrocytes. Thereafter the increase was about two to fourfold each day until the peak was attained.

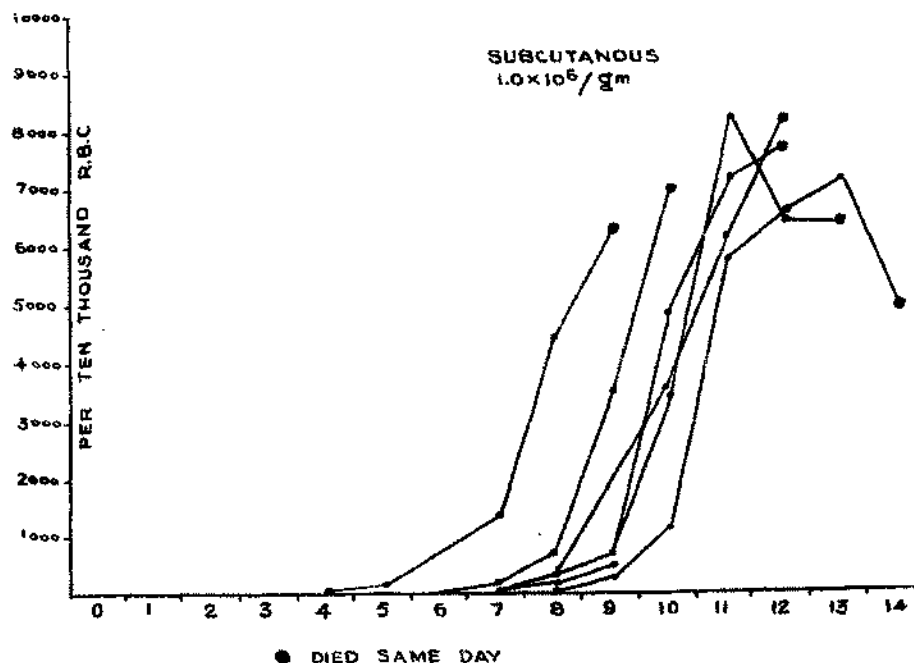
CHART 3.



With a dose of $1 \times 10^6 / \text{gm}$, the peak of infection was attained between 3rd and 5th day though in 50 per cent of cases (7 out of 14) it was on the 5th day. The peak was between the 4th and 5th day when the dose was 0.5×10^6 , whereas it varied between the 5th and 7th day in those which received $0.1 \times 10^6 / \text{gm}$. inoculum. On the day following the highest parasitic infection there was a drop in most cases and the chicks usually succumbed at about this time. However, death during the peak of infection was not uncommon particularly in the group which received the highest dose. Only 2 out of the 50 chicks which received different doses of parasites intravenously, survived for a period beyond the 7th day after the infective inoculum. One died on the 8th and the other on the 9th day.

The peak of infection was attained between the 3rd and the 6th day after patency was established in chicks which received infective dose subcutaneously. The mortality rate also varied accordingly.

CHART 4.



Sporozoite-induced infection.—With one mosquito equivalent dose (1 M.E.), in 4 out of 5 cases patent infection was established on the 6th day. In the other it was on the following day. With a tenth of the dose (0.1 M.E.) the period varied between 6th and the 8th day while with 0.01 M.E., the period was 8th day and beyond up to 12th day. However, in this type of infection, the chicks died within 2 to 6 days after patency was established. The intensity of infection was comparatively much lower than that observed in blood-induced infection.

Brain smear.—Soon after death, brain smears of chicks were examined for tissue forms. These were commonly encountered in those which received sporozoite-induced infection, but none in those which received blood inoculation.

Determination of M.E.D. of quinine.—Seventy-eight chicks were utilized for estimation of the minimum dose of quinine (calculated in terms of mg. base per 50 gm. body weight) which would lower the degree of parasitaemia on the day following completion of the course of treatment to a level which is no greater than 25 per cent of the average of the 4th day count of the comparison group. This also includes 15 chicks which served as the comparison groups. The trials were run in two series.

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In Series I, the dose of infective inoculum was 1×10^6 /gm. injected intravenously. Results are shown in Table I. It will be noted that a dose of 5 mg./50 gm. was effective in producing Class II effect, whereas Schedules II to VI produced Class I effect. A dose of 0.8 mg. reduced parasitaemia comparable to 60 per cent of the control group, whereas parasite counts in Schedules II to IV were much lower than 25 per cent of that of the comparison group. The minimum dose of quinine which was able to effect such results was 1.6 mg./50 gm.

TABLE I.
M.E.D. of quinine in chicks inoculated with 1×10^6 per gm. of parasites.

Schedule number.	Dose of quinine in mg./50 gm.	Number of chicks.	Average 4th day count per 10,000.	Comparison with control group, per cent.	REMARKS.
I	5.0	5	0	...	
II	2.5	5	85	1.3	
III	2.0	10	84	1.3	
IV	1.6	10	90	1.4	M.E.D.
V	0.8	10	3,800	59.4	2 died before observation was complete.
VI	0.4	5	6,000	93	
Comparison group	...	10	6,400	...	5 died before completion of observation.

In view of high mortality rates, particularly amongst chicks in the comparison group, the dose of inoculum was halved in Series II. Observations in uncontrolled infection with a dose of 0.5×10^6 /gm. have already been recorded as mentioned earlier. Results under Series II are shown in Table II :—

TABLE II.
M.E.D. of quinine in chicks inoculated with 0.5×10^6 per gm. of parasites.

Schedule number.	Dose of quinine mg./50 gm.	Number of chicks.	Average 4th day count per 10,000.	Comparison with control group, per cent.	REMARKS.
I	1.6	8	104	1.2	M.E.D.
II	0.8	5	4,950	62.5	
III	0.4	5	6,200	75.0	
Comparison group	...	5	8,100	...	

The findings are similar to that observed in Series I. Though doses lower than 1.6 mg. caused Class I effect, parasitæmia was much higher and comparable to over 50 per cent of the comparison group.

*Determination of M.E.D. in respect of mepacrine, proguanil, chloroquine and amodiaquin (Camoquin).—*The number of chicks utilized for these studies was 112. This included 15 placed under comparison group. Dose of inoculum in all cases was 0.5×10^6 /gm. of body weight of chicks. The trials were conducted in two series. Results obtained with mepacrine and proguanil in Series I are shown in Table III and those under II are shown in Table IV below :—

TABLE III.

M.E.D. of mepacrine and proguanil.

Schedule number.	Dose in mg./50 gm.	MEPACRINE.			PROGUANIL.		
		Number of chicks.	Average 4th day count per 10,000.	Comparison with control group, per cent.	Number of chicks.	Average 4th day count per 10,000.	Comparison with control group, per cent.
I	1.6	4	0	0.0	5	1	...
II	0.8	10	244	3.0	5	4	0.04
III	0.4	10	2,050	24.0	5	28.7	0.3
IV	0.2	8	4,260	51.9	5	182	2.2
V	0.1	...	...	...	5	232	2.8
VI	0.05	...	...	...	3	5,000	60.0
Comparison group	...	5	8,200	...	...	...	...

Only Schedule I under mepacrine produced Class II effect while all others under mepacrine or proguanil caused Class I effect. A dose of 0.4 mg./50 gm. was the minimum dose which reduced parasitæmia to less than 25 per cent of the control group and was thus considered to be the M.E.D. for mepacrine. Similarly,

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the M.E.D. for proguanil was determined to be 0.1 mg./50 gm. Thus Q.E. of mepracrine and proguanil may be calculated as 4 and 16 respectively.

TABLE IV.

M.E.D. of chloroquine and amodiaquin.

Schedule number.	Dose in mg./50 gm.	CHLOROQUINE.			AMODIAQUIN.		
		Number of chicks.	Average 4th day count per 10,000.	Percentage of comparison group.	Number of chicks.	Average 4th day count per 10,000.	Percentage of comparison group.
I	0.8	5	0	...	...	...	...
II	0.4	5	3	0.02	4	0	...
III	0.2	5	20	0.2	4	1.2	0.01
IV	0.1	4	297	3.8	3	75	0.9
V	0.05	3	8,400	109.0	4	1,255.0	16.3
VI	0.025	3	7,850	102.0	4	5350.0	69.4
Comparison group	...	10	7,700	...	...	...	...

Class II effect was produced by a dose of 0.8 of chloroquine while the same effect was observed with 0.4 mg. of amodiaquin. Except in Schedules IV and V under both drugs, reduction in parasitæmia (Class I effect) was a marked feature. With chloroquine in Schedules IV and V, there was no decrease in parasitæmia which seemed to be higher than the comparison group.

The minimum effective doses of chloroquine and amodiaquin may be assumed as 0.1 and 0.05 mg./50 gm. respectively. Hence quinine equivalents may be calculated as 16 for chloroquine and 32 for amodiaquin.

Effect of drugs against sporozite-induced infection.—Twenty-nine chicks were placed under prophylactic trials on sulphadiazine, pamaquin and proguanil. Five birds were placed under comparison group. Drug administration was commenced in the experimental group soon after intravenous injection of 1 M.E. dose. Out of these 29 chicks, 3 died before the 5th day due to causes undetermined. The actual number which survived a period of 6 days or over have

been indicated along with the results in Table V below. The doses adopted under proguanil and pamaquin were equivalent to the two higher schedules used in prophylactic trials in fowls undertaken previously by the authors and found effective (Jaswant Singh *et al.*, *loc. cit.*). Dose of sulphadiazine selected are comparable to 30 and 100 mg./kg. adopted in the previous investigation. Both the regimes under sulphadiazine afforded 100 per cent protection.

TABLE V.
Prophylactic trials with sulphadiazine, pamaquin and proguanil.

Antimalarial.	Schedule.	Dosage in mg./50 gm.	Number of chicks.	Patency established (days).	Protection up to 30 days.	REMARKS.
Sulphadiazine	I	1.25	3	...	3	100 per cent protection.
	II	5.0	3	...	3	100 per cent.
Pamaquin	I	0.125	3	7th-8th	Nil.	...
	II	0.25	4	7th-8th	Nil.	...
Proguanil	I	0.4	4	2 on 9th	2	50 per cent.
	II	0.8	4	13th	3	75 " "
Comparison group	...	...	5	6-7	Nil.	...

DISCUSSION.

Earlier works on the chemotherapeutic activities showed that studies were conducted in such a way that the results could only be expressed qualitatively like slight or profound action, inactive or of doubtful action (Curd, 1943). Thus elaboration of a technique like Roehl test, whereby antimalarials could be evaluated quantitatively, was no doubt a step in the right direction. Although the technique deserves the merit of simplicity, it suffered from several defects. Following the discovery of the antimalarial activity of certain sulphonamides by Diaz de Leon in 1937 (Findlay, 1951), it was soon realized that, though some of them were active against human malaria, they were ineffective against *P. relictum* used for Roehl test in canaries. Thus observations made in this test cannot always be interpreted in terms of the efficacy or otherwise of a compound against human malaria. Secondly, canaries were not always available to all workers. The intramuscular route of inoculation adopted in Roehl test is not considered to be a satisfactory method as patent infection is established only

after 4 to 8 days, thereby facilitating immune mechanism to be operative. Further, the index of activity as determined by chemotherapeutic index (ratio of maximum tolerated dose to the minimum effective dose) often bore no relation to avian and human malaria (Wiselogle, *loc. cit.*).

In view of these discrepancies, Coatney *et al.* (1945) and Curd *et al.* (*loc. cit.*) preferred *P. gallinaceum* in small chicks in which the resultant infection after intravenous inoculation is extremely severe and, if untreated, death invariably followed within a few days. Further, the test is so sensitive that any compound which has even a slight antiplasmodicidal action will delay the death according to its degree of action.

During the present studies the severity of blood-induced *P. gallinaceum* infection in chicks is well demonstrated, parasitaemia reaching as high as 8,000 to 9,000 per 10,000 red blood cells in some cases and between 7,000 and 8,000 in most, whether the infective inoculum was injected intravenously or subcutaneously. However, the prepatent period was considerably prolonged after subcutaneous injection and the peak period varied from 8th to the 13th day, and thus suffered from the same defects as the Roehl test.

There was no difference in the prepatent period after intravenous injections of 1×10^6 /gm.(I) or 0.5×10^6 /gm.(II), and this is obviously due to the very large number of parasites introduced. However, with a dose of 0.1×10^6 /gm. (III), the prepatent period was delayed by a day. Although dose I is more or less similar to that (40 to 50 million parasites per 40 to 50 gm. chicks) used by Curd *et al.* (*loc. cit.*), in the present series it was observed that the peak was attained between the 3rd and the 5th day. Moreover, not uncommonly the chicks died as early as on the 3rd or 4th day. In view of this, dose I was not considered suitable, as the 4th day count was not available in all cases of the comparison groups so necessary to determine the M.E.D. of the antimalarials (Table I).

Again with dose III (0.1×10^6 /gm.) the peak period was spread over from the 5th to the 7th day and there was a delay by a day in the establishment of patent parasitaemia and thus even this was also not considered suitable. On the other hand with a dose of 0.5×10^6 the general pattern of infection though somewhat similar to the higher dosage of inoculum, the chicks did not die until after the 4th day and the peak was between the 4th and 5th day. These results are not altogether dissimilar to those attained by an overall dose of 16 million adopted in U.S.A. for chicks weighing between 43 and 59 gm. (average 50 mg.). However, the dose of inoculum calculated in terms of millions of parasites per gm. of body weight as adopted in the present series would appear to be more accurate and better standardized.

The dose of inoculum adopted for sporozoite-induced infection is the same as used by Coatney *et al.* (*loc. cit.*). In uncontrolled infection the chicks died within a few days after the patency was established at a time when parasitaemia was comparatively low. This is mainly due to blockage of the capillaries by tissue forms of *P. gallinaceum* as determined by examination of brain smear. In blood-induced infection, death during the first week occurred due to the severity of the infection when 70 to 80 per cent of the cells were parasitized.

From Tables I and II it is apparent that the M.E.D. of quinine producing Class I effect is 1.6 mg./50 gm. or 32 mg./kg. whether dose of infective inoculum

used was 1×10^6 /gm. or 0.5×10^6 /gm. The advantage with the latter dose was that none of the chicks in the comparison group died during the period of observation—a phenomenon found to be common with the higher dose. The M.E.D. of quinine determined seems to be in conformity with the findings of Coatney and his colleagues (Wiselogle, *loc. cit.*).

The minimum effective dose of mepacrine capable of producing Class I effect is 0.4 mg./gm. and thus quinine equivalent of mepacrine is 4. The same for proguanil, chloroquine and amodiaquin are 16, 16 and 32 respectively.

Based on series of investigations conducted in U.S.A., Wiselogle (*loc. cit.*) reported that the quinine equivalents are 2 to 6 for mepacrine, 10 for proguanil (against *P. lophura* in ducks), 15 for chloroquine, and 8 to 30 (against *P. gallinaceum*) and 30 (*P. lophura*) for amodiaquin. Thus the results obtained in the present series are very much comparable to those reported above.

During prophylactic trials with pamaquin against *P. gallinaceum* in fowls Jaswant Singh *et al.* (*loc. cit.*) had reported that a dose of 1.25 mg./kg. or higher completely protected all the fowls, whereas in the present series even a dose of 0.25 mg./50 gm., equivalent to 5 mg./kg., proved completely ineffective in chicks.

Although pamaquin has shown prophylactic action against certain avian species of plasmodia, Greenberg *et al.* (1950), Marshall (1946) and Findlay (*loc. cit.*) reported about its ineffectiveness against *P. gallinaceum* in chicks similar to that observed by the present authors. The possible explanations for the different reaction to the same drug by the same species in chicks and fowls have already been discussed by Jaswant Singh *et al.* (*loc. cit.*).

That proguanil is a causal prophylactic antimalarial against *P. gallinaceum* in chicks has been amply demonstrated in the past by Cud *et al.* (*loc. cit.*), Davey (1946a, 1946b) and a host of others. During the present investigation it was also shown that a dose of 0.8 mg./50 gm. (equivalent to 16 mg./kg.) completely protected 4 out of 8 chicks. This is somewhat comparable to the results of trials in fowls conducted previously by the present authors who had reported protection rates of 80 per cent with a dose of 5 mg./kg. and 100 per cent with 7.5 or 15 mg./kg. doses (Jaswant Singh *et al.*, *loc. cit.*).

Greenberg *et al.* (*loc. cit.*) consider that a dose of 1.0 M.E. for a chick is far too high and as such workers who had originally used the dose were unable to demonstrate prophylactic action of many 8-aminoquinolines. Using the same dose of infective inoculum, the present authors have shown ineffectiveness of pamaquin and the causal prophylactic action of proguanil.

Coatney and Cooper (1941) were the first to demonstrate the prophylactic value of sulphadiazine against *P. gallinaceum* in domestic fowls. Subsequently, Davey (1946b) made similar observations against the same species in chicks. During the present studies it was noted that both the schedules were effective. These two schedules were somewhat similar to the two (30 and 100 mg./kg.) out of the five schedules tried by Jaswant Singh *et al.* (*loc. cit.*) against the same species in fowls. While in the previous series a dose of 30 mg. was ineffective, the corresponding dose of 1.25 mg./50 gm. in the present study was able to protect all the 3 chicks.

SUMMARY.

1. A centre has been established at the Malaria Institute of India for rapid screening of antimalarials against *P. gallinaceum* in chicks.

2. Prepatent period, course and intensity of infection and mortality rates have been determined after intravenous injection of different doses of infective inoculum of blood or gland emulsion from mosquitoes.

3. The M.E.D. of the well-known antimalarials like quinine, mepacrine, proguanil, chloroquine and amodiaquin have been assessed and their quinine equivalents have been determined.

4. Prophylactic value of sulphadiazine, pamaquin and proguanil has been assessed.

5. The standards obtained during these investigations will now be of immense help for quantitative evaluation of any new chemical.

ACKNOWLEDGMENTS.

The authors wish to thank the Council of Scientific and Industrial Research for the financial aid for setting up a centre for screening of antimalarials which will not only facilitate rapid screening of new compounds but will infuse fresh enthusiasm for development of synthetic antimalarials in the country. The authors also wish to acknowledge the helpful co-operation of Mr. R. S. Chaudhury, Adviser, Government Poultry Farm and his staff for regular supply of fertilized eggs and also of chicks whenever required.

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PROPHYLACTIC TRIAL WITH LAPINONE AGAINST *P. GALLINACEUM* IN FOWLS.

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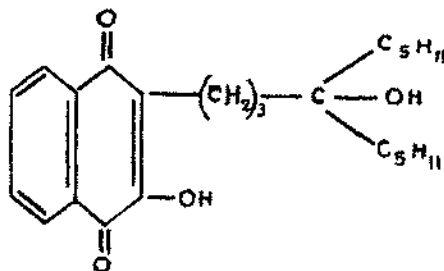
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[June 30, 1952.]

FIESER *et al.* (1948a) synthesized a number of naphthoquinones, some of which have been claimed to possess remarkable activity not only as suppressants but also as prophylactics and curative agents against avian malaria. Though many of them were found to be active against avian malaria, they have not been shown to be so against human malaria (Wiselogle, 1946; Ruhe *et al.*, 1949). The difference in reaction is attributed to their rapid degradation, mainly oxidation in the human body. Several of the isolated metabolites were found to be secondary alcohols, tertiary alcohols or carboxylic acids (Fieser *et al.*, 1948b). One of the synthesized naphthoquinones (Lapinone) with a tertiary alcohol group in the side-chain has been found to be resistant to degradation when given intravenously (Fawaz and Fieser, 1950). Fawaz and Haddad (1951) have demonstrated some definite plasmodicidal activity of lapinone against *P. vivax* malaria when administered intravenously. The chemical formula of lapinone is



The present report records the findings on the use of lapinone against sporozoite-induced *P. gallinaceum* infection in fowls.

MATERIALS AND METHODS.

Avian host.—Observations were carried out in 26 white leg-horn fowls each of an average weight of 0.8 kg. (0.5 to 1.5 kg.). The fowls were obtained from the Government Poultry Farm and were kept on usual standard diet of grains and vegetables.

Parasite and insect hosts.—A strain of *P. gallinaceum* used during these investigations has been maintained in these laboratories through repeated blood passage in fowls Colony-raised *Aedes aegypti* mosquitoes were fed on fowls showing heavy gametocyte counts.

Inoculation.—Suspension of infected glands in serum-saline solution was introduced through the wing vein in each case. The inoculum consisted of sporozoites contained in the glands of two mosquitoes (two mosquito-equivalent dose) for each fowl. The procedure followed was the same as described by Jaswant Singh *et al.* (1952a).

Blood smear examination.—From the fourth day of inoculation, thin blood smears from the experimental fowls were stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944) and were examined daily for parasites for 4 weeks. The parasite counts were made against 10,000 erythrocytes.

Drug.—Lapinone, dark brown in colour and almost tar-like in consistency, was made up in a 10 per cent solution (w/v) in sterile olive oil (slightly warmed). The resulting uniform solution was found suitable for intramuscular injections. The dosage was calculated in milligram per kg. of the body weight. The first dose was injected intramuscularly 45 minutes after sporozoite inoculation. Subsequently, the drug was repeated twice a day for the next 3 days. Three fowls which received no drug served as the comparison group.

Criteria of activity.—Efficacy of the drug was determined by its ability to afford complete protection or prolongation of the prepatent period as compared to the comparison group.

RESULTS.

The dosage schedules employed, with the results, are shown in the Table:—

TABLE.
Prepatent period under different dosage schedules.

Schedule.	Dosage, mg./kg.	Number of fowls.	Prepatent period (in days).	Delayed by days.	Protection.
I ...	2.5	4	7, 10, 9, 8 (7-10)	Nil	Nil.
II ...	5.0	4	8, 9, 10, 9 (8-10)	"	"
III ...	7.5	5	10, 9, 10, 8, 9 (8-10)	"	"
IV ...	10.0	3	9, 8, 10 (8-10)	"	"
V ...	15.0	3	13, 9, 11 (9-13)	1-3 days	"
VI ..	30.0	4	16, 16, 16, 15 (15-16)	5-6 days	"
Control ...	...	3	7, 8, 10 (7-10)	...	...

It will be observed from the Table that in the untreated fowls the prepatent period was between 7 and 10 days from the date of inoculation. Results with Schedules I to IV show no significant variation from observations in the comparison group, whereas the prepatent period was prolonged by 5-6 days under Schedule VI. With a dose of 15 mg. the delay is not marked. Lapinone did not afford complete protection in any of the fowls.

DISCUSSION.

Several members of the hydroxynaphthoquinone series have been found to afford complete protection to chicks receiving inoculation of sporozoites of *P. gallinaceum*. Clarke and Theiler (1948), who had planned their experiments to find the actual phase of the parasite on which the hydroxynaphthoquinones exert their prophylactic action, gave intramuscular injection half an hour before the sporozoite dose. They concluded that their action was against the sporozoites. In similar investigations, Bauer (1934) who had administered naphthoquinone compounds orally 24 hours prior to sporozoite inoculation reported that some of them had prophylactic action.

Davey (1946) has shown that the sporozoites remain in circulation for about 15 minutes in chicks following inoculation with *P. gallinaceum*; and for a maximum of 30 minutes in man following inoculation of sporozoites of human plasmodia (Fairley, 1945). In the present investigation the drug was administered 45 minutes after sporozoite inoculations. Admittedly in this series, action of the drug was not aimed at against the sporozoites in the peripheral circulation, but determining the efficacy of lapinone on the pre-erythrocytic forms of the parasite as was carried out in previous studies on other synthetic antimalarials by Jaswant Singh *et al.* (1952a, 1952b).

From the results it may be noted that even a dose of 30 mg./kg. of lapinone failed to afford complete protection. The prepatent period was, however, prolonged in 2 out of 3 cases receiving a dose of 15 mg. and in 4 out of 4 cases with a dose of 30 mg./kg. Similar delay in the appearance of parasites in the peripheral blood following sporozoite inoculation was reported by Jaswant Singh *et al.* (1952a) in respect of amodiaquin and chloroquine which are purely suppressive drugs. Thus it would appear that lapinone has no action on the pre-erythrocytic forms of *P. gallinaceum*.

SUMMARY.

Lapinone was administered intramuscularly in fowls inoculated with sporozoites of *P. gallinaceum*. It showed no action on the pre-erythrocytic forms, although it suppressed the infection temporarily for a few days in doses of 15 mg. and 30 mg./kg.

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EFFECT OF PARENTERAL ADMINISTRATION OF ANTIMALARIALS IN SIMIAN MALARIA.

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OPINIONS are unanimous on parenteral administration of antimalarials as the method of choice when immediate action is desired in grave emergencies like cerebral malaria or in cases where absorption of the drug from the gastro-intestinal tract is delayed or prevented. Quinine had been used in the past very extensively either in the form of hydrochloride or bishydrochloride, injected by the intravenous or intramuscular route. Even to-day, under such conditions, parenteral quinine therapy has been considered to be the best course of treatment (Black, 1949; Covell, 1949; Jaswant Singh, 1950). However, regarding the choice between intravenous or intramuscular route, there seems to be a remarkable diversity of opinion though most authorities agree that intravenous route should be preferred for reasons to be discussed later.

Although from time to time metallic compounds like antimony or arsenic were tried either alone or in combination with quinine, parenteral therapy has not materially altered since then.

With the advent of synthetic antimalarials, mepacrine dimethanesulphate (Atabrin musonate) or hydrochloride has also been in use in ordinary or in severe cases of malaria. But in view of some toxic manifestations reported by Briercliffe (1935), Shannon *et al.* (1944) and others, mepacrine has not received the same consideration as quinine. While recent preparations like proguanil (paludrine) acetate or lactate have not shown the desired success, chloroquine diphosphate, administered by the intramuscular (Culwell *et al.*, 1950) or intravenous route (Scott, 1950), has been found to be highly promising.

The present report records observations made during a comparative study on the effects of intravenous administration of quinine, mepacrine, NAB, quinarso and chloroquine on the course of parasitæmia in severe infections with *P. cynomolgi* in *rhesus* monkeys. Acute toxic manifestations in doses higher than those used for therapeutic purposes have also been recorded.

MATERIALS AND METHODS.

Twenty-eight healthy North Indian *rhesus* monkeys were utilized for the therapeutic studies and 4 others for comparison purposes. Some of these animals, in which complete clearance of parasites was not attained and infection took a chronic course, were used for toxicity tests some weeks later. In addition, 22 more monkeys, which were utilized for maintenance of the strain of plasmodium and later showed chronic infections, were placed under the toxic dosage schedules.

A strain of *P. cynomolgi* originally isolated by Sinton and Mulligan (1932) and maintained in these laboratories ever since was utilized for these studies.

Each monkey was inoculated with a dose of 100×10^6 parasitized cells per kg. of the body weight of the animal and the day of inoculation was considered '0'-day.

Blood smears, thick and thin, were stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944). Ehrlich's eyepiece adjusted to count 100 erythrocytes of the thin film per field of the oil immersion objective was used for enumeration of parasitized cells. One hundred such fields were counted and the number of such cells per 10,000 erythrocytes was recorded. Before recording negative results, at least 200 fields of thick film were examined using the ordinary eyepiece.

The antimalarials tested in these investigations were dihydrochloride salts of quinine and mepacrine, chloroquine diphosphate (Resochin), novarsenobillon, and also quinarsol which is supplied in ampoules of 2 c.c., each c.c. containing 5 grains (300 mg.) of quinophenyldimethylpyrazolone with 0.115 grain (7.6 mg.) natriumdimethylarsenicum.

The human equivalent doses of these antimalarials were calculated in terms of mg. per kg. of the body weight and were injected intravenously as a single dose in some group. In others, the dose was repeated after 8 hours. In addition, certain higher as well as lower dosage schedules were tried. For calculation, the average human weight was taken as 70 kg.

Drugs were administered by the intravenous route on the 6th day after the infective inoculum, at a time when parasitaemia had attained a level of 10 to 20 per cent of the erythrocytic cell infection. This is a departure from the usual practice adopted for routine therapeutic studies in these laboratories where test samples are administered orally at a time when infection attains 0.1 to 0.2 per cent infection (Jaswant Singh *et al.*, 1951).

For determining the criterion of activity, parasite counts on the day following the treatment were compared with the average 7th day parasitaemia of the comparison group.

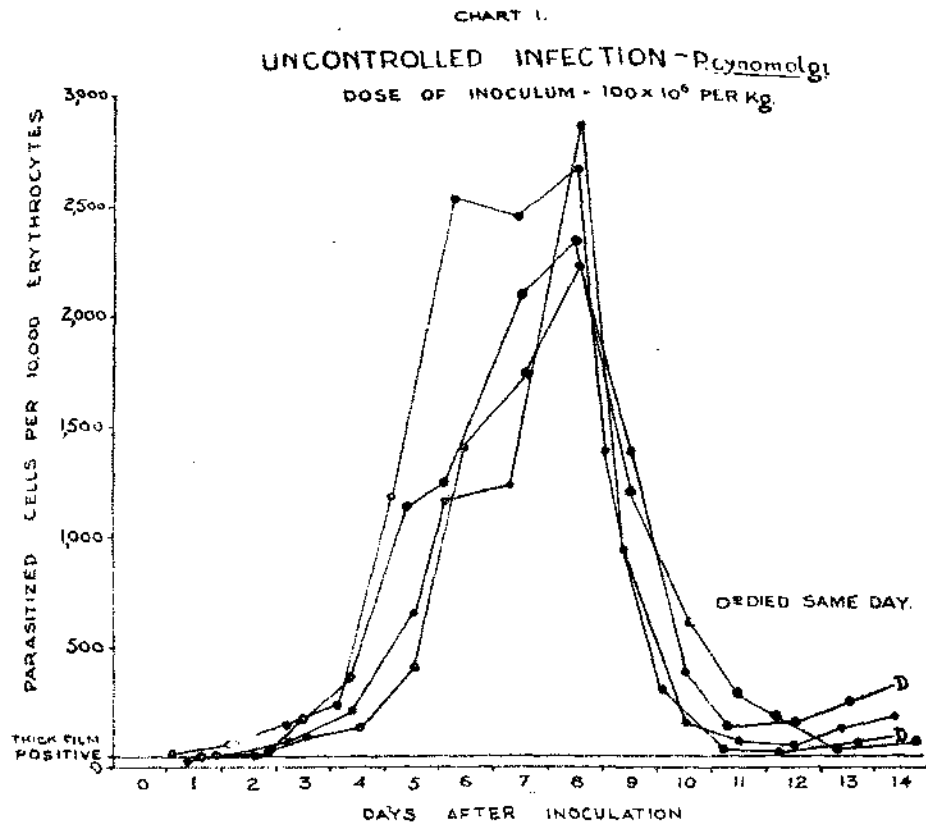
In order to determine sterilization of the blood-induced infection, spleen was removed from those monkeys in which parasite clearance from peripheral circulation was attained after treatment and thick films remained negative for a period of 2 weeks. If no parasitaemia was detectable for a period of fortnight after splenectomy, it was regarded that the monkeys were completely cured.

During acute toxicity studies, only a single intravenous injection was given. The initial doses of quinine and chloroquine were $1\frac{1}{2}$ times more than the highest therapeutic ranges tried, and thereafter the subsequent doses were increased similarly till 100 per cent lethal dose was reached. The initial dose of 7.0 mg. of mepacrine was increased by 1.5 mg. in the succeeding series.

Toxic manifestations were recorded during and after the intravenous injections. The monkeys which died were subjected to post-mortem examination.

OBSERVATIONS AND RESULTS.

Uncontrolled infection.—The prepatent period, course and intensity of infection in the 4 monkeys which were not treated are shown in Chart 1. With a heavy dose like 100×10^6 , patent parasitaemia was established almost immediately, and in most cases parasites were detectable in thin films by the following day. After the 3rd day the rate of increase in parasitaemia was extremely rapid till the peak was attained on the 8th day. Thereafter reduction in parasitaemia occurred by crisis (Chart 1).



In all cases the infection became chronic with concomitant anaemia of severe type and 2 out of 4 monkeys died by the 14th day after the inoculation. After crisis, the total erythrocytic count varied from 0.8 to 2.1×10^9 and haemoglobin percentage was between 14 and 40.

Therapeutic efficiency.—The effect on the course of infection and the reduction in parasitaemia under the single dosage regime is shown in Table I. Similar observations after two doses on the same day appear in Table II.

TABLE I.
Effect of single intravenous dose of antimalarial.

Antimalarial.	Recommended dose in human malaria.	Dose in mg./kg. employed.	Number of animals.	AVERAGE PARASITEMIA PER 10,000 RBC.		Percentage of reduction as compared to 7th day parasitemia in control group.	REMARKS.
				On day of treatment (8th day).	On day following (7th day).		
Quinine	600 mg. (10 grains)	8.6†	2	1,500	90.0	36.5	Complete parasit clearance did not occur and infection became chronic.
Quinarsol*	300 mg. (5 grains) Q. and 7.6 mg. (0.115 grains) As.	4.2†	2	1,635	1,340.0	71.0	
		8.4	2	1,280	680.0	38.0	
Arsenic (NAB)	1,000 mg. (1.0 gm.)	14.2†	2	1,440	1,185.0	62.8	
Mepacrine	100 mg.	1.42†	2	1,426	637.5	33.7	
		5.68	2	1,275	255.0	13.5	Clearance within 72 hours.
		1.42	2	1,365	435.0	22.9	
		5.71†	2	1,520	56.0	2.9	
Chloroquine	400 mg.	11.42	2	1,205	11.0	0.58	Clearance within 60 hours.

*For Quinarsol, dosages used were 4.2 and 8.4 mg. of the quinophenyldimethylpyrazolone and the proportionate dose of sodiumdimethylarsenium.

† These doses in mg./kg. correspond to recommended dose for human cases of an average weight of 70 kg.

Q. = quinophenyldimethylpyrazolone.

As. = sodiumdimethylarsenium.

TABLE II.

Effect of two doses of antimalarials administered intravenously at interval of 8 hours.

Antimalarial.	Dose in mg./kg. equivalent to human adult dose.	Total daily dose.	Number of animals.	AVERAGE PARASITAEMIA PER 10,000 RBC.		Percentage of reduction as compared to 7th day parasitaemia of control group.	REMARKS.
				On day of treatment (6th day).	On day following (7th day).		
Quinine ...	8.6	17.2	2	1,710.0	325	17.2	No clearance of parasites during period of observation of 2 weeks.
Quinarsol ...	4.2	8.4	2	1,400.0	1,175	62.2	
As (NAB) ...	14.2	28.4	2	1,122.5	370	38.6	
Mepacrine ...	1.42	2.84	2	1,385.0	535	28.3	
Resochin ...	5.71	11.42	2	1,627.5	23	1.2	Parasite clearance within 72 hours.

It will be observed from Table I that with schedules comparable to human doses, the therapeutic efficiency was least marked with quinarsol and most dramatic with chloroquine. The reduction caused by NAB, though slightly better than quinarsol, was only half as much as produced by quinine and mepacrine.

By doubling the dose of quinarsol, reduction in parasitaemia was proportional to the twofold increase and became comparable to that caused by quinine. On the other hand, a twofold increase over the human equivalent dose of chloroquine resulted in fivefold reduction. But when the dose of mepacrine was increased to 4 times, the reduction was not proportional but only a little over twofold.

When the human equivalent dose was administered the second time, the parasitic reduction with all antimalarials was more marked, except under quinarsol and was proportional to the increase in the dosage over the single dosage schedules. The reduction was maximum with chloroquine and least with quinarsol.

In cases treated with chloroquine in doses of 5.7 mg. or more, there was complete clearance of parasites from the peripheral blood within a period of 60 to 72 hours, though sterilization was attained only in cases treated with the highest single dose. In all other monkeys treated with quinine, mepacrine, quinarsol, NAB or chloroquine (1.42 mg.), parasitaemia was detectable throughout and infection became chronic after the initial reduction.

In view of the encouraging results observed with quinine, mepacrine and resochin, acute toxicity tests were carried out in respect of these three compounds

only. From Table III it will be observed that the maximum dose of quinine which did not give rise to any toxic symptoms was 13.0 mg./kg., i.e. $1\frac{1}{2}$ times the single therapeutic dose (Table I). Similarly, the maximum tolerated dose of chloroquine was 17.1 ($1\frac{1}{2}$ times the highest therapeutic dose of 11.4 mg./kg.). But an increase by 1.5 mg. over the highest therapeutic dose of atabrin produced some toxic manifestations. L.D.₅₀ for quinine was 17.2 (double the therapeutic dose) as compared to 10 mg./kg. for mepacrine. L.D.₁₀₀ was observed to be 34.2 for quinine, 11.5 for mepacrine and 28.5 for chloroquine.

TABLE III.
Acute toxic effects after a single intravenous dose.

Antimalarial.	Dosage.	Number of animals.	SURVIVAL.		Death within	REMARKS.
			Without toxic symptoms.	Showed toxic symptoms.		
Quinine bihydrochloride	13.0	2	2	...	...	Dose well tolerated.
	17.2	2	1	...	1 (30 minutes)	
	(Schedule increased by 5.6 mg./kg.).	23.0	2	...	1 (5 ")	L.D. 100.
	28.6	2	1	1	1 immediately	
	34.2	2	...	...	2 (5 minutes)	
Atabrin bihydrochloride	7.0	2	1	1	...	L.D. 100.
	8.5	2	...	2	...	
	(Schedule increased by 1.5 mg./kg.).	10.0	2	...	1 (5 minutes)	
	11.5	2	...	...	2 (immediate to 5 minutes).	
Resochin ...	17.1	2	2	...	...	Drowsiness feature was marked.
	(Schedule increased by 5.7 mg./kg.).	22.8	2	...	...	
	28.5	2	...	...	2 (5 minutes)	
	34.2	2	...	...	2 immediately	
	45.6	2	...	...	2 "	

Toxic symptoms due to quinine were found to be depression preceded by transient excitability, followed by rapid breathing and there was mild convulsion. Later, respiration became weak and slow, developing in acute respiratory distress. On auscultation, heart sounds were slightly accelerated at first, then slow, and ultimately became faint. Subsequently breathing was laboured, irregular and finally stopped. Muffled heart beats could be heard for a short period even after the respiration had stopped.

With toxic doses of mepacrine, there was spasmodic contraction of the whole body, restlessness, rapid and shallow respiration followed by death.

The earliest toxic manifestation due to chloroquine was drowsiness. This was followed by twitching of facial muscles, convulsive seizures, hurried respiration, breathlessness, acute air-hunger followed by death.

Besides certain amount of congestion of the brain, no other abnormal features were noticeable during post-mortem examination of the monkeys which died due to heavy doses of quinine, mepacrine or chloroquine.

DISCUSSION.

The object of parenteral administration of antimalarials is to ensure prompt action in real emergency. Quinine and other drugs have been injected by the intravenous or intramuscular route. Many workers have advocated intramuscular route for quinine (Stott, 1915; Stephens *et al.*, 1919; McRobert, 1925) but majority (Bass, 1915; Lane, 1916; James, 1917; Knowles, 1918; Rogan, 1944; Strahan, 1948; Black, 1949; Covell, 1949) consider that absorption from muscles and subsequent concentration in the blood is irregular and slower than by the intravenous route. The defective concentration in the blood and consequently less rapid action against the parasites during an emergency is likely to defeat the very object for which parenteral administration is recommended. Further, some are of opinion that intramuscular injection is likely to cause much local damage leading to necrosis of the muscle tissues, particularly when too concentrated solution of a drug like quinine is injected. It is also believed that when the acid salt is injected into the tissues whose reaction is alkaline there is likelihood of local damages (Acton and Chopra, 1924). Similarly, opinions differ with regard to mepacrine. Field and Niven (1936) believe that intravenous route for atabrin musonate is not necessary as intramuscular route is as rapid.

In view of the different routes, techniques, severity of infection of different strains and advent of newer synthetic antimalarials, proper analysis under standard conditions of the merits of those drugs used parenterally has not been possible. Therefore, during the present investigations due attention was paid to all these factors. The high dose of inoculum, and the commencement of treatment at 10 to 20 per cent erythrocytic cell infection which is one hundred times more than usually employed in these laboratories during therapeutic trials, simulated conditions of severe infection in human malaria when parenteral administration of antimalarials is considered justifiable.

The peak of parasitaemia in uncontrolled infection attained an average of 25.7 per cent cell infection on the 8th day against 18.75 per cent on the 7th day. Thus any possible lowering of parasitaemia on the 7th day in treated animals could only be attributed to the effect of the drug and not due to any other factor.

In the regimes where the recommended human equivalent adult dose was administered, the reduction in parasitaemia due to a single dose of chloroquine was remarkable in that 97.1 per cent of parasites (as compared to the comparison group) were destroyed, as against 63.5 per cent and 66.3 per cent with quinine

and mepacrine, respectively. Action of quinine and mepacrine appears to be somewhat similar. On the other hand, response to the single dosage regime with quinarsol and arsenic was poor, more so with quinarsol.

But when the dose of quinarsol was doubled (8.4 mg./kg.) the reduction was identical to that attained with almost similar dose of quinine (8.6 mg./kg.). Thus it may be interpreted that a single human equivalent dose of quinarsol, though capable of causing slight deceleration in the course of parasitaemia, is inferior to quinine or mepacrine and much more so when compared to chloroquine. It is obvious that the effect produced by quinarsol was mainly due to the quinophenyldimethylpyrazolone content and dose for dose, its activity was similar to quinine. The small amount of arsenic in the form of sodiumdimethylarsenicum did not seem to exert any significant effect. Though NAB treatment was effective in reducing parasitaemia to 62.8 per cent of the control group (reduction by 37.2 per cent), it is about half as effective as quinine or mepacrine and is much below par compared to chloroquine.

The therapeutic efficiency after fourfold increase in the dose of mepacrine (comparable to the human equivalent dose of chloroquine) is at once apparent, but still the reduction was not as dramatic as with chloroquine with which parasitaemia was reduced to 0.58 from 2.9 per cent when the dose was doubled (11.4 mg.). But when it was reduced to 1.4 mg., comparable to the human equivalent dose of mepacrine, parasitic reduction was not proportional, although dose for dose better results were observed with chloroquine than with mepacrine.

When the human equivalent doses in respect of all the antimalarials under discussion were repeated after 8 hours, significantly better results were noticeable in all the cases though much more so with quinine and NAB where reduction was about twofold and thus proportional to the increase in the total amount of the drug. But no great advantage was noticeable in respect of quinarsol and mepacrine when the doses were repeated. On the other hand, the results obtained with chloroquine appeared to be interesting. With a single dose of 5.7 mg., the reduction after 24 hours was 2.9 per cent but when the same dose was repeated after 8 hours, the reduction was 1.2 per cent, i.e. the number of parasites were halved. The same total dose of 11.4 mg./kg., when administered as a single dose, yielded a much greater reduction (fourfold).

Thus the overall picture is that while quinine, mepacrine and chloroquine were highly effective in the recommended human equivalent doses, quinarsol and NAB showed only partial action.

Das Gupta and Siddons (1944) reported that neoarsphenamine was ineffective against *P. knowlesi* with an overall dose ranging from 7.5 to 45 mg. and that the action was partial when the dose was increased to 60 to 90 mg. Against human malaria, arsenic compounds have shown some degree of activity against *P. vivax* but very little action against *P. falciparum*, and as such the rôle of arsenic preparations in treatment of severe cases of malaria is dubious.

de Souza (1939) reported that after quinarsol treatment, clearance of *P. falciparum* occurred earlier than *P. vivax* infection and thus quinarsol treatment in severe cases of *falciparum* malaria should hold some prospect. But during the present series no particular advantage over quinine was observed

when both quinine or quinarsol was administered intravenously in almost equal doses.

Eckhardt (1933) advocated a maximum dose of 0.2 gm. of atabrin intravenously, though Dawson *et al.* (1935) consider that the dose should not exceed 0.1 gm. (1.4 mg./kg. at 70 kg. human weight) and that the intravenous route should be chosen only in real emergency. In the present series it has been observed that with 1.4 mg./kg. dosage the rate of clearance was similar to that attained after quinine (human equivalent dose) administration. The same view is held by Somasundram (1935), who observed that therapeutic efficiency of quinine and atabrin was about the same in parenteral use. But even after a fourfold increase in the dose of mepacrine in the present series, parasitic reduction, though better than observed with quinine, was not so satisfactory as was attained with the same dose of chloroquine. Thus dose for dose chloroquine seemed to outrank all other antimalarials including proguanil lactate, which was tested previously by Jaswant Singh *et al.* (1950), who had observed that with as high a dose as 33 mg./kg. of the compound, total parasite clearance in respect of mild infection of *P. cynomolgi* was not attained earlier than 72 hours.

Toxic manifestations were noticeable in respect of quinine when the therapeutic dose was doubled to 17.2 mg. Mepacrine proved toxic with 7 mg. dosage (5 times the human equivalent dose). On the other hand, no toxic manifestations were detectable in respect of chloroquine until the dose was increased to 22.8 mg. (4 times the recommended human dose). From these it would appear that the margin of safety between the recommended therapeutic and the toxic doses is narrowest with quinine. But various toxic symptoms have been attributed to intravenous administration of mepacrine in human cases. Field and Niven (1936) reported that mepacrine is likely to cause temporary giddiness, while Dawson *et al.* (1935) consider that parenteral atabrin is 20 to 40 times more toxic than by mouth. Similar views were held by Bass (Cushney, 1941). Hay *et al.* (1935) observed that 0.5 per cent of their cases developed mental symptoms. Further, fall of blood pressure has been reported both in cases of quinine and mepacrine. Storm (1935) reported pronounced fall after mepacrine and this was associated with extra systoles. With regard to quinine, most workers are agreed that the fall in blood pressure is due to too rapid administration and that if quinine is properly diluted, 5 to 10 grains in 20 c.c. (Mukarji, 1922; Covell, 1949), and the rate of injection does not exceed 0.5 grain per minute (Brahmachari, 1922; Covell, 1949; Jaswant Singh, 1950) there should be no adverse symptoms.

Thus, taking all points into consideration, intravenous route should obviously be the best course in grave emergencies and the choice is narrowed to quinine and chloroquine, as mepacrine is likely to give rise to more frequent adverse side symptoms than by others. Although proguanil is well tolerated, its action is comparatively slower on account of its ineffectiveness against trophozoite stages (Black, 1949; Davey, 1951). Between the two, chloroquine appears to be vastly superior to quinine with regard to rate of parasite clearance as well as in its margin of safety.

Spicknall *et al.* (1949) and Culwell *et al.* (1950) have reported satisfactory results after intramuscular injection of chloroquine (Resochin) administered in

2.5 to 5.5 mg./kg. dosage. After a single intravenous dose of 400 mg. (5.7 mg./kg.) base of chloroquine in *P. falciparum* infection, Scott (1950) observed that patients became afebrile in an average of 12 hours with a range between 2 and 22 hours. Parasite clearance was attained in about 46 hours (average), the range being 7 to 72 hours.

In view of these and the present observations it is considered that chloroquine has great potentialities in the treatment of severe cases of *falciparum* malaria, though the final judgment can be delivered after the therapeutic efficiency is compared against quinine on an extensive scale involving several strains of the plasmodia.

SUMMARY.

1. A total of 54 *rhesus* monkeys were utilized for studies on the therapeutic efficiency and toxicity of quinine, quinarsol, NAB, mepacrine and chloroquine, administered intravenously.
2. For assessing the therapeutic efficiency, 32 monkeys were heavily inoculated with 100×10^6 parasitized cells (*P. cynomolgi*). Antimalarials were administered on the 6th day after the injection of the infective inoculum at a time when parasitæmia attained a level of 10 to 20 per cent erythrocytic cell infection.
3. The human equivalent dose of each was injected as a single dose in some groups, while in others they were repeated after 12 hours. In some series the doses were varied.
4. Results after a single injection of the human equivalent dose showed the least action with quinarsol and maximum with chloroquine. Efficiency of quinine and mepacrine was on par.
5. By doubling the dose of quinarsol, parasitic reduction was identical to that attained with quinine. By fourfold increase in the dose of mepacrine, the effect was doubled, but similar increase in chloroquine resulted in 5 times better results.
6. After repeating the human equivalent dose, reduction in parasitæmia was proportional in respect of quinine, NAB and chloroquine, while quinarsol showed the poorest result.
7. It was observed from acute toxicity test that the margin of safety between maximum therapeutic dose and toxic dose was wider with mepacrine and chloroquine than with quinine.
8. Taking all points into consideration, the best result was obtained with chloroquine and may at a future date completely replace quinine for parenteral use.

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STUDIES ON *PLASMODIUM BERGHEI* N. SP. VINCKE AND
LIPS, 1948.

***V. On the host range in blood-induced infections.**

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VINCKE AND LIPS (1948) found that the white mouse and rat were susceptible to blood-induced infections of *P. berghei* discovered by them in the tree rat, *Thamnomys surdaster*. Since then a large variety of animals, twenty-one in all, have been successfully infected with the erythrocytic forms by different observers (Table IV). Twenty of these belong to the families *Muridae* and *Muscardinidae* of the order *Rodentia*, while the remaining one is of the family *Pteropodinae* of the order of *Chiroptera*. Vincke and Lips (*loc. cit.*) reported that rabbits and guinea-pigs were not susceptible to the infection. Deeliens and Lamy (1951), however, were successful in infecting new-born rabbits, but not similar guinea-pigs. Durbin (1951) reported that lambs, pigs, puppies, kitten and chicks were not susceptible to blood-induced *P. berghei*. The present communication deals with attempts to infect three monkeys, one cat, two rabbits, two guinea-pigs, three bandicoots, one mongoose, and a pigeon, all of which were unsuccessful.

MATERIAL AND METHODS.

The strain of the parasite was the same as previously described in Part I of these studies (Ramakrishnan and Satya Prakash, 1950). The guinea-pigs and the rabbits were obtained from a local colony; bandicoots and monkeys were trapped. The monkeys (*Macarus mulatta*) Nos. 117, 119 and 120 used in the experiment were small and a few months old, each weighing 1.5 to 2 lbs. (Table I).

The estimation of dosage and the method of inoculation were as already described by Ramakrishnan and Satya Prakash (1950). The route of inoculation was either intravenous or intraperitoneal. Rat blood with high

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parasitaemia was used and the dose of infection is indicated in the tables against each animal. Blood smears were obtained daily from all the animals and stained by the J. S. B. technique (Jaswant Singh and Bhattacharji, 1944) and were examined for several days before inoculation to eliminate natural infections and subsequently for prolonged periods or till death.

Splenectomy was performed under Nembutal anaesthesia (15 mg. per 20 gm. body weight) with aseptic precautions and the wound closed with catgut sutures.

RESULTS.

TABLE I.

Protocol of monkeys inoculated with rat blood containing P. berghei.

Monkey number.	Number of inoculations.	Route of inoculation.	Approximate number of parasitized erythrocytes inoculated. (Millions)	Period of observation in days after first inoculation.
117	4	3 I.V. 1 I.P.	1660	16
119	2	2 I.V.	1070	65
120	3	2 I.V. 1 I.P.	4320	32

I.V. = Intravenous.

I.P. = Intraperitoneal.

Monkeys.—After a week's observation, 0.5 c.c. of the blood of monkey No. 117 was injected intraperitoneally into two normal white rats which were kept under observation for twelve and twenty-two days respectively. Blood smears, both of the monkey and the rats, examined daily, were negative. This showed that repeated inoculations of large doses of parasites failed to infect the monkeys; the negative sub-inoculations in the albino rats confirmed it and proved that the parasites were not present in the monkey's blood even at sub-patent levels after a week of the inoculation.

Guinea-pigs.—Guinea-pig No. 1 was inoculated thrice intraperitoneally at intervals during a period of thirteen days, and the total approximate dose of infection was 702 millions parasitized erythrocytes. Blood smears, examined daily during the thirteen days, were negative. On the thirteenth day, just before the animal died, 3 c.c. of its blood was injected intraperitoneally into guinea-pig No. 2. The blood of this animal was negative till the third day of inoculation when it died. Post-mortem smears of its organs and heart blood were also negative to parasites.

Rabbits.—Two intraperitoneal injections of the infected rat blood containing approximately 630 millions parasitized erythrocytes were given to a rabbit at an interval of a week. Blood smears examined daily for two weeks from the first inoculation were negative.

Cat.—A cat was given an intravenous injection of rat blood containing approximately 1870 millions parasitized erythrocytes. Blood smears were examined daily for three weeks and were negative.

Mongoose (Herpestes mungo).—Two intraperitoneal inoculations containing a total of approximately 570 millions parasitized erythrocytes in rat blood were given to the mongoose at an interval of one week. This was followed a week later by an intravenous injection of infected rat blood, and the animal died during the process. Throughout the period of observation of two weeks, daily blood smears, as well as post-mortem organ and heart blood smears, were negative.

Pigeon.—Daily blood smears from the pigeon were negative during an observation period of twenty-five days after two intravenous inoculations of 150 millions and 13 millions parasitized rat erythrocytes respectively at an interval of fifteen days.

*Bandicoot (Bandicoota indica)**.—Three bandicoots were inoculated and examination of the daily blood smears as well as post-mortem smears of the heart blood and organs were negative. Bandicoot No. 3 was splenectomized after the first four inoculations (sixty-one days from the first) and was given three further inoculations subsequently. The details are indicated in Table II:—

TABLE II.

Protocols of three bandicoots inoculated with rat blood containing P. berghei.

Bandicoot number.	Number of inoculations.	Route of inoculation.	Total approximate dose : parasitized erythrocytes (millions).	Period of observation in days.
1	1	I.P.	250	7
2	1	I.P.	150	9
3	7†	5 I.P. and 2 I.V.	2242	95

† The last three inoculations were after splenectomy.

I.P. = Intraperitoneal.

I.V. = Intravenous.

* The authors are grateful to the Director, Indian Museum, for the identification of the specimen.

The last inoculation was given at 10.55 hours on the twenty-first day of the splenectomy. In order to determine the period during which the inoculum remained viable in the circulation of the bandicoot, 0.20 c.c. to 0.35 c.c. of its blood was drawn and sub-inoculated intraperitoneally at different intervals into a series of rats (*Rattus rattus*), blood smears of which were examined daily. The details are summarized in Table III below :—

TABLE III.

Details of sub-inoculation of blood from bandicoot No. 3 into rats.

<i>R. rattus</i> number.	Amount of blood sub-inoculated in c.c.	Interval between last super-infection and sub-inoculation.	Result of sub-inoculation.
299	0.30	5 minutes	Positive in 7 days.
300	0.30	1 hour	" " 10 "
301	0.25	2 hours	" " 10 "
302	0.25	3 "	Died on the 4th day.
303	0.30	4 "	Positive in 10 days.
304	0.35	5 "	" " 14 "
305	0.35	6 "	Died on the 11th day.
306	0.35	24 "	" " 11th "
295	0.20	4 days	Negative in 28 days.
296	0.20	4 "	" " 12 "
297	0.20	5 "	Positive in 22 days.
298	0.20	5 "	Died on the 13th day.

The positive sub-inoculations in rats indicated that the infected rat erythrocytes were viable and in circulation up to a observed period of five days, and that the parasites in them were infective to rats throughout this period.

DISCUSSION.

Malaria parasites in general display a very high degree of host specificity more so those of the higher animals. A few exceptions to this rule have, however, been recorded in which simian parasites have been successfully passaged into man and *vice versa*. *P. knowlesi* was successfully passaged by blood to man (Knowles and Das Gupta, 1932), but not to rabbits, guinea-pigs, rats, mice, squirrels and pups (Khazan Chand and Harbhagwan, 1934). Taliaferro and Taliaferro (1934) inoculated human blood containing *P. falciparum* to a howler monkey which became infected and underwent a spontaneous cure shortly afterwards. *P. malariae* was successfully passaged to a chimpanzee by Rodhain (1948). The avian malaria parasites, however, exhibit a lower degree of specificity than the mammalian ones. The canary can be infected with almost all the species of avian malaria parasites (Russell *et al.*, 1946).

In respect of host specificity, *P. berghei* would appear more to resemble the avian plasmodia than the other mammalian ones. The known hosts for blood-induced *P. Berghei* are reviewed in Table IV appended. As indicated earlier, a large number of animals belonging to different genera of the two families *Muridae* and *Muscardinidae* of the order *Rodentia*, as well as one of family *Pteropodinae* of the order *Chiroptera*, have been found to be susceptible to blood-induced infections of *P. berghei*. The degree of susceptibility differs in these different animals, as for instance in mice, rats and squirrels (Ramakrishnan and Satya Prakash, 1950: 1951). Rodhain (1949) described the infections in the cotton rat as comparatively mild, while Adler, Yoeli and Zuckerman (1950) showed that the infection in the hamster ran a severe course.

Animals like the rabbit, guinea-pig (family *Leporidae*) and the bandicoot (family *Muridae*) which also belong to the order *Rodentia* were found to be resistant to blood infections of *P. berghei*. The mongoose (*Herpestes mungo*), an animal belonging to the order *Carnivora*, was not found to be susceptible to the infection.

It is of interest in the case of *P. berghei* that the white mouse, highly susceptible to blood-induced infections, exhibited a natural immunity when sporozoites were inoculated (Vineke, 1950). Sporozoites of *P. berghei* found in a single specimen of *A. stephensi* (Ramakrishnan and Satya Prakash, 1950) as previously described were inoculated intraperitoneally into a white mouse and no infection could be detected during an observation period of thirty-five days. Similar differences in susceptibility of man to sporozoite- and trophozoite-induced infections of *P. knowlesi* are also known. Ray (1951) caused three batches of five specimens of *A. annularis* harbouring sporozoites of *P. knowlesi* to bite three human volunteers*. After biting the human volunteers, each mosquito was dissected and the residual sporozoites were inoculated into three monkeys all of

* One of the volunteers had never experienced any malaria infection at any time while the other two had malaria prior to 1943, 8 years previously and underwent a radical cure.

which took the infection. Blood films of the volunteers were examined periodically and not one of them developed the infection in the course of ten months of observation. About the same period, two white mice were inoculated each with two mosquito equivalents of *P. knowlesi* sporozoites, and no infection could be detected in them during two months when their blood smears were examined daily. Man, as already stated, is susceptible to blood-induced infections of *P. knowlesi* but not to sporozoites. White mice on the other hand are not susceptible either to sporozoite-or blood-induced infections of *P. knowlesi*. Coulston and Huff (1947) studied sporozoite-induced infections of *P. relictum* in canaries and pigeons and found that while cryptozoites were found in both the birds, blood infection became evident in the former and not in the latter. On the other hand, blood-induced infection of *P. relictum* produced high parasitemia in the pigeon. They concluded that susceptibility of a host to a *plasmodium* could not be judged solely by the result of blood-induced infections or the degree of parasitemia resulting therefrom.

As already stated, the bandicoot—a close relative of mice and rats—was not found to be susceptible to blood-induced infections of *P. berghei*. The sub-inoculation of blood from bandicoot No. 3 indicated that the inoculated parasites were viable and infective up to a period of five days. It would appear that either the plasma of the bandicoot or its erythrocytes, or both, were inhospitable to the parasites. Unless the development of the parasite was grossly interfered with in the bandicoot, the parasites must have multiplied five times during the period as the length of the schizogony cycle for *P. berghei* was shown to be twenty-four hours (Ramakrishnan and Satya Prakash, 1950). Since the bandicoots were found to be refractory to the infections, it could only mean the merozoites of each successive generation infected the rat erythrocytes introduced in the inoculum. This would also indicate that the bandicoot plasma was not inimical to the merozoites but the erythrocytes were inhospitable.

Experiments of McGhee (1950) on the other hand indicated exactly the reverse in respect of mice and *P. lophurae*, an avian infection. He injected erythrocytes from a mouse which was not susceptible to the avian infection, into the circulation of an infected chick embryo and found that the parasites successfully invaded the mouse erythrocytes also. This would indicate that in the mouse the plasma and not the erythrocytes was inhospitable to the parasite unless the cell underwent changes in the new environment of the embryo circulation.

In extensive studies on the host specificity of human *Plasmodia*, Bass (1922) inoculated infected blood into a large variety of animals with negative results. He attempted *in vitro* culture of the parasites in the sera of the different animals and man and found growth only in the latter. This was probably due to some inimical factors in the animal sera.

SUMMARY.

Monkeys, rabbits, guinea-pigs, bandicoots, mongoose, cat and pigeon were found not to be susceptible to blood-induced infections of *P. berghei*.

It was inferred that the erythrocyte of the bandicoot and not its plasma was inhospitable to the parasites. The host specificity of plasmodia in general is discussed.

TABLE IV.

Vertebrate hosts known to be susceptible to blood-induced infection of *P. berghei*.

Name of the host.	Order.	Family.	Observer.
<i>Thamnomys surdaster</i> ...	Rodentia	Muridae	Vincke and Lips (1948).
<i>Rattus rattus</i> ...	"	"	" "
<i>Mus musculus</i> ...	"	"	" "
<i>Sigmodon hispidus</i> ...	"	"	Rodhain (1949).
<i>Pelomys frater</i> ...	"	"	Vincke (1950).
<i>Rattus concha</i> ...	"	"	"
<i>Rattus tullebergi</i> ...	"	"	"
<i>Rattus calbino</i> ...	"	"	"
<i>Lophuromys rita</i> ...	"	"	"
<i>Seccosomus</i> sp. ...	"	"	"
<i>Dendromus oomilio</i> ...	"	"	"
<i>Tatera myasee</i> ...	"	"	"
<i>Mastomys concha</i> ...	"	"	"
<i>Legallia</i> sp. ...	"	"	"
<i>Mesocricetus auratus</i> ...	"	"	Adler, Yoeli and Zuckerman (1950).
<i>Microtus guntheri</i> ...	"	"	"
<i>Sciurus palmarum</i> ...	"	"	Ramakrishnan and Satya Prakash (1950).
<i>Meriones shawi</i> ...	"	"	Sergent and Poncet (1950).
<i>Clethrionomys glareolus britannicus</i> ...	"	"	Bray (1951).
<i>Craphiarrus murinus</i> ...	"	Muscardinidae	Vincke (1950).
<i>Rousettus leachi</i> ...	Chiroptera	Pteropodinae	van Riel (1950).

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STUDIES ON *PLASMODIUM BERGHEI* N. SP. VINCKE AND
LIPS, 1948.

***VI. Reaction of blood-induced infection in albino mice to DARAPRIM†,
2:4-diamino pyrimidine.**

BY

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[June 30, 1952.]

JASWANT SINGH *et al.* (1951) have reviewed the trends in chemotherapeutic research leading to the synthesis of an antimalarial compound, Daraprim 50:63 (2 : 4 diamino-5-*p*-chloro-phenyl-6-ethyl pyrimidine). Falco *et al.* (1951) observed the antimalarial activity of daraprim against *P. gallinaceum* and *P. berghei* and found it 200 times more active than proguanil against the latter.

* The investigation was partly financed from a grant by the Indian Council of Medical Research.

† Received through the courtesy of the Wellcome Laboratories of Tropical Medicine, London, in bottles containing 500 tablets of 10 mg. base per tablet.

Ramakrishnan *et al.* (1951) have reported the reaction of *P. berghei* to quinine and sulphadiazine, and the present communication is an extension of this work with daraprim.

METHODS AND MATERIAL.

The strain of parasite and the albino mice* were from the same sources as reported before (Ramakrishnan and Satya Prakash, 1950). Estimation of dose, inoculation and enumeration of parasites were carried out as described previously (Ramakrishnan *et al.*, *loc. cit.*).

The dose of inoculation was 5 million parasites per mouse, given by the intraperitoneal route. Drug was commenced only when the parasitaemia became patent and had reached a count of 200 or more per 10,000 erythrocytes, which occurred in most cases on the third or fourth day after inoculation.

The dose of daraprim in every case was calculated and recorded per 20 gm. body weight in terms of the base content. The drug was easily soluble in water and progressive dilutions were made to prepare small doses that were used in the trials. Fresh solutions were made for each course of treatment and the requisite quantity administered orally to the animal by means of a pipette. In all cases the total quantity of the drug per mouse was divided into six equal doses and administered twice daily (10 a.m. and 4 p.m.) for three consecutive days. The mice were weighed every day, but the amount of drug to be given per mouse was based on the weight recorded on the first day of drugging.

Eight animals distributed in different batches were not treated and served as controls. The course and intensity of infection were similar to that reported previously (Ramakrishnan *et al.*, *loc. cit.*).

Radical cures were confirmed in each case by negative sub-inoculation following an observation period of ten days when no parasites could be detected. The M.E.D. was considered to be that dose of the drug which when administered on the lines detailed above caused class II effect and continued to keep peripheral blood free from parasites for a minimum period of three consecutive days as described earlier by Ramakrishnan *et al.* (*loc. cit.*).

RESULTS.

The data are given in Tables I and II. *P. berghei* was found to be sensitive to very small 'homoeopathic' doses of daraprim. As small a dose as 1×10^{-6} mg. per 20 gm. (2×10^{-4} mg./kg.) weight of the mouse was found to be sufficient to lower the parasitaemia to significantly low levels. During the preliminary trials of the drug, doses ranging from 0.0002 to 0.02 mg. were tried and all the dosages were effective, and cleared the peripheral blood of parasites producing a class II effect. During further trials, lower concentrations of the drug were also found to be active, the parasitaemia showing a temporary deceleration in the place of normal acceleration. It was not considered feasible or useful to administer drugs in doses lower than 1×10^{-6} mg. which, as already stated, had an effect on the parasitaemia.

* Some of the mice were received through the courtesy of the Directors, Haffkine Institute, Bombay, and the Indian Veterinary Research Institute, Mukteshwar.

TABLE I.

Preliminary observations.

Total dose of daraprim (mg. per kg. body weight).	Number of mice.	TOTAL NUMBER OF PARASITES PER 10,000 ERYTHROCYTES ON DAYS COMMENCING FROM DAY OF DRUGGING.											
		1	2	3	4	5	6	7	8	9	10	11	12
1.0	1	252	68	5.0	1.0	0	0	0	0	0	0	2	7
3.0	2	267	136	0.0	0.0	0	0	0	0	0	2.5	88	486
3.5	2	243	50	3.5	0.5	0	0	0	0	0	0	0	0*
4.0	2	337	95	35.0	0.5	0	0	0	0	0	0	0	0†
5.0	2	291	94	0.0	0.0	0	0	0	0	0	0	0	0†
7.5	2	315	54	0.5	0.0	0	0	0	0	0	0	0	0†

* Positive on the 13th day.

† Sub-inoculations negative.

TABLE II.

Determination of M.E.D.

Total dose of daraprim (mg. per kg. body weight).	Number of mice.	TOTAL NUMBER OF PARASITES PER 10,000 ERYTHROCYTES ON DAYS COMMENCING FROM DAY OF DRUGGING.											
		1	2	3	4	5	6	7	8	9	10	11	12
0.00005	2	311	267	246	99.0	6.0	13	26.0	89.0	173.0	212	558	472
0.0001	2	294	220	16	1.5	0.0	2	2.5	4.0	9.0	63	159	344
0.0002	2	341	183	100	53.0	30.0	64	243.0	502.0	440.0	340	574	504
0.0004	2	261	256	136	65.0	42.0	145	374.0	357.0	348.0	411	675	618
0.0005	7	263	228	27	0.3	0.0	0	0.0	0.5	14.0	139	194	194
0.01	2	263	72	12	1.5	0.5	0	0.0	0.0	0.5	21	121	126
0.1	2	242	68	0	0.0	0.0	0	1.0	1.5	21.0	26	231	205

Treatment with doses ranging from 1×10^{-6} mg. to 0.07 mg. per 20 gm. weight cleared the peripheral blood of parasites for three to nine consecutive days, at the end of which parasitaemia became patent again. Animals treated with doses of 0.08 mg. and above cleared the peripheral blood of parasites in forty-eight to eighty-four hours from the commencement of treatment. When the animals remained negative for ten days, the radical cure was confirmed by negative sub-inoculations. The M.E.D. of the drug for the three classes of effects (Shannon, 1945-46) were as below :

M.E.D. for class I effect 1×10^{-6} mg. per 20 gm. (probably lower).

" " " II " 1×10^{-5} mg. " "

" " " III " 0.08 mg. " "

The time taken for parasite clearance was found to vary with the dose of the drug and is compared with similar data on quinine and sulphadiazine from previous studies as indicated in Table III :—

TABLE III.

Clearance of P. berghei from peripheral blood in treated mice.

Drug.	Dosage range (mg./kg.).	CLEARANCE IN HOURS FROM COMMENCEMENT OF DRUGGING.	
		Asexual.	Sexual.
Quinine ...	4,800 to 6,000	24-72	24-96
Sulphadiazine ...	3 to 30	48-72	48-72
Sulphadiazine ...	60 to 150	48	48
Daraprim ...	0.0005 to 0.07	72-96	48-120
Daraprim ...	0.08 to 7.5	48-60	48-84

It is seen from this table that except in the case of quinine the period in which the parasites were cleared was not significantly different. As in the case of *P. knowlesi* treated with daraprim (Jaswant Singh *et al.*, *loc. cit.*) the gametocytes took a slightly longer time for clearance than the asexual parasites.

Dosage schedules temporarily suppressing the parasites were re-analysed as in Table IV below in terms of the duration for which the suppressive effect lasted.

TABLE IV.
Suppression of parasitaemia with daraprim.

Dose (mg. per kg. body weight).	Duration of suppression in days.
0.0005	4
0.01	3
0.1	4
1.0	6
3.0	7
3.5	9

The larger the dose, the longer was the period of suppression. It would appear that when the total dose of the drug administered was between 0.0005 mg. and 0.1 mg. per kilo it was excreted within three to four days. Higher doses took a longer time for elimination.

DISCUSSION.

P. berghei has been found to exhibit a phenomenally high degree of sensitivity to daraprim. Falco *et al.* (*loc. cit.*) reported that some of the 2:4-diamino pyrimidines were highly active against *P. berghei*, and daraprim was 200 times more active than proguanil. A high sensitivity of the same strain of parasite to sulphadiazine and its tardy response to quinine were reported by Ramakrishnan *et al.* (*loc. cit.*), who found the M.E.D. for class II effect of the former drug to be 0.12 mg. per 20 gm. and its quinine equivalent to be 800. The M.E.D. of quinine, sulphadiazine and daraprim for the three classes of effects are shown in Table V :—

TABLE V.
M.E.D. for quinine, sulphadiazine and daraprim in *P. berghei* malaria in mice.

Drug.	M.E.D. (mg./kg. body weight).		
	Class I.	Class II.	Class III.
Quinine ...	1,200	4,800	6,000*
Sulphadiazine ...	1.5	6.0	60.0
Daraprim ...	0.00005	0.0005	4.0

* Class III effect was not obtained even with this dose.

From the above it is seen that the ratio of M.E.D. for class II and class III effect is 10 in the case of sulphadiazine and 8,000 in the case of daraprim. This was contrary to expectations as one would have considered that the ratio would be small in respect of a drug to which the parasite was so highly sensitive. At this stage any explanation of this phenomenon must be purely speculative and it is hoped that further investigations on the drug metabolism, blood level studies and rate of excretion of the drug may throw some light. The last factor, namely, rate of excretion as inferred purely from biological criteria, has been referred to above. It was seen that higher the dosage schedule, the longer did the suppressive effect last. As judged by the period during which the parasites remained sub-patent when dosage schedules were from 0.0005 to 0.1 mg. per kg., the drug was eliminated below effective levels from the blood in three to four days as at the end of the period the parasites reappeared. The rate of excretion was not in any way dissimilar to that of sulphadiazine as reported earlier by Ramakrishnan *et al.* (*loc. cit.*).

No toxic symptoms of any description were noticed in any of the animals under the different dosage schedules tried. The daily weight of the mice showed no reduction in any of the treated animals.

SUMMARY.

1. *P. berghei* was found to be extremely susceptible to the new synthetic antimalarial, daraprim.
2. Deceleration of parasitaemia was evidenced even with such minute doses as 10^{-6} mg. per 20 gm. body weight while 10^{-5} mg. and 8×10^{-2} mg. produced class II and class III effects respectively.
3. The quantity of the drug for effecting a radical cure was far higher than the suppressive dose, as compared with similar doses of sulphadiazine. This is inexplicable with the present knowledge and studies on excretion rate and blood threshold of the drug may help elucidation.
4. With the doses producing class II and class III effects, parasite clearance was evidenced during the same period within 48 to 120 hours even though doses required to produce class III effect were higher. No significant difference was noticed in the action of the drug on the asexual and sexual forms.

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***VII. Spleen size in albino mice and rats with blood-induced infections.**

BY

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[April 23, 1952.]

A CHARACTERISTIC feature of plasmodial infection in the vertebrate host is the enlargement of spleen. Ever since Dempster (1848) described the manual method of palpation of the malarial spleen in human beings, it has continued to remain the method of choice both to the diagnostician and the epidemiologist. A great deal of information about malarial splenomegaly has accumulated chiefly through four types of investigations, namely (a) epidemiological surveys, (b) post-mortem studies in human malaria, (c) observations in the course of malaria therapy for the general paralysis of the insane, and lastly (d) through experimental malariology in animals.

The present communication deals with the analysis of post-mortem spleen measurements in albino mice and rats infected with *P. berghei*. The former were found to be highly susceptible to the infection, while the latter were less so, and some developed chronic infections (Ramakrishnan and Satya Prakash, 1950b).

MATERIAL AND METHODS.

The strain of *P. berghei*, the albino mice and rats were from the same sources as previously reported by Ramakrishnan and Satya Prakash (1950a, 1950b). Specimens of *R. rattus* were obtained from the local municipal authorities. Most of the animals were of unknown ages, but full grown and more than twenty-four weeks old; a few albino rats were born in the local colony and were of known age groups. The sex distribution was random. The estimation of dose and degree of infection and the method of drugging were as described previously by Ramakrishnan and Satya Prakash (*loc. cit.*) and Ramakrishnan, Satya Prakash and Krishnaswami (1951). The dose of infection for a few mice and all rats was approximately 8×10^5 parasitized erythrocytes. In the case of other mice, however, the dose was 2 million or 5 million parasites. All inoculations were given by the intraperitoneal route.

The data analysed accrued over a period of two years from routine post-mortem measurement of spleens of all animals used in the course of different

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experiments. The spleens removed at post-mortem were individually lowered gently into a known volume of water contained in a measuring cylinder calibrated to one-fifth of a millilitre. The volume of water displaced by the spleen was read off directly. The above procedure was carried out as soon as possible after the death of the animal, and any showing the slightest sign of putrefaction was discarded from the analysis.

ANALYSIS AND RESULTS.

The data comprise measurements of spleen of one hundred and twenty-five mice and two hundred and forty-nine rats. Uninfected animals which died naturally without any apparent cause provided the spleens for determining the average normal size. The spleen volumes of normal animals are shown in Table I. All the averages have been corrected to the first decimal figure.

TABLE I.
Spleen volume of normal white mice and rats.

Animal.	Number examined.	Average spleen volume in c.c.
White mice ...	32	0.2
White rats ...	58	0.4

The smallest spleen volume of normal mice and rats was 0.1 c.c., while the largest was 0.4 c.c. and 1.4 c.c. respectively. The average spleen size of normal mice was determined to be 0.2 c.c. and that of normal rats 0.4 c.c.

Acute untreated infections.—The analysis of spleen volumes of infected mice and rats that died during the acute stage is shown in Table II.

TABLE II.
Spleen volumes of mice and rats dying of acute untreated infection.

Animal.	Dose : Number of parasitized erythrocytes.	Interval in days between inoculation and death.	Number examined.	Average spleen volume in c.c.	Per cent variation from normal.
Mice ...	8×10^5	6-25	4	0.6	plus 200
Mice ...	$5 \times 10^{**}$	6-35	6	0.2	0
Rats ...	8×10^5	1-25	63	2.5	plus 525

* The dose was 5×10^6 parasites.

Mice inoculated with a lower dose showed 200 per cent increase in the average spleen volume over the normal, while in those infected with a much higher dose the spleens were not enlarged. In the rats on the other hand, where the infection was milder, the spleen for identical infections showed an enlargement more than five times the normal.

The lowest spleen volume recorded among mice infected with 8×10^5 parasitized erythrocytes was 0.4 c.c., while with the higher dose of infection it was 0.1 c.c. Among the infected rats it varied from 0.8 to 5.0 c.c.

The spleen volumes of sixty-three rats were analysed in terms of intervals between the days of inoculation and death of the animals as shown in Table III.

TABLE III.

Spleen volume in rats with acute infection.

(Dose of infection 8×10^5 parasitized erythrocytes.)

Interval in days between inoculation and death.	Number of animals.	Average spleen volume in c.c.	Per cent increase over normal.
1-5	3	1.4	250
6-10	28	1.6	300
11-15	25	2.0	400
16-20	6	2.6	550
21-25	1	5.0	1,150
TOTAL	63	2.5	525

It was found, as expected, that there was a progressive increase in the size of the spleen during the acute primary stages, the degree of enlargement being proportional to the duration of patent parasitæmia.

Latency.—Spleen volumes of animals that died during different periods of latency are analysed in Table IV. Previous studies reported by Ramakrishnan,

Krishnaswami and Satya Prakash (1951) indicated that a large percentage of animals that developed a chronic infection retained the parasitæmia at sub-patent levels up to a minimum period of six months.

TABLE IV.

Spleen volume in rats with latent infection.

Duration of latency in days.	Number of animals.	Average spleen volume in c.c.	Per cent increase over normal.
6-30	6	1.1	175
31-60	5	1.3	225
61-90	8	1.0	150
91-100	3	1.1	175
101-150	2	1.3	225
151-200	4	0.8	100
TOTAL	28	1.0	150

From the above it is seen that in rats which were able to overcome the primary acute phase, the spleens remained enlarged during latency, but the increase in size was not the same as during the acute phase of the infection. The average increase in the chronic infections was 150 per cent above the normal, while it was as high as 525 per cent during the acute stage. It would appear that after the crisis the enlarged spleen reduced in size to less than half and remained so as long as the latent infections persisted.

Inadequate treatment.—During the course of experiments with quinine and sulphadiazine reported earlier by Ramakrishnan, Krishnaswami and Satya Prakash (*loc. cit.*), fifty-seven mice were given different doses of either drug, all of which were less than the minimum effective dose required to clear the

peripheral blood of parasites. The analysis of their volumes is shown in Table V.

TABLE V.

Spleen volumes of mice dying with inadequate treatment during the acute infection.

Interval in days between inoculation and death.	DOSE OF INFECTION IN TERMS OF PARASITES.			
	2 million		5 million	
	Number of animals.	Average spleen volume in c.c.	Number of animals.	Average spleen volume in c.c.
1-5	...	...	5	0.2
6-10	2	0.8	9	0.3
11-15	1	1.0	15	0.4
16-20	1	0.8	5	0.3
21-25	2	1.4	9	0.3
26-30	2	0.4	4	0.4
31-35	...	...	2	0.2
TOTAL	8	0.8	49	0.3
Per cent enlargement over normal.	...	166.6	...	50

With an inoculum of 2 million parasites followed by inadequate treatment, the increase in size of the spleen was 167 per cent as compared to a 200 per cent in untreated infections initiated by a lower dose of infection namely 8×10^5 parasitized erythrocytes (Table II). But when the dose of infection was 5×10^6 parasites with incomplete treatment, the spleen showed only a 50 per cent enlargement as compared to no enlargement in similar untreated infections (Table II).

Suppressive treatment.—Eleven mice were given sufficient antimalarials which rendered the peripheral blood free of parasites for a temporary period during which they died and their spleen volumes are analysed in Table VI. Other mice that survived under identical conditions of infection and treatment showed patent parasitaemia after an interval.

TABLE VI.

Spleen volumes of white mice dying during suppressive treatment.
(Dose of infection 5 million parasites.)

Latency in days.	Number of animals.	Average spleen volume in c.c.
1-5	2	0.75
6-10	5	0.4
11-15	4	0.4
TOTAL	11	0.5

When mice infected with 5 million parasites were treated with sufficient antimalarials to suppress the parasitaemia in the peripheral blood, the spleen was enlarged to a less extent (150 per cent over the normal) than in untreated mice in whom the infection was initiated by a lower dose of infection namely 8×10^6 parasitized erythrocytes. On the contrary, as noted above, in mice which were infected with 5 million parasites and not treated, the spleen showed no enlargement.

Radical cure.—Fifteen mice infected with 5 million parasites were treated with sulphadiazine in doses far in excess of the minimum effective suppressive dose. The peripheral blood was free of parasites for a continuous period of fifteen days' observation. Sub-inoculations of blood from a few of these animals into *R. rattus* were negative, confirming the radical cure. The animals died or were sacrificed at different periods within three months following the fifteen days of observation. As the animals were housed in a common cage after the cure, it was not possible to analyse the spleen volumes in relation to the interval between dates of cure and death.

The average spleen volume of the fifteen mice was found to be 0.3 c.c. It was concluded that after a radical cure of a single blood-induced infection in mice the spleen did not return to normal within three months.

The sex and age factor.—The available data were analysed to determine if the spleen volume had any relation to sex and none could be found. As previously stated, only nineteen rats in the whole series were of known ages varying from twelve to twentyseven weeks. All of them died during the acute

phase in seven to thirteen days after the inoculation and the analysis of their spleen volumes is shown in Table VII.

TABLE VII.

Spleen volume in white rats of known ages dying during untreated acute infections.

(Dose of infection 8×10^5 parasitized erythrocytes.)

Age in weeks.	Interval in days between inoculation and death.	Number examined.	Average spleen volume in c.c.
12	8-11	4	1.3
16	10	1	0.8
17	9	2	1.1
20	8-12	3	1.0
22	7	1	2.0
23	7-13	5	1.4
24	13	2	1.3
27	7	1	0.8
Total ...	...	19	1.3

Age as such did not seem to be correlated with the difference in the average spleen volume. The range of individual spleen measurements was about the same in each age group.

DISCUSSION.

The main features of the analysis presented in the report are : (a) the same strain of parasite was used to infect two closely related hosts, one of which showed a much higher degree of susceptibility to the infection than the other, and (b) all the animals were given a single known dose of infection in an identical way.

The degree of enlargement of the spleen in a plasmodial infection is known to depend primarily on the parasite and the host, each of which may be modified by a number of variables, namely, species, strain, dose and virulence in the case of the former and race, age, sex, nutrition, the degree of susceptibility and the extent of therapy in the latter.

The degree of splenomegaly in mice with single infections varied when the doses differed. Indeed, with the higher dose of infection, no change in the spleen volume was noticed. Taliaferro and Mulligan (1937) considered that acute

malaria, especially of *P. falciparum*, was associated with destruction of various splenic cells which may arrest hyperplasia and prevent an increase in the size of the spleen. They produced experimental evidence of this in acute infections of *P. knowlesi* in monkeys. Scheube (1903) reviewed the literature and indicated that in rare cases the spleen in malaria instead of being enlarged may be considerably reduced in size.

Many epidemiological studies have been carried out to discern the relationship between splenomegaly and the different species of *Plasmodia* in the same host and opinions were different and inconclusive. Boyd *et al.* (1926) in their Brazilian studies concluded that *P. vivax* was a more efficient agent than *P. falciparum* in producing greater enlargement of the spleen. Christophers (1929) considered that splenomegaly was greatest with *P. vivax* and *P. malariae*. Sweet (1933), on the contrary, found that there was no difference in the average enlarged spleen of those with *P. malariae* infections and others with *P. falciparum* in Madras area. He also found higher average enlargement of spleens in a community associated with *vivax* malaria. Hill *et al.* (1943) were of opinion that *P. falciparum* produced a greater enlargement of the spleen than *P. vivax* infections. In the course of studies in relation to malaria therapy, Kitchen (1941) believed that in primary *falciparum* attacks in highly susceptible individuals, splenomegaly did not attain the proportions noted in *vivax* malaria.

In the course of animal experiments when splenomegaly was measured *in vivo* by rendering the organ extra-abdominal by surgical procedure, Coggeshall (1937) and Afridi (1938) came to the conclusion that *P. cynomolgi*, comparatively more benign to the monkey, produced a greater degree of splenic enlargement than *P. knowlesi* which produced invariably fatal infections in the same animal.

It would be seen from the above that infections due to species which were more benign and chronic in their effects tended to produce greater degree of splenomegaly, although opinions differed especially in the epidemiological studies. It was possible that among other variations the strains, though of the same species, were different.

In the present analysis the strain and the species of parasite remained the same, but the hosts, though closely related, were different. The difference in the pathogenicity of the infection to the two hosts has already been described by Ramakrishnan and Satya Prakash (1950a). In the mice which were highly susceptible to infection, the spleen on the average showed a 200 per cent increase in volume, while for the same dose of infection in rats (to which the infection was more benign) that died of the acute infection, the spleen showed an average enlargement more than twice that in mice or 525 per cent. This high degree of enlargement in the rats did not persist once the acute phase was over, and the spleen in the chronic cases, though enlarged, was only about a third the size of the average acute spleen.

Chemotherapy of malaria infections affects the degree of splenic enlargement. Taliaferro and Taliaferro (1949) in crucial experiments concluded that quinine and spleen were independent and complementary to each other in combating the infection. The degree of splenomegaly would depend on the extent of medication in a community. James (1929), who rightly considered that recognition of splenomegaly in malaria is the greatest aid to the epidemiologist, mentioned

that it had not made headway in the U.S.A. and Holland, evidently from a widespread belief that the results greatly underrated the endemicity. The question in so far as the U.S.A. were concerned was re-examined by Darling (1926), who concluded that the apparent deficiency of spleen examinations was attributable to an oversight of small degrees of splenomegaly. Spicer (1945) in the same country confirmed this and concluded that inclusion of spleens palpable only on deep inspiration were useful in the determinations of spleen rates even under hypoendemic conditions. Swellengrebel and de Buck (1938) confirmed the findings of Darling in respect to Holland. Boyd (1949) considered that this was due to the fact that in both the countries in comparison to the rest of the world the consumption of quinine had been heavy.

It was seen from Table V that mice infected with 2 million parasites and inadequately treated showed a greater average enlargement of the spleen than similar ones in which the infecting dose was higher. The eleven mice which were submitted to suppressive doses of drugs showed an average spleen size which was greater than the normal (Table VI) but was less than in similar untreated infections (Table II). The degree of enlargement with suppressive treatment was, however, more than with non-suppressive treatment (Table V). The fifteen mice in which the infections were radically cured showed that the spleen remained enlarged for at least three months of such a cure.

The individual spleen measurements of the animals in the different categories, namely, normal, acutely and chronically infected and treated to different degrees, showed a wide range and have been indicated in the text. Apart from other factors there appeared to be an individual factor with regard to the spleen volume.

The above findings confirm many of the well-known phenomena of splenic reaction to malaria. James (*loc. cit.*) emphasized the value of spleen rates and measurements to the epidemiologist in wholly untreated populations. The analysis presented here re-emphasizes this point. The epidemiologist of to-day cannot depend on spleen rates and measurements to the same extent as in the past for his interpretation of the true endemicity in a community. He has to take into consideration among other factors the extent to which antimalarials are used by the population. The difficulties are progressively greater due to the increasing and wide availability of antimalarials.

SUMMARY.

1. Analysis of spleen volumes, in both normal and infected albino rats and mice, has been presented. The variation in sizes in different phases of the infection, treated and untreated, and its importance in epidemiological studies have been discussed.
2. In acute untreated infections, the splenomegaly was found to progress with the duration of parasitaemia. The degree of enlargement was comparatively higher in rats.
3. During latency, following the acute phase in rats, the spleen reduced in size but never to the normal.
4. The enlargement of spleen in animals receiving suppressive doses of antimalarials was of the same order as in untreated infections, while in animals

receiving sub-effective doses the degree of enlargement varied with the quantum of infection.

5. The spleen remained enlarged up to a period of three months after a radical cure.

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THE VALIDITY OF ESTIMATION OF ANOPHELES DENSITIES
ON THE BASIS OF HAND COLLECTION ON A TIMED BASIS
FROM FIXED CATCHING STATIONS.

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INTRODUCTION.

STUDIES on the measurement of malaria naturally include an appraisal of the densities of the vector species of anophelines as much for an understanding of the epidemiology of the disease as for an immediate assessment of the efficacy of control measures.

The density of anophelines has been variously defined as the number per room, per catch, per house, per trap or per collector with or without reference to any specified period of time. Apart from differences in the units of collection specified above, there are also variations in the method of collection such as by hand or after spraying. Again the traps used may or may not contain a human or animal bait.

None of the methods has universal application and the methods used often vary with the actual problem on hand. Mosquitoes also vary much in their resting and feeding habits and in their responses to external stimuli. The resting habits influence numbers found in daytime shelters, the feeding habits, the numbers which collect at night in the proximity of the hosts of predilection or enter baited traps and the responses to external stimuli the numbers attracted to other types of traps.

Among the methods employed for measuring *Anopheles* densities, the collection of adult mosquitoes from daytime resting places, such as human, animal or mixed dwellings, is the most common if at least for the ease with which such collection can be made.

In the estimation of the number of anophelines per room, per house or per catch, collections are usually made to depletion. In defining densities on the basis of 'per man-hour', although catches are not made to depletion, the time spent in each catching station, at all events under the conditions prevalent in India, is usually adequate to obtain a substantial proportion of the actual mosquito population resting in it at the time of collection. This, at best, has been a presumption till now. The data herein presented furnish a fair idea how far the above presumption is valid.

PLAN OF WORK.

Six villages in Poona District, viz. Manjri, Manjri Pathar, Tupewadi, Moshi, Chikhli and Kelagaon, were selected and in each village 12 collecting stations were established after an initial reconnaissance. The stations were typical dwellings in the villages and consisted of one cattleshed, two mixed dwellings (those housing both cattle and men under the same roof) and nine human dwellings. The walls of all the stations were of mud or of brick plastered with mud and the roofs either of tile or wood or thatch. Only two stations had a zinc sheet roof. All the stations had doors and one or more windows but either with tight fitting shutters or capable of being covered to prevent egress of mosquitoes at the time of spraying.

Four well-trained insect collectors were detailed for the work and each insect collector was assigned three collecting stations in each village. The work of the insect collectors was always under close supervision. In each station, a hand collection of mosquitoes was first made for exactly 30 minutes using suction tubes and flashlights, and immediately after the hand catch, white cloth sheets were spread completely covering the floor and white paper sheets placed on all other horizontal surfaces on which knocked-down mosquitoes might fall. Then the doors and windows were shut and all the other openings and crevices closed with paper, gunny cloth, etc., and the room was thoroughly sprayed with pyrethrum extract (0.2 per cent pyrethrin in kerosene) using hand pumps. The doors and windows were kept closed for about 5 minutes after the spraying. The mosquitoes found knocked down on the sheets were then carefully picked up, put into test tubes and later counted and identified. The work in each village commenced usually at about 07.30 hours and was completed by 13.00 hours. The studies were made during the month of March, April, May, June and July 1951. Each village was visited at intervals of a fortnight and the collections repeated. In the first fortnight in March, only 3 villages were visited and in the month of June only four. On a few occasions a few catching stations were missed either because they were locked or because the householders did not permit collection. When any of the insect collectors was not available, his place was filled by an equally efficient substitute. In all 620 observations were made.

RESULTS.

In Table I are shown the total numbers of all mosquitoes, by species and sex, collected in the entire series of observations. *Anopheles culicifacies* Giles, 1901, the local malaria vector, was the predominant species and accounted for more than half of the total anophelines collected. Of the total number of this

species collected by hand, 20.5 per cent (253 out of 1,237) were males, while in the number collected by hand and by subsequent spray, 20.7 per cent (811 out of 3,914) were of this sex. Similarly, males were 18.5 per cent in the hand catch (407 out of 2,201) and 17.9 per cent in the total catch (1,392 out of 7,785) in respect of all species of anophelines. In the case of culicines the percentage of males was 31.2 (479 out of 1,536) and 32.6 (1,609 out of 4,940) respectively.

Hand collection limited to half an hour in each station has in the total experience of these observations yielded 31.6 per cent of the *culicifacies* population actually present. For all anophelines taken together the proportion of the total collected by hand collection limited in time as above was 28.3 per cent, for culicines it was 31.1 per cent and for all mosquitoes 29.4 per cent.

The entire data in respect of *A. culicifacies* only were subjected to a detailed statistical analysis, adopting the intra-correlation technique to see how far the variations due to stations, insect collectors and period of collection affected the numbers collected by hand only and by hand and spray together. The data are summarized in Table II, which shows the actual number collected in each station on each occasion, the hand collection represented by H and the total collection by T.

The analysis of variance is given below :—

ANALYSIS OF VARIANCE.

Due to				Degrees of freedom.	Sum of squares.	Mean square.
Collectors	...	...	...	8	4543.71	567.90
Fortnights	...	...	...	9	8021.25	891.25
Villages	...	...	...	5	6380.72	1276.14
Collectors × fortnights	...	...	...	34	2071.95	87.39
Fortnights × villages	...	...	...	40	23330.23	583.26
Villages × collectors	...	...	...	39	6059.87	155.38
Collectors × fortnights × villages	...	...	...	92	21382.85	232.42
Total	...	...	...	227	72690.58	...
Between stations	...	...	...	392	70693.50	180.34 M ₂
Total	...	...	...	619	143384.08	...
Within stations	...	...	...	620	52881.50	85.29 M
TOTAL	...	...	...	1,239	196265.58	...

The above analysis furnishes an estimate of variance between stations (Mx) and within stations (M). The intra-class correlation between 'H' and 'T', as

defined by $r = \frac{Mx - M}{Mx + (k-1)M}$ (where k is the number of values in each station, i.e. 2), is found to be 0.3578. To test the significance of 'r', 'z' transformation

is made according to the formula, $z = \frac{1}{2} \log_e \frac{1 + (k-1)r}{1-r}$. The value of 'z' thus found is 0.3733 and since 'z' is a normal variate, to be significant it should exceed twice its standard error $\frac{1}{\sqrt{n - \frac{3}{2}}}$, where n = number

$$\frac{1}{\sqrt{n - \frac{3}{2}}}$$

of observations. With $n = 620$, the standard error is found to be ± 0.0402 . Thus the estimated value of 'z' which is 0.3733 is more than 9 times its standard error showing that the intra-class correlation between 'H' and 'T' is highly significant. It can reasonably be assumed that under the conditions of the experiment the pyrethrum spray knocked down the entire mosquito population inside the catching station. And the number caught by hand, exhibiting as it does a significant correlation with the total number of mosquitoes, may be deemed to be a reliable measure of the total mosquitoes present in each catching station.

DISCUSSION.

The utility of any particular method of collection of adult anophelines has to be considered from three points of view, viz. (1) its statistical validity, (2) the ease of adoption under conditions normally obtaining in malaria organizations serving comparatively wide territories, and (3) the feasibility of the determination, in some measure, of the total mosquito population prevalent in an area from the data obtained by the particular method of collection.

The use of traps for collection of adult anophelines of the oriental species has not given satisfactory results in the comparatively few cases where it has been tried. Other methods commonly adopted consist of hand catch on a timed basis or to depletion by hand catch. Ribbands (1946) has criticized the value of results obtained by hand catch of daytime resting mosquitoes. Even after catching to depletion he found that, on the average, approximately 28 per cent of anophelines fail to be caught as determined by a further catch after spraying. Secondly, he has found variations extending from 50 to 90 per cent in the proportion captured by hand of the total population. Thirdly, males and unfed females are, according to him, liable to be missed by hand catch to a greater extent than fed females. His data refer to catches made on 60 days and the total number of anophelines caught was only 494. The numbers are apparently too small and do not lend themselves to any detailed statistical analysis. At all events, he has not subjected his data to any such analysis. Senior White and Venkat Rao (1946) refer to Ribband's work quoted above and have furnished data relating to their own experience in India. Their data confirm Ribband's conclusion that approximately 28 per cent of the total population fail to be captured by hand (even when this was apparently done to

depletion). They further conclude that, though their data throw no light on Ribband's contention regarding the wide variations from day to day, the 'individual' factor must form so large a part that they are inclined to doubt whether the effort of making further extractions from their figures would be worth while.

The data presented in this paper relate to as many as 3,914 specimens of *A. culicifacies*, collected on 620 different occasions. The variable elements among these observations consist of catching stations, villages, individuals making the collections and the period of collection. The statistical analysis shows that though collection by hand limited to a period of half an hour in each catching station yields, on the average, only about 32 per cent of the total *culicifacies* population (28.3 per cent for all anophelines, 31.1 per cent for culicines and 29.4 per cent for all mosquitoes), the correlation between the hand catch and the total catch in a large series of observations is positive and significant in the case of *culicifacies*. That the variations are wide is shown by a not-too-high correlation. Its significance, however, shows that the variations are within the limits of random sampling. It may be pointed out that while the insect collectors in this organization are well trained, none of them has such outstanding qualifications as to constitute a very highly selected type of personnel. But it should be emphasized that for the purposes of this experiment the observations were subjected to strict supervision. Given these prerequisites, the data show that the method of collection by hand on a timed basis may well yield an index of the total mosquito population which is prevalent in any catching station. While in the experience of Ribbands (*loc. cit.*) and Senior White and Venkat Rao (*loc. cit.*) catching by hand to depletion yields as much as 72 per cent of the total population, catching by hand for half an hour has yielded, in the authors' experience, only about 32 per cent of the total *culicifacies* population. While, therefore, catching by hand to depletion would yield more mosquitoes than catching on a timed basis, the authors' observations show that catching by hand on a timed basis yields results which are well within the limits of sampling variations as a measure of the total mosquito population in the daytime indoor resting places.

As regards the second factor, viz. the ease with which the method can be adopted, admittedly, hand catch on a timed basis is the simplest. Though catching to depletion yields a much higher proportion of the total mosquito population, that method is liable to be associated with 'individual' factor to a much greater extent than catching on a timed basis. The limit of time is precisely determined in the latter method and for that purpose every insect collector is provided with a watch. It is possible, no doubt, to set a limit in the case of catching to depletion as well, say, for instance, that no more mosquito is caught over a given period of time. Even so, the time that has to be spent in a particular catching station will be widely variable even if the collector is most efficient and conscientious, especially when densities are high.

In the experience of one of the authors' (T. R. R.) the time taken to collect to depletion in certain special types of huts constructed for studies on *culicifacies* was about 3-4 hours in each hut. From the practical point of view, no organization can provide the requisite personnel necessary for catching to depletion in every catching station. While no method can be adopted by reason merely of its simplicity if it has no statistical validity, the data presented here show

a fair degree of statistical validity and confirm the authors' presumption that hand catch on a timed basis furnishes a most ready measure of densities of anophelines in indoor daytime resting places.

Spray catching is no doubt likely to yield much more certain results than catching by hand either on a timed basis or to depletion and is also comparatively rapid but the application of an insecticide is likely to interfere with the subsequent behaviour of anophelines the extent of which cannot be adequately determined. It may be argued that if determination of anopheline densities by spray catch is resorted to at long intervals of time the effects of the spray on the behaviour of anophelines in the interval may be taken to be negligible. This will, however, defeat the purpose that most organizations have of getting an immediate measure of mosquito densities. Adult *Anopheles* densities are generally collected for two main purposes, one during the conduct of malaria surveys and the other for estimating their prevalence to furnish an immediate measure of the efficacy of the insecticidal application for malaria control. For the former purpose, inasmuch as collections by traps have proved unsatisfactory, hand collection is generally resorted to. After spray catch, mosquitoes cannot be brought back to the laboratory in a live condition which is necessary for an estimation of the oöcyst rate. Hand catch is, therefore, the main method that is available for such purpose and as hand catch to depletion is not feasible, collection by hand on a timed basis can alone be resorted to. An estimation of density of anophelines to furnish a measure of efficacy of the insecticide application will have to be made under different conditions of association between the vector species and man. This takes one on to the third factor specified earlier, viz. how far the anophelines present in indoor daytime resting places are a clue to the total *Anopheles* population in any area. Various aspects of mosquito behaviour relating to place of feeding, resting habits, host of predilection, longevity and age composition, etc., have all a bearing on this factor and are therefore to be reckoned in determining the best way in which mosquito densities are to be assessed for ascertaining the duration of the efficacy of an insecticidal application. This discussion is deferred to a later paper. The collection of mosquitoes in indoor shelters is, however, commonly adopted for the purpose of assessing the degree of association of the vector species with man. A comparison is made both between sprayed and nearby unsprayed villages and between a few randomly selected sprayed dwellings and a few structures specifically left unsprayed. The present studies have shown that so far as the comparison of densities in two or more indoor stations within the same village is concerned, the hand catch limited to half an hour in each station is valid. The question whether the densities in two different villages could be compared was outside the purview of the present investigation. But as a routine practice in the authors' organization, malaria control is inaugurated in any district only after a prior survey extending over at least one year, during which period densities of *Anopheles* are carefully recorded. Prior data are therefore available to indicate the degree of natural variations in densities between villages. This can be duly allowed for when a comparison is made between the densities of sprayed and unsprayed villages after control measures are instituted. Hence this method of collection by hand for half an hour in each station and the expression of densities *per man-hour* can be usefully adopted for this purpose also.

Rajindar Pal (1951) states that the density index as expressed *per man-hour* has serious drawbacks of personal factors and that total collections yield more

accurate results in this respect. In making this statement two issues have been mixed up. Data has been furnished to show that the density expressed as 'per man-hour' truly reflects the density of the total mosquito population in the particular catching station. It is not claimed that the total anopheline population in any particular catching station or in a group of stations truly reflects the total mosquito population in the village as a whole. No worker has so far described any method for this purpose with any proof of its validity and in the authors' view there is a definite lacuna in this aspect of epidemiology of malaria. Broadly stated, in malaria control schemes in which residual insecticides, such as D.D.T., are applied in smaller dosages and the period of a second application of the insecticide has to be determined, appraisal of mosquito densities at comparatively frequent intervals will be necessary. It would be unsafe in such cases to use another insecticide to collect the resting mosquitoes for it is not known what influence the latter insecticide would have on the subsequent densities of mosquitoes. Hand collection would be the method of choice in such cases and the authors' data show that hand collection limited to half an hour in each station gives a reliable measure of the total mosquito population present in the catching stations.

The authors' experience, furnished by these data, is contrary to that of Ribbands (*loc. cit.*) according to whom males are likely to be missed in the hand collections. The percentages of males collected by hand catch in the present data is practically the same as in the total catch for all mosquitoes, culicines, all anophelines and *culicifacies*.

SUMMARY.

A special study was made in Poona District, India, to determine the validity of hand collection of mosquitoes on a timed basis for estimating mosquito densities. Six villages were selected in each of which 12 typical dwellings were chosen as routine collecting stations. Visits were paid to these stations at intervals of a fortnight. The resting mosquitoes were collected first by hand with a suction tube and flashlight for exactly 30 minutes in each station and subsequently the rooms were sprayed with pyrethrum extract, and the knocked-down mosquitoes collected from specially placed white ground sheets were identified and enumerated. In all 620 observations were made over a period of 5 months. The variable factors in this study were between stations, villages, insect collectors, and the different periods of time when collections were made. In all 3,914 *culicifacies* were collected out of which 1,237 were collected by hand. The numbers were subjected to a statistical analysis adopting the intra-class correlation technique. The intra-class correlation between the hand captures and the total captures (sum of hand captures and the subsequent spray captures) was found to be 0.3578. Using the 'z' transformation, the value of 'z' was found to be 0.3733 with a standard error of ± 0.0402 , indicating a highly significant correlation. The significance of this finding is discussed and it is concluded that the use of hand captures for half an hour from fixed catching stations yield a reliable measure of the densities of mosquitoes prevalent in the catching stations and that this method was adequate both in the conduct of malaria surveys and in appraisal of mosquito densities after institution of control measures.

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TABLE I.

Summary of mosquito collections made in 620 observations in
Poona District—March to July 1951.

Species.	HAND COLLECTION.*			TOTAL COLLECTION, I.E. SUM OF HAND COLLECTION AND COLLEC- TION MADE AFTER PYRETHRUM SPRAYING.		
	Males.	Females.	Total.	Males.	Females.	Total.
<i>A. annularis</i> ...	10	29	39	16	82	98
<i>A. barbirostris</i> ...	0	0	0	0	4	4
<i>A. culicifacies</i> ...	253	984	1,237	811	3,103	3,914
<i>A. fluviatilis</i> ...	0	10	10	3	26	29
<i>A. pullulus</i> ...	1	0	1	1	0	1
<i>A. splendidus</i> ...	1	0	1	1	3	4
<i>A. stephensi</i> ...	9	26	35	21	65	86
<i>A. subpictus</i> ...	89	627	716	354	2,459	2,813
<i>A. theobaldi</i> ...	1	5	6	1	5	6
<i>A. burkhudi</i> ...	42	99	141	183	565	748
<i>A. vagus</i> ...	1	13	14	1	63	64
<i>A. varuna</i> ...	0	1	1	0	1	1
Crushed and unidentified ...	...	...	...	...	17	17
Total anophelines ...	407	1,794	2,201	1,392	6,393	7,785
Culicines ...	479	1,057	1,536	1,609	3,331	4,940
Total mosquitoes ...	886	2,851	3,737	3,001	9,724	12,725

* Total time spent 310 man-hours.

TABLE II.

Number of *A. culicifacies* adults collected in each collecting station.

Fortnight	1st fortnight, March 1951.			2nd fortnight, March 1951.				
Insect collector	A.	B.	C.	A.	B.	C.	E.	I.
Village	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.
1. Moshi	3-8	0-4	1-1	2-3	2-2	0-1	...	...
	1-1	1-1	0-4	0-0	3-11	6-8	...	...
	...	...	...	1-7	0-1	5-7	...	...
	...	...	...	0-2	1-1	8-52	...	...
2. Kelaguan	2-6	0-25	7-0	0-1	8-29	5-16	...	...
	4-16	0-0	0-0	6-13	0-3	1-3	...	...
	5-6	1-2	4-6	0-1	1-1	2-4	...	...
	...	8-8	0-1	5-8	0-0	0-0	...	...
3. Chikhli	3-21	4-9	4-13	0-1	1-3	3-20	...	...
	1-2	0-3	1-1	6-10	1-3	1-1	...	...
	7-16	0-0	0-0	3-7	0-0	0-0	...	...
	3-9	1-1	4-15	2-7	0-2	4-8	...	...
4. Manjri Pathar	...	...	...	3-56	4-7	0-0	2-44	...
	...	...	...	3-5	3-4	19-29	6-27	...
	...	...	...	...	3-25	2-17	10-43	...
	...	...	...	...	...	...	20-56	...
5. Manjri	...	...	...	0-0	1-15	5-6	1-4	0-1
	...	...	...	8-18	1-1	1-2	9-33	3-49
	...	...	...	1-1	0-1	...	...	...
6. Tupewadi	...	...	...	3-3	1-2	0-9	0-2	...
	...	...	...	19-26	2-5	3-5	15-16	...
	...	...	...	2-6	0-0	0-0	7-19	...

H = Number collected by hand during a search of 30 minutes.

T = Total number collected by hand (H) and the number collected subsequently after pyrethrum spray.

A, B, C, D, E, F, G, H and I refer to individual insect collectors.

TABLE II—contd.

Fortnight	1st fortnight, April 1951.						2nd fortnight, April 1951.				
Insect collector	A.	B.	C.	D.	E.	I.	A.	C.	D.	E.	I.
Village	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.
1. Moshi	0-0	0-0	...	0-0	...	...	0-1	23-38	3-8	0-4	...
	3-4	0-2	...	1-1	...	...	0-9	1-3	5-12	1-4	...
	1-5	0-0	...	0-1	...	...	0-1	3-12	0-0	0-0	...
	0-1	0-0	...	1-1	...	...	...	...	...	...	...
	1-21	5-12	...	0-0	...	1-5	5-8	3-17	3-6	...	...
2. Kelagaon	0-0	0-0	...	1-5	...	...	2-4	2-4	0-0	...	...
	5-6	0-0	...	1-1	...	...	4-9	0-2	0-0	...	...
	2-2	...	...	0-2	...	...	0-0	9-12	2-6	...	...
	5-33	...	0-1	0-0	0-5	...	1-20	0-0	1-3	0-0	4-7
3. Chikhli	2-2	...	2-3	0-0	1-1	...	0-1	0-1	0-1	0-6	...
	0-0	...	9-16	0-0	2-4	...	0-0	...	0-0	...	...
	...	...	...	...	...	...	0-2	...	...	...	...
	2-4	...	29-30	96-317	3-30	0-8	0-4	21-29	4-6	0-0	0-3
4. Manjri Pathur	14-81	...	9-73	3-7	44-90	0-16	9-12	0-1	0-0	2-14	...
	...	...	17-26	...	2-2	...	...	2-5	2-4	...	...
	...	...	...	...	...	...	...	8-18	...	...	...
	0-3	...	3-6	0-4	1-1	1-25	0-0	0-0	0-3	0-0	0-0
5. Manjri	2-3	...	6-18	11-15	11-19	...	1-4	5-13	2-3	1-8	4-62
	...	...	1-1	0-0	0-0	...	...	0-0	1-1	...	...
	0-0	...	0-0	0-0	2-11	...	0-0	0-1	0-0	3-3	1-2
6. Tupewadi	0-6	...	3-7	3-3	2-4	...	0-5	0-5	5-26	2-5	4-11
	3-22	...	3-5	4-21	10-17	...	...	...	...	...	...

H = Number collected by hand during a search of 30 minutes.

T = Total number collected by hand (H) and the number collected subsequently after pyrethrum spray.

A, B, C, D, E, F, G, H and I refer to individual insect collectors.

TABLE II—contd.

Fortnight	1st fortnight, May 1951.					2nd fortnight, May 1951.				
Insect collector	A.	C.	D.	E.	I.	A.	B.	C.	D.	I.
Village	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.
1. Moshi	0-0	0-1	0-4	0-3	0-5	1-1	3-4	0-0	7-12	...
	2-3	0-1	3-4	0-1	...	0-0	1-7	0-0	0-0	...
	0-0	4-12	0-1	...	...	...	0-0	0-10	0-0	...
	...	...	...	...	...	...	...	...	...	...
2. Kelagaon	5-34	7-22	0-2	3-29	0-0	5-15	0-15	1-8	0-15	...
	0-1	...	4-6	2-8	0-2	0-0	1-2	8-27	1-2	...
	5-16	...	...	1-4	...	3-6	0-0	...	1-1	...
	...	...	...	...	...	...	...	...	...	...
3. Chikhli	0-16	0-0	0-3	2-3	...	1-2	0-0	1-5	0-0	...
	3-7	0-0	0-0	0-1	...	0-0	1-1	1-3	0-0	...
	0-0	1-4	9-18	2-7	...	0-0	0-0	1-1	0-0	...
	4-6	0-0	5-10	11-12	...	0-0	2-4	1-1	3-8	...
4. Manjri Pathar	1-4	0-1	0-0	3-5	...	0-0	0-0	0-0	0-1	...
	0-0	1-9	0-0	11-17	...	0-0	1-1	0-0	3-3	...
	...	...	...	...	...	...	...	...	...	...
	0-1	0-0	0-13	3-3	1-1	0-0	0-2	0-1	0-0	1-4
5. Manjri	0-0	13-16	16-22	36-57	10-94	0-2	1-3	2-2	0-2	...
	...	0-0	0-1	...	...	0-0	0-0	0-0	...	...
	0-0	1-2	0-0	5-5	...	0-0	0-2	0-1	3-22	0-0
6. Tapewadi	1-1	0-2	0-0	3-19	...	0-0	0-0	0-0	14-20	5-6
	0-1	1-3	1-1	0-1	...	...	...	0-0	0-0	...

H = Number collected by hand during a search of 30 minutes.

T = Total number collected by hand (H) and the number collected subsequently after pyrethrum spray.

A, B, C, D, E, F, G, H and I refer to individual insect collectors.

TABLE II—contd.

Fortnight ...	1st fortnight, June 1951.				2nd fortnight, June 1951.				
Insect collector ...	A.	B.	C.	D.	A.	B.	C.	D.	I.
Village ...	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.	H-T.
1. Moshi	0-0	0-0	0-0	0-0	0-0	0-1	0-0	1-1	0-0
	0-0	0-0	0-0	1-1	0-0	4-5	2-6	0-0	...
	...	0-0	0-1	0-0	0-1	0-0	4-14	0-1	...
	...	...	...	...	...	...	...	...	...
2. Kelagaon	0-0	3-8	1-1	0-0	0-0	0-31	4-30	0-3	...
	0-0	0-1	1-2	0-2	0-1	0-0	0-0	0-0	...
	0-0	0-0	0-0	1-1	...	1-1	2-2	0-0	...
	...	...	...	...	...	...	...	...	...
3. Chikhli	0-0	0-0	3-12	0-0	0-0	0-1	0-0	0-0	0-0
	0-1	0-0	0-9	0-0	1-1	0-0	...	...	...
	0-0	0-2	0-2	1-3	...	...	...	...	...
	...	...	...	...	...	...	...	...	...
4. Manjri Pathar	2-2	0-0	0-0	0-0	...	...	0-0	...	...
	13-28	3-5	0-1	0-0	0-1	0-0	1-2	0-3	...
	0-0	2-7	0-1	0-3	0-0	3-4	2-5	3-5	0-4
	...	...	...	...	0-1	0-0	0-1	3-3	...
5. Manjri	...	...	...	...	9-15	2-4	0-0	0-0	0-47
	...	...	...	...	...	2-4	10-14	...	...
	...	...	...	...	...	...	...	...	...
	0-0	0-0	0-0	0-2	...	...	...	...	...
6. Tupowadi	...	1-1	...	...	...	...	...	...	...
	...	...	...	...	...	...	...	...	...

H = Number collected by hand during a search of 30 minutes.

T = Total number collected by hand (H) and the number collected subsequently after pyrethrum spray.

A, B, C, D, E, F, G, H and I refer to individual insect collectors.

4. Manjri Pathar	1-4	4-9	...	...	1-1	...	0-3	...	4-5	...	0-6	0-0	1-1	...
	0-1	3-14	...	...	7-15	...	2-28	...	0-3	...	3-11	3-6	0-3	...
	0-4	0-3	...	...	4-7	...	1-10	...	2-2	...	0-2	4-4	0-0	...
	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5. Manjri	0-0	0-2	...	0-0	0-0	...	...	4-24	0-0	...	0-0	0-0	0-0	0-0
	...	2-2	...	4-8	...	...	...	...	0-0	...	0-2	2-2	5-10	...
	...	2-6	...	0-0	...	...	...	...	0-0	...	0-0	...	0-0	...
6. Tupewadi	...	2-7	...	1-4	2-2	...	...	...	2-2	...	7-12	1-1	0-2	...
	...	0-0	...	0-3	2-10	...	...	...	3-6	...	0-7	1-4	5-15	...
	...	...	...	...	...	...	...	...	0-3	...	0-3	0-0	1-25	...

H = Number collected by hand during a search of 30 minutes.
 T = Total number collected by hand (H) and the number collected subsequently after pyrethrum spray.
 A, B, C, D, E, F, G, H and I refer to individual insect collectors.

SOME OBSERVATIONS ON RURAL MALARIA CONTROL USING RESIDUAL INSECTICIDES IN WETTABLE POWDER FORM.

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[June 13, 1962.]

INTRODUCTION.

THIS paper records some of the observations made in the course of rural malaria control with the use of residual insecticides in the form of wettable powder. The observations were first made in Kanker Town, the headquarters of a former Feudatory State in Madhya Pradesh, whose limits lie between 20°6' and 20°34' N. latitude and 80°41' and 81°48' E. longitude. They were further extended to some villages in the neighbourhood. The average annual rainfall in this area is 49 inches and rice is the staple crop. While in the town of Kanker itself houses are constructed with brick walls and tiles or terraced roofs, in the villages the houses mostly consist of thatched huts.

EPIDEMIOLOGY.

The spleen rate in the age group 5 to 12 in August 1949 was 25 per cent in Kanker Town. In most of the surrounding villages the spleen rate varied from 10 per cent to 50 per cent. But in a few villages it exceeded 50 per cent. The malaria season is from August to December. The principal vector is probably *A. fluviatilis*, though *A. culicifacies* may also play a subsidiary rôle. These presumptions are based upon authors' experience in the neighbouring foothill areas, whose physiographical features are the same as in Kanker area.

MALARIA CONTROL.

In 1949, all the houses in Kanker Town were sprayed with DDT wettable powder 50 per cent twice during the malaria season, the first round of spray in August and the second in October 1949. The dosage used was 56 mg. per sq. ft.

In 1950, this programme was repeated. In 1951, Kanker Town and 29 surrounding villages were sprayed with benzene hexachloride 50 per cent wettable powder in a dosage of 11 mg. of gamma isomer per sq. ft. During this year too, two rounds of spray were carried out, the first in July-August and the second in September, with an interval of six weeks between the two rounds. The total population of the area under spray during 1951 was 11,500, the population of Kanker Town itself being 4,231 (1951 census).

ASSESSMENT OF RESULTS.

(i) *Dispensary figures.*—It will be seen from Table I that while the total attendance from all diseases has dropped considerably in 1950, the number of

TABLE I.
Dispensary figures—Kanker.

Years.	Total attendance for all diseases.	Malaria cases.	REMARKS.
Average of 5 years			
1944 to 1948 ...	55,167	6,400	
1949 ...	57,568	5,364	
1950 ...	30,559	5,016	
1951 ...	22,096	3,249	

malaria cases shows only a very small decrease. In 1951, however, both show a considerable reduction. The fallacy of dispensary figures is well known based as they are on out-patient diagnosis and in some cases even what one may call absentee diagnosis, that is, the diagnosis being made in the absence of the patient himself. Sometimes malaria is expressed as a ratio of the total cases treated for all diseases in order to make allowances for the changing popularity of the local medical officer. A considerable rise or drop in the number of cases treated in the dispensaries usually occurs soon after there has been a change in the medical personnel. On this account, the ratio of malaria to total cases is sometimes considered a better index. If this yardstick is used in this instance, during 1950 and 1951 one would assume an increase in the incidence of malaria, since the ratio is higher during these years than in the previous period. Actually, however, this will not be a valid estimate. The large decline in the total cases is a measure of the considerable reduction in the incidence of malaria and possible collateral benefits as well.

(ii) *Spleen rates.*—In Kanker Town, the spleen rate in age group 2 to 10 years dropped from 25 per cent in August 1949 to 9 per cent in August 1950, 6 per cent in March 1951 and 5 per cent in May 1952, out of a total approximate number of 500 children examined. In the neighbouring villages under control, the spleen rate was 28 per cent in March 1951 prior to control and 25 per cent

in April 1952 after one season of control. Kanker Town has had three years of malaria control and the spleen rates have been brought down to the low figure of 5 per cent. The surrounding villages have had only one year of malaria control and the spleen rate shows no evidence of reduction yet. *Prima facie*, this would show that the reduction in the transmission of malaria has not been of any higher order than usually obtained in malaria control by anti-larval methods. In the latter type of control the first year or two do not show any reduction in spleen rates. The use of residual insecticides is on the other hand followed by spectacular reduction in the spleen rates even after one season of control. Instances are too numerous in the literature to merit specific mention. In the present experiment, however, the spleen rates do not show any significant reduction. This presumably calls for a more intense programme of spraying either by an increase in dosage or by a larger number of rounds of spray, or both when benzene hexachloride is used.

(iii) *Infant parasite rates.*—In Kanker Town, the infant parasite rate was 0 per cent (out of 27) in July 1951, 1.9 per cent (out of 51) in November 1951 and 2.3 per cent (out of 43) in February 1952. In 8 nearby villages under control, the infant parasite rate in the corresponding periods was 2.4 per cent (out of 41), 10 per cent (out of 60), and 23 per cent (out of 73). In 6 comparison villages left unsprayed, similar figures were 0 per cent (out of 27), 60 per cent (out of 51) and 32.5 per cent (out of 43). In the above computations, an infant found positive was not included in subsequent examinations in order to avoid the fallacy of counting a parasitic relapse as a fresh infection. The data regarding infant parasite rates clearly reveal the very considerable elimination of malaria transmission in Kanker Town. Hence the large number of malaria cases recorded in the dispensary records even after successful malaria control should be taken to be due to errors in diagnosis inherent in clinical diagnosis in out-patient practice and the tendency on the part of medical officers in malarious tracts to reckon most illnesses as malaria, either directly or indirectly. One other explanation that is possible is that either the figures are swollen by malaria patients from neighbouring villages or the urban people contract the infection in the neighbouring villages and suffer from the disease in the town. While the first contingency may occur to some extent, it will only add to the dispensary figures by a small fraction. There is no evidence for the second factor inasmuch as the spleen rates in Kanker Town have remained exceedingly low. There still remains the possibility of the adults of Kanker Town contracting the infection in villages, but the number that thus go out to the villages and spend a night, must be a very small fraction. On the whole, therefore, the conflicting data in Kanker Town showing an almost total reduction in the infant parasite rates but only a small reduction in the number of malaria cases treated, should be attributed to the inherent errors in diagnosis in out-patient dispensaries. Such errors, however, are liable to get less and less with a more pronounced reduction in the transmission of malaria by repeated malaria control measures in successive years.

The very high infant parasite rate of 60 per cent in the comparison villages at the height of the malaria season while the spleen rate is only between 10 and 50 per cent, requires further study in the light of the hypothesis put forward by Macdonald (1950) with regard to the limit of the parasite rate reached in a community exposed to a specific load of infection. But the basic fact remains that

malaria transmission is of a very high order in this area judging from the infant parasite rate. The present experiment also shows that in an area of such high transmission, spraying with benzene hexachloride in a dosage of 11 mg. of gamma isomer per sq. ft. only twice during the malaria season is not adequate to eliminate active malaria transmission during the season. The infant parasite rates have remained as high as 10 per cent at the height of the season. Presumably there will be need to increase the dosage as well as to increase the number of rounds of spray. That there must be at least 3 or even 4 rounds of spray, is shown by the fact that since the stoppage of the second round of spray in October 1951, the infant parasite rates in the sprayed villages have reached as high as 23 per cent in February following, while even in the unsprayed villages the infant parasite rate at that period was only 32.5 per cent. The actual reduction in malaria transmission in the villages has indeed been of a small order which has been reflected by a non-reduction in the childhood spleen rate. Out of the 17 infants who were positive for malaria infection, 77 per cent showed *vivax* infections.

For administrative reasons it has not been found possible to carry out entomological investigations along with this experimental control. Although Gabaldon (1949) says that after insecticidal spraying more attention should be paid to malaria prevalence than to anophelometric indices, the almost complete reduction in the transmission of malaria in Kanker Town and its slight reduction in the surrounding villages in 1951, would have been better understood in the light of the behaviour of anophelines in the two localities if it had been possible to collect data in this respect.

SUMMARY.

1. DDT indoor residual spray applied in a dosage of 56 mg. per sq. ft. twice during the malaria season over a period of two years, followed by benzene hexachloride in a dosage of 11 mg. gamma isomer twice during the season of the third year have brought about an almost complete elimination of malaria transmission in Kanker Town. Both insecticides were used in the form of water wettable powder.

2. Dispensary statistics, however, do not run parallel to spleen rate and infant parasite rate but during the third year they seem to fall in line, although they are still too high, judging from other indices of malarimetry.

3. Two rounds of application of benzene hexachloride water wettable powder in a dosage of 11 mg. per sq. ft. in the endemic villages surrounding Kanker Town have been followed only by a small reduction in the transmission of malaria taking the total season's experience as a whole. At the height of the season, malaria transmission was reduced to one-sixth and yet at the end of the season the total damage to the community has not shown reduction except to a small extent. This indicates a definite need for increasing the number of rounds of spray with benzene hexachloride and possibly for an increase in the dosage as well.

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ORGANIC SYNTHETIC INSECTICIDES EMPLOYED IN MALARIA CONTROL.

BY

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REVOLUTIONARY advances in the control of insects and pests affecting man have been made in recent years. The most outstanding and spectacular progress has attended the exploitation of organic synthetic compounds as insecticides. Some of the noteworthy reviews are published by Andrews and Simmons (1948), Upholt (1950), Wharton (1950), and King (1951).

The most extensively used insecticides consist principally of chlorinated hydrocarbon compounds, such as D.D.T., B.H.C., chlordane, and dieldrin.

The determination of the most effective dosage for successful malaria control has however presented wide variations in the experience of workers in different parts of the world. While, malariologists in the west advocate and practise a single large application of 200 mg./sq. ft. of D.D.T., workers in India have preferred the use of 50 to 100 mg./sq. ft. repeating the application twice or even three times during the malaria season. Experience regarding the rate of deterioration of D.D.T. from sprayed surfaces also seems to vary with different dosages and formulations. Hadaway and Barlow (1948, 1949, 1951 and 1952) consider that solid particles of insecticides are sorbed into inner layers of mud surfaces. Downs *et al.* (1951) link up the question of D.D.T. deterioration with the phenomenon of catalytic decomposition of D.D.T. by soil constituents, more especially its iron content. Some published and unpublished data in India show that the rate of deterioration for any given dosage of D.D.T. is fairly uniform throughout the country and that a single larger application, such as 200 mg./sq. ft. has a tendency to a correspondingly higher rate of deterioration than smaller dosages (Viswanathan and Gadre, 1950; Jaswant Singh *et al.*, 1951). The relative efficacy of D.D.T. formulations either in the form of wettable powder or as an emulsion is another feature which has presented variations in the experience of different workers. While the majority of workers both in the west and in India are

agreed that the use of D.D.T. in the form of a wettable powder yields better results on mud-plastered walls (Barlow and Hadaway, 1948; Sundararaman and Peffly, 1949; Kruse and Kochady, 1950; Jaswant Singh *et al.*, *loc. cit.*), a contrary experience has also been reported by some workers (Personal communication from Dr. Viswanathan). It would, therefore, appear desirable that further work on the rate of deterioration of D.D.T. from sprayed surfaces, made up of different soil composition, should be linked up with a study of different formulations. The particle size of D.D.T. is another relevant matter requiring further study.

The relative efficacy of D.D.T. and B.H.C. has also been subject of different experience by different workers. Fresh deposits of B.H.C. give more complete kills than D.D.T. as has been shown by experiments in Mexico (Bordas and Downs, 1951), Uganda (Wilkinson, 1951) and Malaya (Bertram, 1950). D.D.T. is, however, more effective as regards the persistence of residual effect. In fact, World Health Organization has recommended re-application of B.H.C. after three months and D.D.T. every six months (Malaria Conference, Equatorial Africa, 1951). Trials in Africa indicate that B.H.C. is perhaps superior in its residual insecticidal action to D.D.T. against *A. gambiae*. (Thomson, 1949, 1950; Davidson, 1949, 1951), though good results against that species have also been recorded with D.D.T. (Hocking, 1947). Some attempt at an explanation for such discrepancies has been made with reference to the rate of loss of D.D.T. from the sprayed surface due to the process of sorption and also to the phenomenon of excito-repellency.

The most effective dosage and the frequency of application of B.H.C. has yet to be precisely worked out in India by large-scale field trials. Viswanathan *et al.* (1949) tried B.H.C. on experimental basis in the villages of Bombay State and found that D.D.T. had a slightly more persistent residual action than B.H.C. Jaswant Singh *et al.* (*loc. cit.*) tried B.H.C. on experimental basis in the villages of Punjab State and found that under local conditions, there was little to choose between D.D.T. 50 mg. and B.H.C. 10 mg. gamma isomer per sq. ft.

Evidence tends to show that the use of D.D.T. and B.H.C. in combination in the form of wettable powder would yield better results than the use of either insecticide separately (Pal, 1951a; Jaswant Singh *et al.*, *loc. cit.*; Dicke and Paul, 1951; Van Tiel 1952). This requires further confirmation by large-scale field experiments.

Chlordane and toxaphene have been used either alone or in combination with D.D.T. in some countries, notably in Italy, where the development of resistance of *Musca domestica* was noticed after the application of D.D.T. as a residual insecticide (Mosna, 1949, 1950; Missiroli, 1950). Apparently such combination of insecticides may take care of resistant strains. That there is actual synergism between D.D.T. and chlordane, has been shown by Dicke and Paul (*loc. cit.*). It may, however, be stated that there have been no reports of any such development of resistance among flies in the Indian sub-continent (Busvine, 1949; Pal, 1951b).

Dieldrin is steadily coming into greater prominence on account of its increased toxicity to insects. It has not, however, been extensively used anywhere so far. Pal *et al.* (1951) tried dieldrin on experimental basis in the villages of Punjab State and found that under local conditions, dieldrin was

effective for about 8 weeks against mosquitoes when applied at the rate of 50 mg. per sq. ft.

By and large, D.D.T. has been usefully employed in rural malaria control in many parts of the world against several vector species manifesting different behaviour such as host predilection, density in indoor daytime resting places, anthropophilic index, etc. It has been used successfully as much against *Anopheles* which principally rest outdoors but feed indoors at night as also against those which rest indoors by day and feed outdoors by night. It is obvious that results will be most efficacious with respect to purely domestic species resting indoors by day and feeding indoors at night. Effective malaria control is attributed both to an actual reduction in *Anopheles* density and to the phenomenon of interception. While there are some records in literature of the development of resistance in some culicines (Missiroli, 1947; Mosna, 1947; 1948; Deonier and Gilbert, 1950; Deonier *et al.*, 1950; and King, 1950) and some evidence based on laboratory and field studies about a few species of anophelines such as *A. sacharovi* in Greece (Hess, 1952), *A. albimanus* in Panama (Trapido, 1952) developing slight resistance, there is no evidence of development of resistance with respect to any of the Indian *Anopheles* vector species, even in areas where malaria control has been continuously in progress on a large scale over a period of six years. *Culex fatigans*, however, appear to have developed a certain degree of resistance to D.D.T. (Pal *et al.* 1952a).

The employment of synergists with residual insecticides is yet in the early stage of experimentation. Synergism used somewhat in the loose sense, would seem to consist of (a) actively and directly improving the insecticidal action, (b) retarding the decomposition of the insecticide from the sprayed surface, and (c) interfering with catalytic agents which are supposed to assist in the decomposition of D.D.T. American workers have screened nearly 2,400 compounds out of which D.M.C. (1-1-bis p-chloro-phenyl-ethanol) and 17 other chemicals have been demonstrated to have marked synergistic action (Summerford *et al.*, 1951). In India Ranganathan *et al.* (1949) have reported the synergistic action of B. Methyl anthraquinone. These compounds improve the effectiveness of D.D.T. against resistant flies.

A more recent development in organic synthetic insecticides is the synthesis of compounds closely related to pyrethrum as for example, allethrin (Schechter *et al.*, 1949). It appears to be as non-toxic to animals as natural pyrethrins. The toxicity of synthetic and natural pyrethrins incorporating different synergists such as N-propyl isome, pipernoyl butoxide and cashewnut shell oil has been investigated on *Culex fatigans* and *A. stephensi*. As space spray, allethrin compares favourably with the natural pyrethrum, but the residual toxicity was found to be poor (Pal *et al.* 1952b).

Recent trends would seem to indicate that a useful line of further research does not lie so much in the discovery of more toxic insecticides as in the most effective and economic use of the well-known insecticides.*

* Specifications for the largely used insecticides have been prescribed by the World Health Organisation (Technical report series 34 and 54). Storage of insecticides in the form of water wettable powder even for long period does not involve any risk of chemical decomposition but its physical properties such as suspensibility and wettability are partially affected. Storage of solutions or emulsion concentrates on the other hand is likely to be followed by some chemical decomposition especially in the case of the latter. Storage decomposition is determined more by the container than by the solvent, which has a protective action. Lindane is particularly susceptible to decomposition in metal containers.

In conclusion it may be emphasized that this paper does not attempt at any exhaustive review of the several aspects of the insecticides used in malaria control but rather seeks to present briefly the salient features and to bring out succinctly a few important aspects having a practical bearing on economical and successful malaria control which require further planned investigation through large-scale field experiments. A chart giving such salient features is attached for ready reference.

ACKNOWLEDGEMENTS.

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THE RELATIONSHIP BETWEEN THE PHYSICAL FORMS OF D.D.T. AND THEIR BIOLOGICAL EFFECTIVENESS ON WATER SURFACES.

BY

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INTRODUCTION.

THE lack of residual action of D.D.T. when used on water surfaces to control mosquito larvæ has not been fully explained, although Upholt (1947) has suggested the adsorption of D.D.T. by the organic materials of a pond-bottom mud complex, and the redistribution and ultimate precipitation of D.D.T. due to wind and wave action. Furthermore, efforts to extend larviciding residuum either by increasing the dosage of D.D.T. without killing fish or by changing the type of application have been unsuccessful.

The extension of D.D.T. residuum is a practical problem as D.D.T. mosquito larviciding is a valuable adjunct to the residual spray treatments against adults and should continue to play an important role in the control or eradication of disease-vector species of mosquitoes. It was the object of this investigation to study the basic or fundamental aspects of the problem involved in the lack of residuum from D.D.T., in the hope that the information obtained might prove useful in developing a more effective larviciding method. Reference to literature (Ferguson *et al.*, 1949) showed scanty data on such important questions as the behaviour of D.D.T. crystals or formulations on water surfaces, and the effects of the environmental complexes which occur in natural bodies of water.

* These investigations were carried out during the tenure of a post-doctorate research fellowship of the National Institute of Health (1948-49) at the Communicable Disease Centre, Public Health Service, Federal Security Agency, Savannah, Georgia, U.S.A.

TECHNIQUE.

A laboratory technique has been developed whereby mosquito larvae can be exposed to a treated water surface for a limited period of time and removed with a minimum of disturbance of the surface film (Pal, 1951).

The basic device used for this technique was a 14 inches $\times$ 17 inches $\times$ 2½ inches white-enamelled tray with a glass partition placed across it from side to side in such a manner that when the tray was filled with 6,000 c.c. of tap water the surface was divided with the barrier extending about ½ inch below the water level (Plate I, fig. 1). The partition was adjusted so that a unit area of water surface at one end of the tray equalled 104.5 square inches or 1/60,000 of an acre. Slight variations in the unit area arose due to lack of absolute uniformity in size and shape of photographic trays.

The ends of the glass barrier were sealed to the tray with a solution of one part clear pine resin and four parts flexible collodion, and then covered with a thin coat of paraffin to add rigidity to the joints and to reduce any creeping action of the surface films.

Films of 1 : 4 pine resin to collodion or paraffin were tested for impermeability to oil solutions of D.D.T. by sealing the short stem of J-shaped glass tubes with test films, filling the tubes with 0.6 per cent D.D.T. in No. 2 fuel oil, and inserting them in 250 c.c. of water so that the D.D.T. solution in the long stem of the tubes was about 1 inch above the water level. After 3 days under these test conditions, the oil solution had not penetrated any of the films in quantities sufficient to affect 4th-instar larvae placed in the water for 24 hours. The use of Triton B-1956* (an alkylated aryl polyether alcohol) in the D.D.T. No. 2 fuel oil solution did not materially change the results. Sufficient oil penetrated the films in 4 days to affect test anopheline larvae somewhat. However, in view of the stringent 3-day tests with full strength oil solutions at positive pressure against thinner films than those actually used in sealing the barrier, it is believed that a thick pine resin-collodion film used as a seal prevented the spread around a glass barrier of a thin surface film of D.D.T. in fuel oil. The glass barrier was further supported at the ends by slotted wooden blocks.

In position, the lower edge of the barrier was 1½ inches to 1¾ inches above the bottom of the tray, permitting the free manipulation of a small wire net in introducing and removing larvae. The net has a rectangular basket ½ inch deep, ¾ inch wide, and 2 inches long, fabricated from galvanized 16-mesh/inch screen. It is fitted with a stiff wire handle long enough to reach under the glass partition to any point in the tray.

In use, 6,000 c.c. of water was placed in the tray and 0.06 c.c. of a test larvicide solution was applied with a pipette to the surface of water on one side of the barrier (measuring 104.5 sq. inches).

Tests were made by introducing larvae under the glass barrier to the treated side of the tray where they were left for an exposure period of 35 minutes. In the removal process, a needle was used to stimulate those larvae on the water surface to go to the bottom where they were easily collected with the net. They were washed in tap water and then transferred to a finger bowl and fed for 24

* Spreading agent produced by Rohm & Haas Co., Philadelphia, Pa.

PLATE I

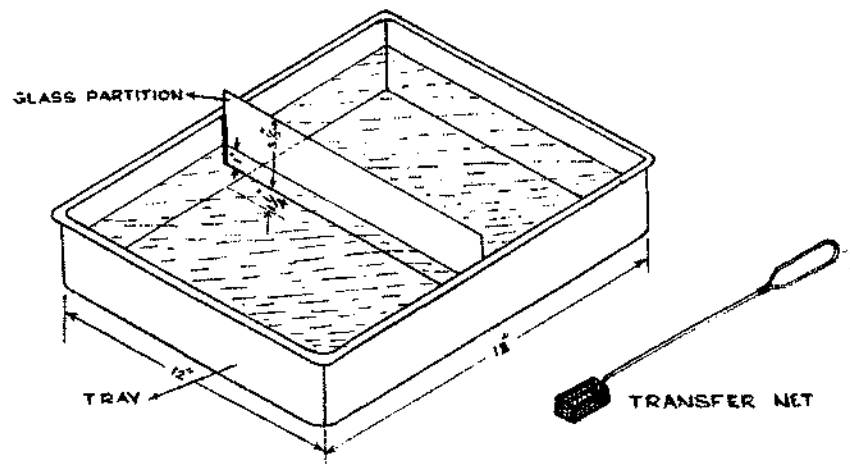


Fig. 1

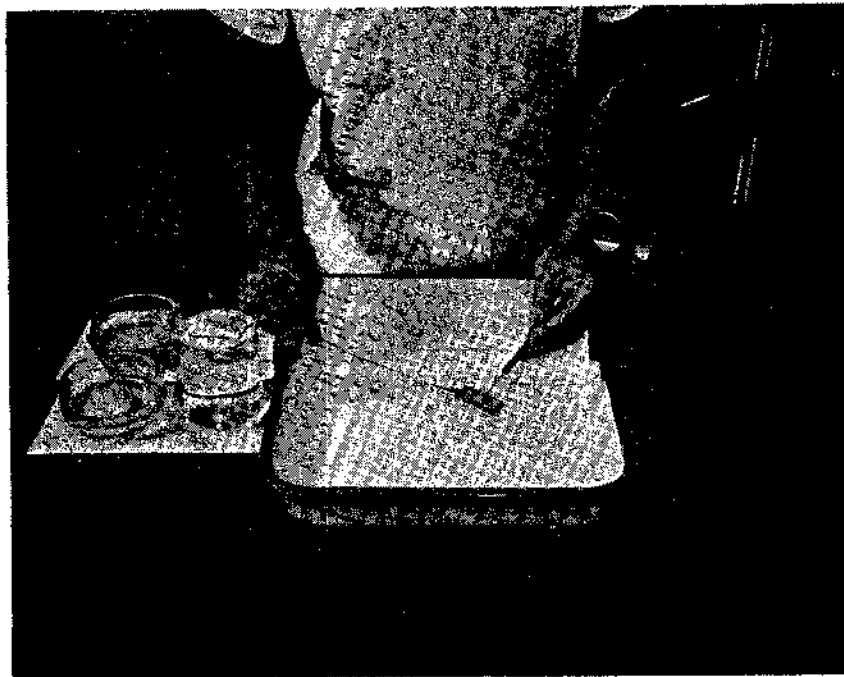


FIG. 2

Equipment used in the barrier technique for larvicidal studies showing white-enamelled tray, glass barrier with supporting wood blocks, transfer net, containers for washing and holding test larvae, and microscope for film observations.

hours. After this holding period, the rate of mortality was recorded. Controls were handled in an identical way except that neither side of the control tray was treated. Under these test conditions, mortality was rarely encountered in control larvæ during the 24-hour holding period. In these tests, 10 to 12 larvæ were used for each exposure. Replicate testing trays were set up with untreated check trays in the ratio of 3:1 or 6:1.

A second technique using glass crystallizing dishes, 4 inches high and 7½ inches in diameter, was also employed. The mosquito larvæ were exposed and removed directly through the treated surface in these tests. Observations on mortality in comparison with controls were made similarly to those described above.

The barrier technique was used when successive tests were to be made on a given surface film to determine progressive changes in the absence of mechanical agitation. The second technique was used when mechanical agitation of the test insecticide was immaterial.

BIOLOGICAL EFFECTIVENESS OF D.D.T. APPLICATIONS ON WATER SURFACE.

As dry floating D.D.T. crystals.—Three milligrams of recrystallized D.D.T. (0.4 pound per acre), applied in the form of a single mass of crystals floating on the surface of water, did not give any 24-hour mortality of *Anopheles quadrimaculatus* larvæ with exposure of 35 minutes on the first to fifth day after application. However, when applied in the form of fine crystals well distributed on the surface of water, the same amount gave 24-hour mortalities of 50 per cent or greater for 5 days. These results are based on five replicates.

In No. 2 fuel oil solution.—0.06 c.c. of 0.6 per cent D.D.T. solution in fuel oil (0.05 lb. D.D.T. per acre) was applied to the surface of water in each of the 12 test trays. Each test film was then exposed daily to groups of ten 4th-instar larvæ of *A. quadrimaculatus* for periods of 35 minutes. Results (Chart 1) indicated that under the experimental conditions:

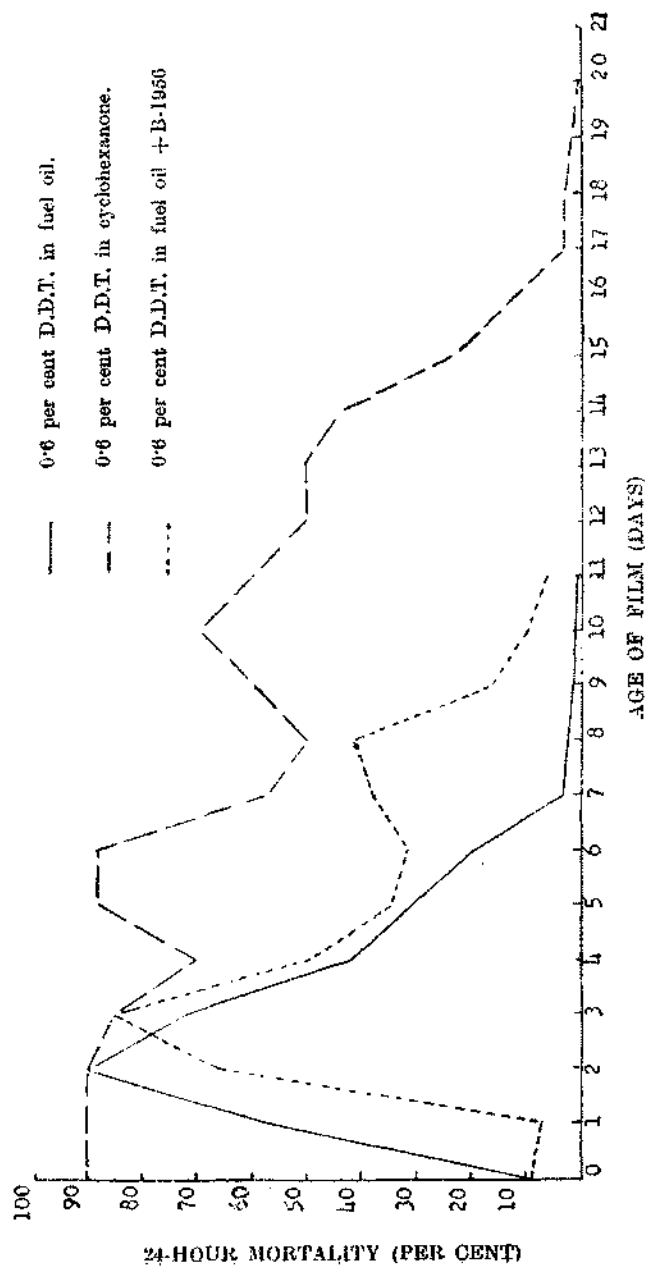
- (a) The surface film of D.D.T. in fuel oil was not very effective immediately after application.
- (b) The peak of effectiveness was reached in about 2 to 3 days after which it declined and became ineffective 5 to 8 days after application.
- (c) The duration of maximum effectiveness was relatively short.

Five replications of D.D.T. solutions in No. 2 fuel oil samples of different colour showed slight variations in their residual effect, the variation being from 3 to 7 days*.

In No. 2 fuel oil with 5 per cent Triton B-1956 added as a spreading agent.—Addition of Triton B-1956 surface-active agent seemed to delay the maximum effectiveness of the surface applications for 24 hours longer than fuel oil solutions alone and prolonged the residual effectiveness for a corresponding length of time

* The chemistry of fuel oil is complex and it is very difficult to determine which constituents of the fuel oil may have a harmful effect on D.D.T. This problem is beyond the scope of the present investigation.

CHART 1.



Average 24-hour mortality (per cent) of 4th-instar *A. quadrimaculatus* larvae after 30-35 minutes direct exposure to larvicidal films of 0.6 per cent D.D.T. solution in cyclohexanone, No. 2 fuel oil, and No. 2 fuel oil with 0.5 per cent Triton B-1956 added as a spreading agent.

(Chart 1). These tests showed a secondary peak of effectiveness on or about the 8th day which did not occur with fuel oil alone. In overall effectiveness, however, the addition of Triton B-1956 did not seem to affect either the toxicity or the duration of activity under laboratory conditions.

In various other larvicidal oils.—0.6 per cent D.D.T. solution in Velsicol* AR-30, AR-50, AR-60 and NR-70, and in PD 544C†, a fairly representative series of the methylated naphthalenes, when tested under similar conditions gave an appreciable residual effect in the laboratory (for about 4 days longer than the fuel oil samples; Table I).

TABLE I.

Average 24-hour mortality (per cent) of 4th-instar A. quadrimaculatus larvæ after 35-minute exposure to larvicidal films of 0.6 per cent D.D.T. solutions in various larvicidal oils applied at the rate of 1 gallon of solution per acre (0.05 lb. of D.D.T. per acre). Results are averages of three replicates.

Solvent.	Age of treatment in days.							
	0	3	4	6	7	8	11	13
Velsicol AR-30 ...	30	...	100	100	90	...	100	50
Velsicol AR-50 ...	100	...	100	70	70	...	70	10
Velsicol AR-60 ...	50	...	100	50	80	...	60	10
Velsicol NR-70 ...	30	...	100	80	80	...	100	30
PD 544C ...	30	...	100	100	100	...	90	50
Fuel Oil Sample A ...	75	85	...	...	100	10	...	...
Fuel Oil Sample B ...	8	72	40	15	3	...	...	...

* Products of the Velsicol Corp., Chicago, Ill.

† Product of the Socony Vacuum Corp., New York, N.Y.

In pure chemical solvents.—Solutions containing 0.6 and 5.0 per cent D.D.T. in various solvents, such as aliphatic, aromatic, and halogenated hydrocarbons, esters, and ketones, were applied to the surface of water and tested under similar conditions. Residual effectiveness was greatly prolonged in the case of cyclohexanone (Chart 1), whereas in pentachlorethane, carbon disulfide (and fuel oil) it was comparatively poor (Table II). Equal amounts of the pure solvents in control tests did not show any larvicidal effect after one day.

In liquid petrolatum and paraffin-wax jelly.—At 0.05 lb. D.D.T. per acre, a jelly consisting of equal parts of paraffin-wax and a 5 per cent D.D.T. solution

TABLE II.

Average number of days during which 50 per cent or higher mortality was obtained against 4th-instar A. quadrimaculatus larvæ after exposure of 35 minutes to surface films of 5 per cent and 0.6 per cent solutions of D.D.T. in various solvents, applied at the rate of 1 gallon of solution per acre (dosage of 0.4 and 0.05 lb. of D.D.T. per acre).

Solvent.	TIME INTERVAL (DAYS DURING WHICH 50 PER CENT OR GREATER EFFECTIVE- NESS OBTAINED).	
	5 per cent solution.	0.6 per cent solution.
Cyclohexanone ...	49	15
Isopropanol ...	49	10
Chloroform ...	42	10
Xylene ...	37	10
Ligroin ...	35	8
Ethylacetate ...	35	8
Carbon disulfide ...	28	8
Pentachlorethane ...	15	8
Fuel oil with SSA*	9-15	4-7
Fuel oil without SSA* ...	9-15	4-0
		Composite figure for different samples.

* SSA—Surface-active agent Triton B-1956 (0.05 per cent added as a spreading agent). Fresh preparation tested in controlled conditions of temperature and humidity. Air movement variable.

in liquid petrolatum gave a slightly prolonged residual effect extending up to 19 days. When tried in the field, effective control was obtained for only 4 days after which early 3rd-stage larvae began to appear, and 13 days after treatment many pupae were noted (Table III).

TABLE III.

Field test with 5 per cent D.D.T. solution in liquid petrolatum and paraffin wax jelly applied May 27, 1949 at the rate of $\frac{1}{2}$ gallon per acre (0.05 lb. D.D.T. per acre). Number of anophelines and culicines expressed per dip.

Before treatment	May 31, 1949	June 3, 1949	June 9, 1949
Culicine 1.3 ...	0.07	0.23	2.00
Anopheline 3.5 ...	0.7	1.48	4.67
Number of large larvae ...	Young small larvae.	Largest larvae early 3rd stage.	Many anopheline pupae.

PHYSICAL, CHEMICAL AND ENVIRONMENTAL FACTORS.

Rate of crystallization and crystal habits of D.D.T. on the surfaces of water.—In order to explain the progressive changes in D.D.T. residual effectiveness, studies were made on the rate of crystallization and crystal habits of D.D.T. from 0.6 per cent solutions in fuel oil and cyclohexanone, the two solvents giving extreme results.

The surface of the water was examined periodically both under direct and ultraviolet light, and photographs were taken of a portion of the surface film transferred to a glass slide (Plate II, Fig. 1).

The general physical picture encountered when D.D.T.-fuel oil solutions were applied to the surface of water was as follows:

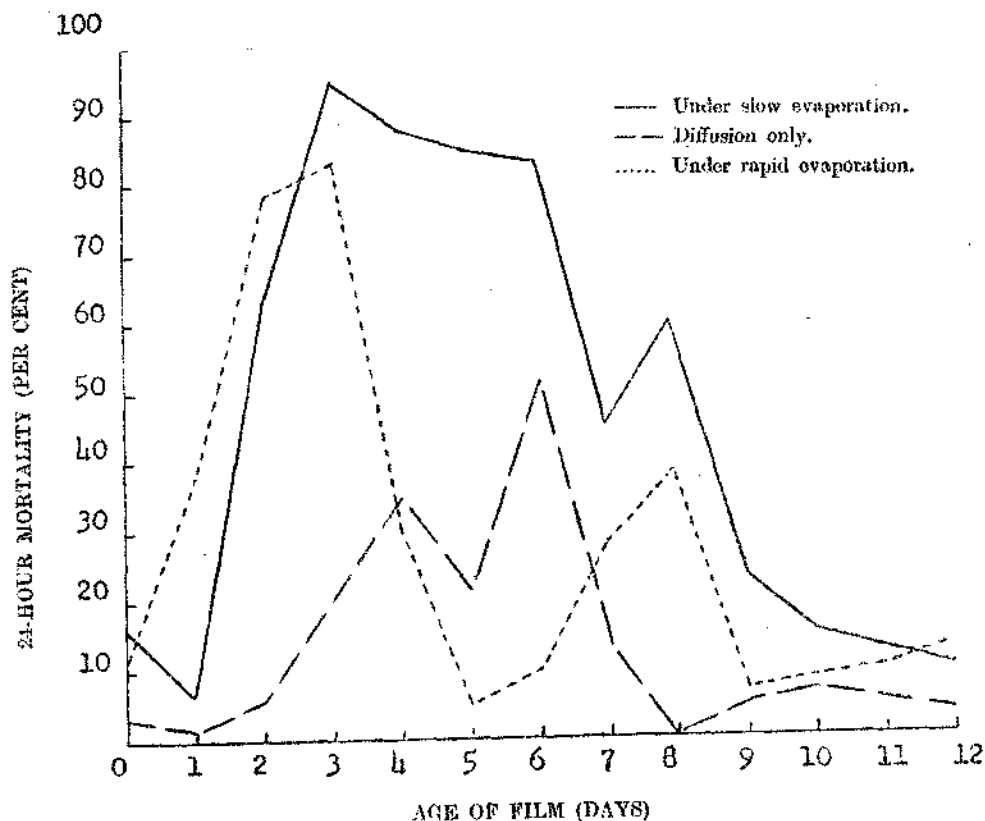
- The D.D.T.-oil solution spread as a thin surface film.
- Within 1 to 2 hours the film broke into lenses still connected by a continuous film of multi-molecular thickness.
- Within 1 day the surface film broke up into well-dispersed lens aggregates.
- During the next 2 to 3 days the oil gradually evaporated and wet (oil-coated) crystals averaging 170μ in length were formed.
- Portions of the crystals then either floated to the side or sank during the next 4 to 8 days.

In contrast with volatile solvents such as acetone and cyclohexanone the surface film crystallized within 1 to 4 hours after application and produced relatively small crystals averaging $68\ \mu$ in length.

Size differences in D.D.T. crystals from different solvents were noted, but on morphological grounds they could not be correlated with specific solvents. Though the individual crystal sizes with solvents of low volatility were larger than those from solvents of high volatility.

Diffusion.—In course of studies on the barrier technique, the question was raised whether larvæ held on the untreated side of the test trays were affected by surface treatment on the other side. In 12 replications a very low effectiveness was obtained on the untreated side 1 to 5 days after application of 0.6 per cent D.D.T.-fuel oil and Triton B-1956 solution at the rate of 0.05 lb. D.D.T. per acre. Kills rose to a peak of about 50 per cent mortality on the 6th day and then declined rather rapidly (Chart 2). The effects

CHART 2.



Average 24-hour mortality (per cent) of *A. quadrimaculatus* 4th-instar larvae after 30-35 minute exposures by diffusion and by direct contact under slow and rapid evaporation with larvicidal films of 0.6 per cent D.D.T. solution in fuel oil with 0.5 per cent Triton B-1956 added as a spreading agent.

PLATE II

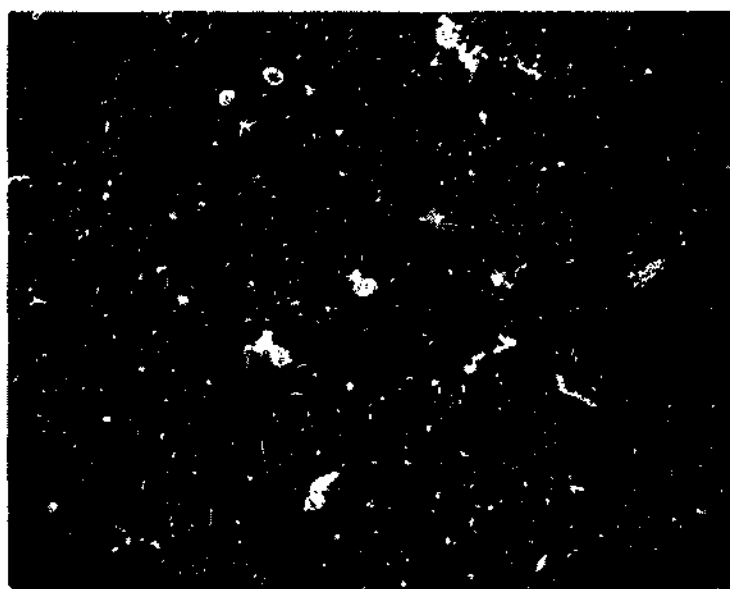
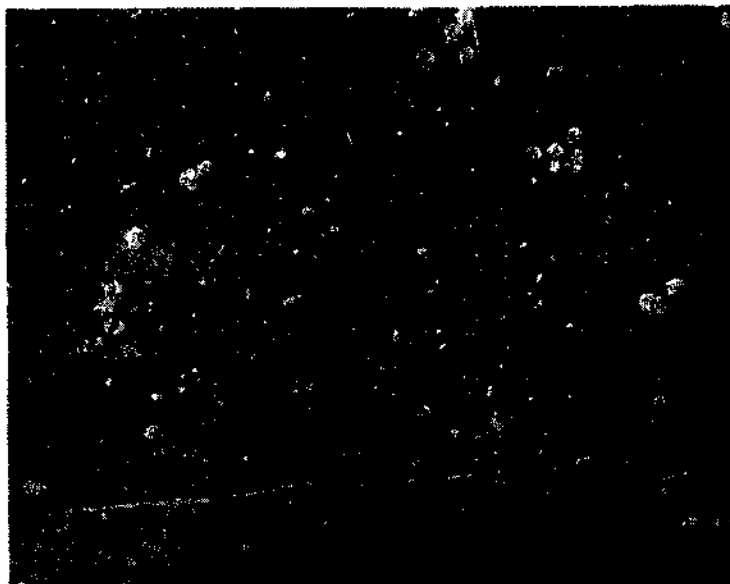


FIG. 1.—D.D.T. crystals on water surface (A) from 0.6 per cent D.D.T.-No. 2 fuel oil film, and (B) from 0.6 per cent D.D.T.-cyclohexanone film. Note dispersion of crystals in lens aggregates. In (A) average size 170μ . Average size in (B) 68μ .

of diffusion appeared 4 to 6 days after the application of the surface film, when the crystals were observed on the treated side of the barrier. This point is given further consideration in studies on the rate of evaporation and biological effectiveness of surface films.

Chemical disappearance.—The results previously described indicated a differential biological activity of D.D.T. when applied as a larvicide in different solvents (Table II). Consequently, a series of chemical determinations* was made to ascertain the amount and distribution of D.D.T. present in the experimental containers (glass crystallizing dishes and enamelled trays) at various intervals after application.

In one series, D.D.T.-solvent films were placed on water which was allowed to evaporate to complete dryness. Coatings, composed of D.D.T. mixed with the mineral content of the water, left behind in the containers were washed with acetone to secure the sample for quantitative determinations and in no case was more than 40 per cent of D.D.T. recovered. Apparently some change, possibly chemical rearrangement or decomposition and/or volatilization, had occurred so that the full amount of D.D.T. was no longer detectable in the chemical determination.

The maximum amount of D.D.T. recovered from any experimental container was between 20 and 40 per cent of the amount applied in xylene, chloroform, cyclohexanone, or ethyl acetate; however, it was less than 20 per cent in the case of pentachlorethane, ligroin, and fuel oil solutions (Table IV).

TABLE IV.

Quantitative estimation of D.D.T. from test trays 3 weeks after treatment (after complete evaporation of water, surface film not exposed to larvæ).

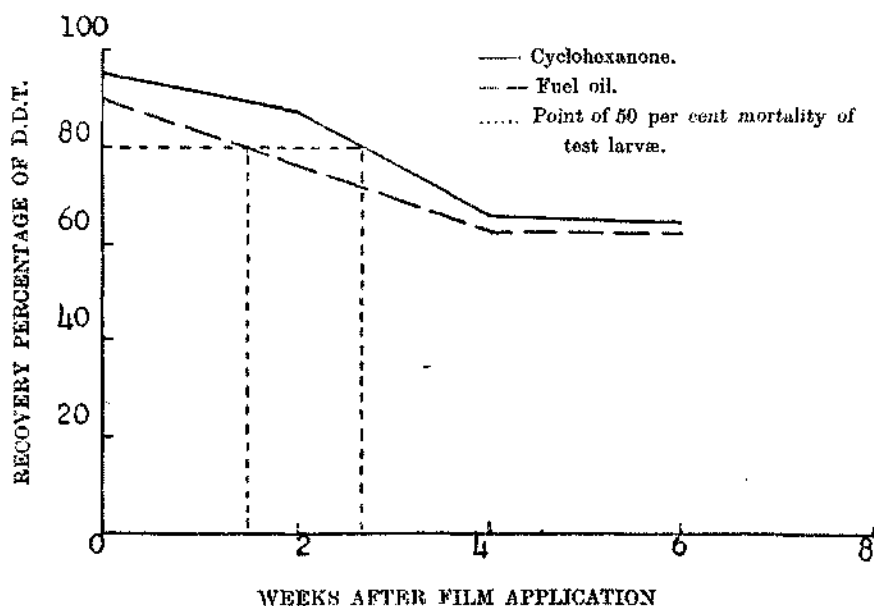
Solvent.	Total amount of D.D.T. applied (mg.)	Chemical determination of D.D.T. (mg.)	Recovery percentage.
Xylene	9	3.5	39
Chloroform	9	3.08	34
Cyclohexanone	9	2.4	27
Ethylacetate	9	2.2	24
Pentachlorethane	9	1.5	17
Ligroin	9	1.28	14
Fuel oil without SAA† ...	1.08	0.08	7
Fuel oil with SAA† ...	1.08	0.0	0

*The chemical estimations were made by a combined method of Bent and Schechter-Haller with slight modifications. The authors are obliged to Dr. W. T. Sumnerford for running these tests.

† Surface-active agent—Triton B-1956, Rohm & Haas Co., Philadelphia, Pa.

In the second series of experiments, 0.06 c.c. of 5 per cent D.D.T. solutions in either cyclohexanone or fuel oil (0.4 lb. D.D.T. per acre) were applied on tap water and on distilled water. Sufficient water was used in the test containers to prevent complete evaporation. After 0-, 2-, 4- and 6-week periods, containers were selected for quantitative determination of D.D.T. and were thoroughly washed with benzene (shaking the solution for 4 to 8 hours). With cyclohexanone, chemical estimations made soon after application gave 96.3 per cent recovery of D.D.T.; 2 weeks after application 87 per cent recovery and 4 to 6 weeks after application about 70 per cent recovery. With fuel oil, the chemical recovery of D.D.T. was 90 and 80 per cent at 0 and 2 weeks after application, and 64 to 66 per cent at 4 and 6 weeks (Chart 3).

CHART 3.



Recovery percentage of D.D.T. in test trays 0 to 6 weeks after treatment when applied as 5 per cent solution of D.D.T. both in fuel oil and in cyclohexanone at the rate of 1 gallon per acre on the surface of water. The surface film was undisturbed and unexposed to larvae. The water was not allowed to evaporate completely. Short dash lines show relationship between D.D.T. recovery rates at time of 50 per cent mortality of larvae.

The above results appear to indicate a gradual loss of D.D.T. from containers in 0 to 6 weeks. No apparent difference was disclosed by tests with either tap or distilled water or with either enamelled trays or glass dishes.

Under the experimental conditions with cyclohexanone, biological effectiveness dropped below 50 per cent mortality 3 weeks after application; with fuel oil it dropped to that level 10 days after application. In both cases, the D.D.T. recovered at corresponding intervals was about 80 per cent of the amount applied. This amount of D.D.T. in a fresh application would produce 90 to 100

per cent mortality. Besides the chemical loss of D.D.T., it appears as though some further factors are responsible for the loss of biological effectiveness of D.D.T.

Sunlight.—Fresh solutions of 5 per cent and 0.6 per cent D.D.T. in pentachlorethane, No. 2 fuel oil, and cyclohexanone were prepared and divided into three samples in clear-glass bottles. The first sample of each solution was stored in dark; the second exposed to room light; the third exposed to sunlight. After two weeks under the above conditions, 0.06 c.c. portions of each sample (0.05 and 0.4 lb. D.D.T. per acre) were tested for biological effectiveness against *A. quadrimaculatus* 4th-instar larvae.

The results (Table V) indicated a decrease in biological activity of solutions exposed to sunlight for 2 weeks. It is of interest to note that the above results conform to recent chemical studies (Fleck, 1949) which speak of catalytic decomposition of D.D.T. by exposure to ultraviolet light.

TABLE V.

Average number of days during which 50 per cent or higher mortality was obtained against 4th-instar A. quadrimaculatus larvae after exposures of 35 minutes to surface applications at the rate of 1 gallon per acre of various 0.6 and 5 per cent D.D.T. solutions stored under different conditions.

Solvent.	Stored in dark.		Exposed to roomlight.		Exposed to sunshine	
	5 per cent.	0.6 per cent.	5 per cent.	0.6 per cent.	5 per cent.	0.6 per cent.
Pentachlorethane	8	8	8	8	7	3
No. 2 fuel oil ...	15	10	13	8	0	2
Cyclohexanone	21	10	...	10	7	2

Rate of evaporation.—It was noted that the residual effectiveness of oil films in test trays varied with the evaporation rate. Trays subject to a continuous breeze not strong enough to be accurately measured with the available anemometers, but of sufficient intensity to blow a match flame to horizontal (rapid evaporation), showed (Chart 2) an abrupt drop in biological activity 3 to 5 days after application, whereas with negligible air movement (slow evaporation) residual activity did not show a definite break until 6 to 9 days after application (Chart 2). In all trays the greatest peak of larvicidal activity was reached on the third day after treatment, but the mortality level was lowest in trays exposed to rapid evaporation. It seems that air movement was not only con-

ductive to crystallization but also caused surface disturbances which influenced the distribution of crystals on the surface of water.

Effect of simulated conditions of rain.—Preliminary studies on the effect of rain on larvicidal films were made in the laboratory by artificially producing the action of rain on treated water surfaces.

Tests on the effect of rain were made in special barrier trays. Film was applied only on one side of the barrier tray; while the other side was equipped with an overflow drain pipe to maintain constant water levels. Two types of trays were used: (a) Enamelled trays, $14 \times 17 \times 2\frac{1}{2}$ inches with an overflow drain pipe $1\frac{1}{4}$ inch high, and (b) stainless-steel trays $14 \times 17 \times 6$ inches deep with an overflow pipe 2 inches high. The stainless-steel trays were used in the later evaluations as they effectively prevented splashing of water and oil films over the sides of the tray and the barrier.

The mechanism for producing rain consisted of a shower-head water source located 28 inches above the larvicidal film. The shower head was supplied with water under constant pressure by a small electrically driven centrifugal pump. The desired pressures were maintained by a by-pass valve in the supply line. With water supplied to the pump under a constant head of $1\frac{1}{4}$ inches and operating the pump at 7 lb. pressure per sq. inch, a total flow of 0.55 gallon per minute of water was produced through 34 holes of 0.04-inch diameter in the shower head. However, only about 0.50 gallon per minute actually landed in the tray. A 15-minute operation of machine was comparable to 7 inches of rain.

Surface films of 5 per cent D.D.T. No. 2 fuel oil applied at the rate of 1 gallon per acre tested for 35 minutes against ten 4th-instar *A. quadrimaculatus* larvae before and after a 7-inch rain (0.5 inches/minute) showed marked reduction in the 24-hour mortalities in the latter case. (Table VI).

TABLE VI.

Average number of days that 50 per cent or higher mortality was obtained for 4th-instar A. quadrimaculatus larvae after exposure of 35 minutes to surface application of D.D.T. exposed to simulated rain conditions. Five per cent solution in No. 2 fuel oil was applied at the rate of 1 gallon of solution per acre.

Enamelled tray. (Loss by overflow splashing.)		Stainless steel. (No loss by overflow splashing.)	
Without rain.	After rain.	Without rain.	After rain.
7	0	8	1
7	1	8	2
12	4	8	1

DISCUSSION.

It would be evident from the data presented above that the maximum biological effectiveness of D.D.T. surface films was observed to coincide with the appearance of crystals from the films. Crystallization of D.D.T. is apparently

dependent upon the volatility of the solvent, as the D.D.T.-cyclohexanone film attained maximum effectiveness rapidly in comparison with the less volatile fuel-oil film.

The maximum biological effectiveness is apparently not only dependent upon the appearance of crystals but also upon their distribution on the water surface. With a single mass of dry D.D.T. crystals practically no kill was obtained; whereas when the same amount was fairly well dispersed as crystal fragments, erratic kills were obtained for 5 days. In this case, however, the dry crystals tended to drift to the sides of the experimental trays. Distribution of crystals from the fuel oil film was not uniform because the crystals were formed in the scattered lens-aggregates. The crystals were held on the treated surface by a film of oil and gave biological effectiveness of over 50 per cent for 3 to 7 days. The crystals from cyclohexanone were formed uniformly and remained well distributed over the entire treated surface and gave biological effectiveness of over 50 per cent for 1 to 21 days.

The residual effectiveness of a D.D.T. surface application may be dependent upon the persistence of the crystals and their unit-density on the surface of water, which would be influenced by size, traces of solvents left after evaporation, and surface disturbances.

Dry D.D.T. crystals were large in size and sank rapidly as compared to smaller crystals obtained from fuel oil and cyclohexanone solutions. The coating of solvent on the crystals helps in their floatation and also increases the permeability into cuticle.

Environmental disturbances, such as air movement and rain, tend to redistribute and sink the crystals present on the surface of water regardless of size or hydrophobic solvents. This point was emphasized by partial failure of paraffin wax and D.D.T. liquid-petrolatum jelly in field tests versus laboratory tests.

When the crystals of D.D.T. sink to the bottom their deposition on the "bottom-mud complex" thereafter is due to chance. It may be that once the crystals settle to the bottom, the nature of the bottom soils determines the extent to which the D.D.T. is inactivated. This inactivation of D.D.T. may be due to various factors, physical or chemical (Upholt, *loc. cit.*).

Besides these physical changes, D.D.T. showed an apparent deterioration in solution which was accelerated by exposure to sunlight. When D.D.T. solutions are exposed in the field as very thin films on the surface of water, this reaction may be of greater significance than when held in bulk. Even in bulk storage the deterioration was marked.

The duration for which D.D.T. formulations remain in solution form on the surface of water may, therefore, be a factor of importance, because it may not only affect initial effectiveness but may also affect loss of residual effectiveness, as D.D.T. apparently undergoes chemical changes more rapidly in solution than in crystal form. Cyclohexanone evaporates soon after application leaving behind crystalline D.D.T. which, comparatively, may take a much longer time to deteriorate to the same degree than would the same amount of D.D.T. held in solution in fuel oil.

It would appear that the duration of residual effectiveness of D.D.T. on water surface can be extended by using solvents: (a) which cause the least chemical decomposition of D.D.T.; (b) produce a continuous and stable surface film; (c) produce crystals (coated with the solvent) fairly rapidly.

This investigation suggests some very interesting problems for further studies. For example, the deterioration of D.D.T. whether in dry or solution form; the chemical rearrangements which it undergoes on storage or when exposed to sunlight; and the relationship between the different solvents and the deterioration rate of D.D.T.

SUMMARY

Fundamental studies on the behaviour of D.D.T. crystals and formulations on water surfaces have been made with a view to studying the factors influencing the residual action of D.D.T. as a larvicide. The typical behaviour of surface applications supports the hypothesis that crystallization of D.D.T. is conducive to maximum biological activity. The lack of larvicide residuum is probably due primarily to the redistribution or dispersal of D.D.T. crystals and the removal of these crystals from the surface of water by wind, rain, and other environmental conditions. Another factor which may be important in the loss of residual action is the chemical rearrangement and deterioration of D.D.T. in solution in different solvents and the catalytic action of sunlight to accelerate this process.

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THE RELATIONSHIP BETWEEN THE PHYSICAL FORMS OF D.D.T. AND THEIR BIOLOGICAL EFFECTIVENESS ON SOLID SURFACES.*

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INTRODUCTION.

PARKIN AND GREEN (1945) have reported that DDT in kerosene solution is more toxic than dry DDT deposits, but that a supersaturated solution of DDT in kerosene is not so toxic as crystalline DDT deposits. Conflicting observations by different workers on the toxicity of DDT crystals of various sizes in water suspensions have been recorded. According to Vanderplank (1947), 'large' crystals had longer persistent effect, though they were less toxic than 'small' crystals, while McIntosh (1946, 1947) has reported that large crystals have shorter persistent effect, but are more toxic. Hadaway and Barlow (1951) on the other hand observed an inverse relationship between the crystal size and toxicity in an aqueous suspension. Differences in effectiveness have also been attributed to differences in the amount of insecticide picked up and retained by the insect.

The relationship between the physical forms of insecticides and their biological effectiveness may influence the type of formulation which should be used under varying conditions in control programmes. The need for careful investigation of the problem is therefore obvious. The fate of DDT crystals formed from solutions and their biological and physical properties have been studied. Some of the important results are described in this paper.

TECHNIQUE.

A special technique was developed to test the biological effectiveness of residual deposits of insecticides (Pal, 1951). Test panels (glass, wood and mud plaster), 6 × 6 inches, were sprayed with a DeVilbiss atomizer using a standard method of application. Each sprayed panel was placed between two wooden boards, 18 × 8 inches, so that a circular area of the panel, 5 inches in diameter, was available for exposure to test insects (Plate III, Fig. 1 and 2). Another

*These investigations were during the tenure of a post-doctorate research fellowship of the National Institutes of Health (1948-1949) at the Communicable Disease Centre, Public Health Service, Federal Security Agency, Savannah, Ga., U.S.A.

glass panel (unsprayed) was then moved over the treated area so that the two glass panels were 0.5 cm. apart. A known number of house-flies (usually 25) was introduced on to the treated surface by means of a glass plunger tube through a movable perforated square panel of plywood. The insects were able to move freely on an horizontal plane, but remained in contact with the sprayed surface. The number of insects knocked down were recorded after every five minutes. A second series of tests was made by exposing insects for a specified period of time, usually 15 minutes, and then transferring the insects to holding cages where they were kept under observation for 24 hours. Mortality was then recorded for each experiment.

This technique proved fairly sensitive in detecting differences in knockdown and mortality rates for various deposits. It allowed test insects to remain constantly in contact with the surface film for the desired exposure period and the treated surfaces were well protected from any extraneous disturbance other than the normal biological activity of the test insects.

RATE OF CRYSTALLIZATION AND CRYSTAL HABITS.

The rate of crystallization and type of DDT crystals, formed from different solvents representing aliphatic, aromatic, and halogenated hydrocarbons, alcohol esters, and ketones, were studied. A 5 per cent solution of DDT in each solvent was sprayed on glass panels at the rate of 100 mg. of DDT per sq. ft. and the surface film was examined 4, 8, 12 and 16 weeks after application. The deposits showed a great tendency to form highly viscous gummy droplets which even in the case of highly volatile solvents such as acetone and isopropanol (Table I) did not crystallize 8-16 weeks after spraying.

The crystallization rate of DDT did not show any general similarity within related groups such as aliphatic, aromatic, and halogenated hydrocarbons, or within a particular group of solvents; for example, from chloroform and from chlorobenzene solutions, DDT crystallized 12 and 4 weeks respectively after application.

The typical morphology of DDT crystals from different solutions was studied with stereoscopic-dissecting and compound microscopes under ordinary, polarized, and ultraviolet light. Individual crystals from different solvents varied from 7 to 528- μ along the longest axis, and were distinguishable according to shape into the following five broad types (Plate IV and V):—

- I. Dendritic crystals extending horizontally from an angular base.
- II. Flat-fibrous or needle-like crystals.
- III. Spherulitic crystals with thick or thin branches.
- IV. Interspersed masses of both needles and prisms.
- V. Fragments of crystals.

Different solvents produced specific types of crystal habits, although in certain cases more than one crystal habit may be interspersed (Table I). Differences in DDT concentration, type of surfaces, dust particles, temperature conditions, etc., also influenced the rate of crystallization and crystal habits.

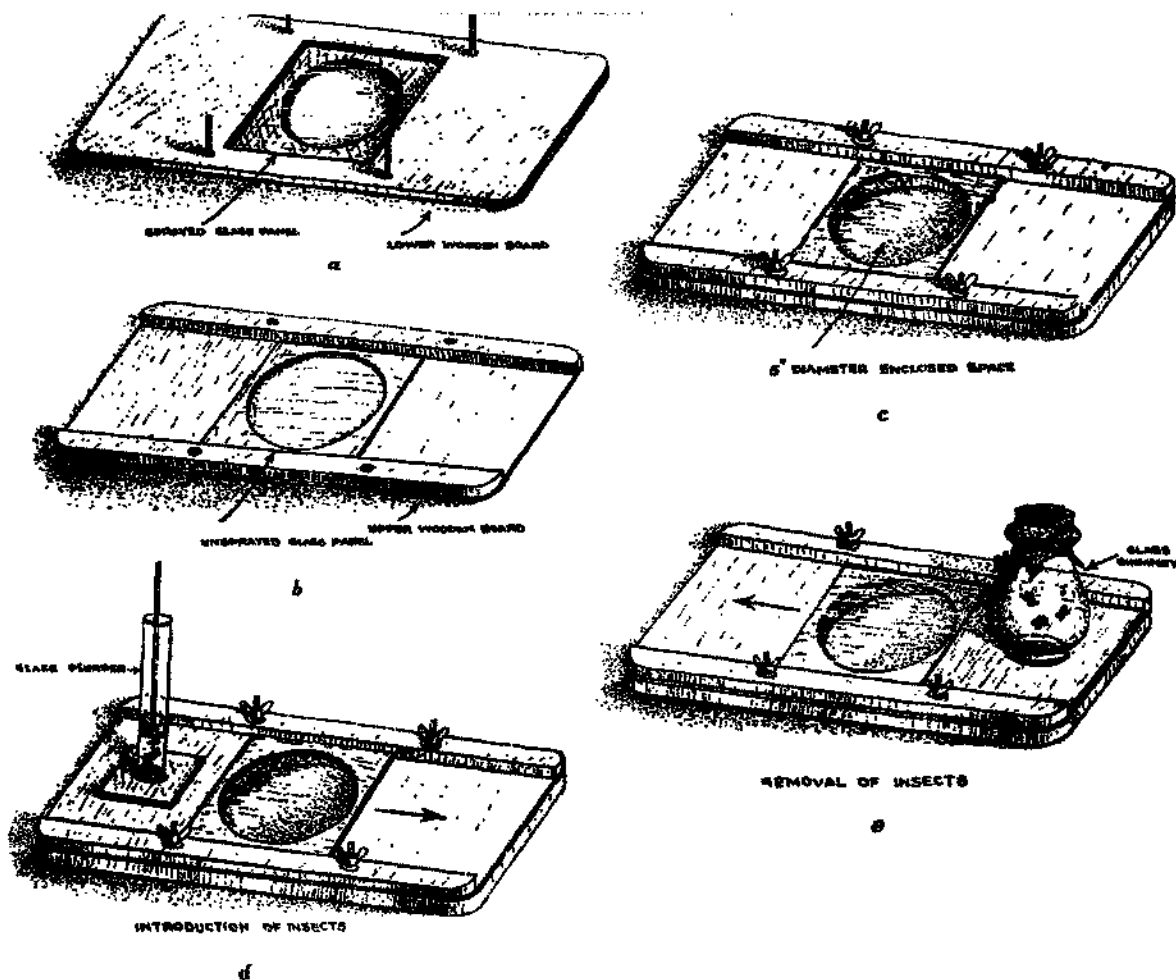


Fig. 1

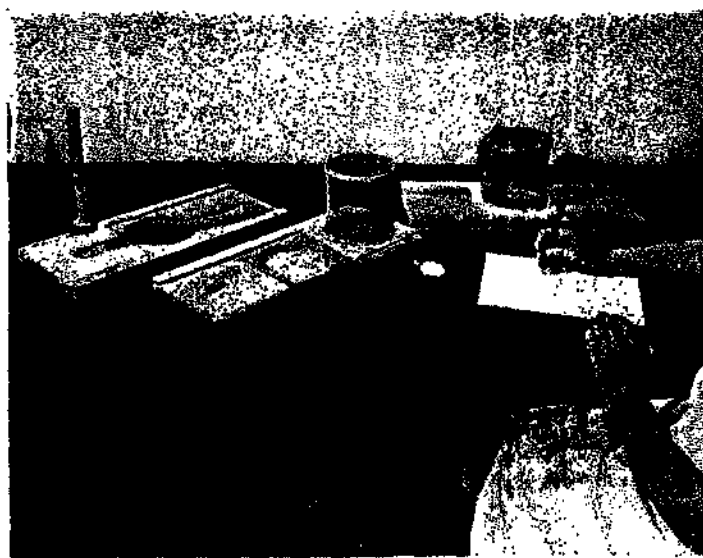
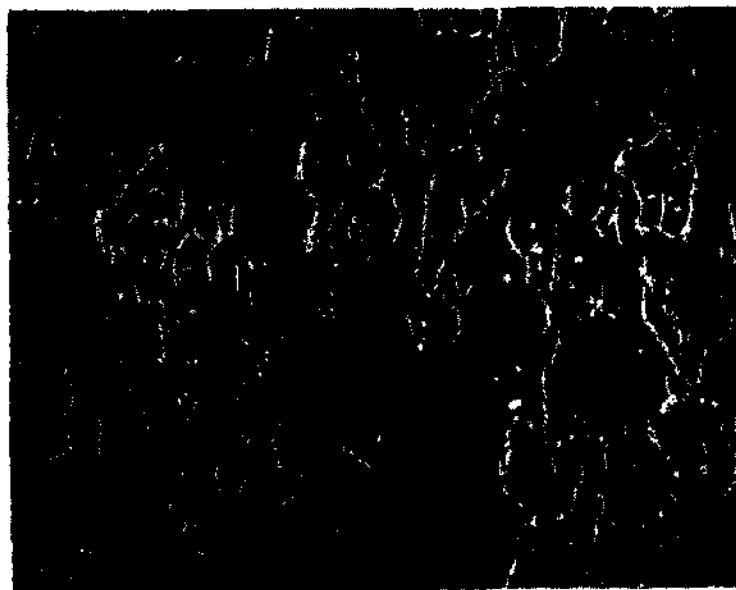


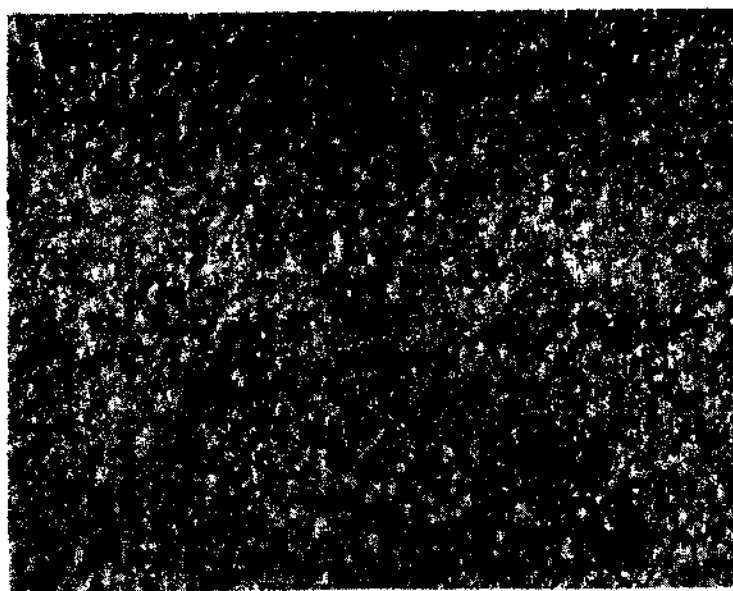
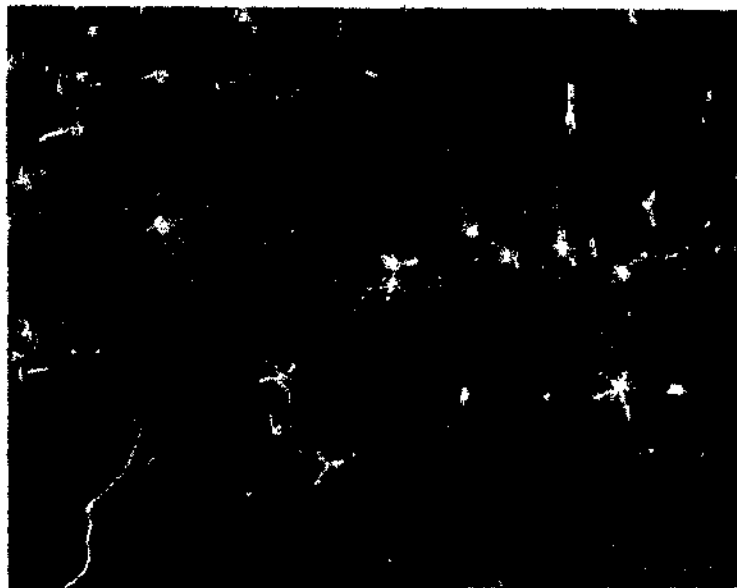
Fig. 2. Apparatus used for testing the biological effectiveness of insecticidal deposits on sprayed panels showing procedure in placing insects on the treated panels, observing them during exposure period, removing insects to holding cage for 24 hours' mortality.

PLATE IV



Typical dendritic DDT crystals from xylene emulsion (above) designated as type I in table I, and (below) flat fibrous or needle-like DDT crystals from deobase solution designated as type II in Table I.

PLATE V



Typical spherulitic DDT crystals from xylene solution (above) designated as type III in Table I. and (below) DDT crystal fragments from water-wettable DDT designated as type V in Table I.

TABLE I.

The rate of crystallization and the morphology of DDT crystals from 5 per cent DDT solutions in various solvents applied at the rate of 100 mg. of DDT per square foot on glass panels by means of a DeVilbiss atomizer. Four replicates of each determination.

Chemical grouping.	Solvent.	Vapour pressure mm. at 25°C. *	CONDITION OF DDT WITH AGE OF FILM ** (WEEKS).					Types of crystal habit.
			1	4	8	12	16 or over.	
Aliphatic hydrocarbon	Ligroin (B.P. 60-90)	§	A	D	D	...	...	II and III.
	Diobase	§	A	D	D	...	...	II.
Aromatic hydrocarbon	Benzene	96.45	A	B	D	D	...	III.
	Xylene	13.92 app.	A	B	C	D	...	III.
	Camene	14.10	A	B	C	C	D	III.
Halogenated carbon. hydro-	Chloroform	100.1	A	B	C	D	...	III and IV.
	Trichlorethylene	73	A	B	C	D	...	III and IV.
	Pentachloroethane	†	A	B	D	...	...	IV.
	Chlorobenzene	12.1	A	D	D	...	...	III.
	Dichlorobenzene	§	A	A	C	C	D	III.
Ketones	Methyl ethyl ketone	99.5	A	D	D	...	...	III.
	Cyclohexanone	§	A	A	C	D	...	III and IV.
	Acetone	229.2	A	A	D	...	...	IV.
Esters	Ethyl acetate	95.7	A	D	D	...	...	I.
Alcohol	Isopropanol	§	A	A	D	C	D	III and IV.
Miscellaneous	Carbon disulfide	366.11	A	D	D	...	...	III.
	Xylene emulsion	§	D	D	D	...	...	I.
	Wettable powder	§	D	D	D	...	...	V.

* Interpolated data given.

** The descriptive categories of film condition are divided as follows: (A) Mostly supersaturated droplets, only few crystals, (B) Approximately equal proportions of supersaturated droplets and crystals, (C) Few supersaturated droplets, mostly crystals, (D) All crystals.

§ The types of crystal habit are described in the text.

§ Data not available.

† Data not available for 25°C. Vapour pressure was 39 mm. at 70°C.

**BIOLOGICAL AND PHYSICAL PROPERTIES OF SUPERSATURATED*
DROPLETS VS. CRYSTALS OF D.D.T.**

(a) *Supersaturated droplets vs. crystals.*—A series of glass panels sprayed with 5 per cent DDT-deobase solution at the rate of 100 mg. of DDT per sq. ft. were tested for biological activity after 0, 1, 5 and 15 days of spraying.

With progressive aging after the first day, the DDT-deobase residual deposits on glass panels showed comparatively increasing biological effectiveness in the case of female mosquitoes although the differences do not appear to be significant (Table II). The surface deposits 1 and 5 days after spraying showed very few crystals, most of the deposits remained in the form of supersaturated droplets, and after exposure to insects, however, most of the D.D.T. crystallized;

TABLE II.*

The time (minutes) required for 50 per cent knockdown and the 24-hour mortality (per cent) of house-flies after 15-minute exposures to deposits of 100 mg. DDT per sq. ft. at various stages of crystallization after application as 5 per cent DDT-deobase solution on glass panels. Results are averages of three replicates.

Age of film (days).	Time required for 50 per cent knockdown (minutes).	24-HOUR MORTALITY (per cent).	
		Male.	Female.
0	5	100	100
1	30	94	70.5
5	45	100	87.5
15	30	100	97

Significant
10 per cent
level only.

* The term supersaturated is used for composition of unknown physical state which may actually be an eutectic mixture, false gel, or supercooled solution rather than a true supersaturated solution.

this was due to mechanical disturbance of the droplets caused by the activity of the test insects.

In another series of tests, two sets of glass panels were sprayed in a similar manner as described above. One set was exposed to insects on the 1st and 5th day after spraying and the other on the 5th and 15th day after spraying. The panels showed comparatively greater biological effectiveness when tested for the second time (Table III). Progressive crystallisation of supersaturated droplets seems to increase the biological effectiveness of D.D.T. surface deposits.

TABLE III.*

The 24-hour mortality (per cent) of house-flies after 15-minute exposures to deposits of 100 mg. DDT per sq. ft. applied as a 5 per cent DDT-deobase solution on glass panels. Both first and second exposures were made on the same panels. Results are averages of three replicates.

Panel.	Age of residue (days).	Cumulative number of exposures to insects.	24-HOUR MORTALITY (per cent).	
			Male.	Female.
A	1	1	94	70.5
	5	2	97.5	78
B	5	1	100	87.5
	15	2	100	97

Significant

Significant

* The data (Tables II and III) was statistically analysed by the formula

$$\chi^2 = \frac{P_1 Q_1 + P_2 Q_2}{100n_1 + 100n_2}$$

where $n_1 = n_2 = 75$

p_1 = percentage for 1st case

$Q_1 = 100 - P_1$

p_2 = percentage for 2nd case

$Q_2 = 100 - P_2$

and it was found that each of the following differences in per cent mortality are significant (5 per cent level)

after day 1 and 5
" " 5 and 15

Explanation for the low toxic activity of supersaturated droplets.—More information was necessary concerning the proportion of DDT in supersaturated droplets. Loss in the weight of a residual application of a 5 per cent DDT-xylene solution was determined after it had dried sufficiently to form such droplets. Glass slides, 2 × 3 inches, were carefully weighed before and after spray application and again after 24 hours. The loss in weight of the spray deposit was found to be as high as 92.2 per cent of the total spray applied (Table IV). On the basis of the low vapour pressure of DDT, this loss may be almost entirely due to the evaporation of xylene, indicating a final mixture of DDT xylene well above the proportions of a saturated solution.

TABLE IV.

*The loss in weight of spray deposits from a 5 per cent DDT solution in xylene during a 24-hour period after application to glass panels.
No DDT crystal formation occurred.*

Initial weight of panel (gm.).	Panel weight immediately after spraying (gm.).	Total weight of spray (gm.).	Panel weight 24 hours after spraying (gm.).	Loss of weight (gm.).	Per cent loss.
11.8485	11.8595	0.0110	11.8495	0.0100	90.9
11.8462	11.8562	0.0100	11.8472	0.0090	90.0
11.0820	11.0930	0.0110	11.0830	0.0100	90.9
10.1120	10.1245	0.0125	10.1130	0.0115	92.0
10.6222	10.6337	0.0115	10.6231	0.0106	92.2
11.1434	11.1500	0.0066*	11.1434	0.0066*	100.0*

* Solvent check consisted of xylene without DDT

Further investigations were made to determine the wetting power for the insect tarsi of supersaturated droplets in comparison to droplets of fresh solution. The wetting power of a liquid for a solid can be measured by the angle which the surface of the liquid makes with the plane of the solid at the point of contact—the contact angle. If the wetting is complete or perfect (for example the tarsus of a fly when dipped in an oil solution), the contact angle is approximately

0°. On the other hand, when the wetting is incomplete (as the tarsus of a fly when dipped in water), the contact angle is finite.

In order to measure the wetting power of droplets at various intervals after spraying, the tarsus of a fly, mounted on an applicator stick attached to a rack-and-pinion device, was lowered or raised so that it was brought into contact with the droplets of DDT-deobase in a normal manner.

The glass panels with droplets were mounted on the horizontal platform of a micro-projector (Pal, 1950) and were so arranged that the droplets approximately bisected the field of a horizontally-mounted microscope objective lens. The enlarged profile of a droplet was projected through the lens and was reflected on an observation plane. Here it was continuously observed, and the contact angle formed between the tarsus and the droplet was measured from the projected profile. With progressive aging of spray droplets for the first 7 days, the wetting ability of the droplets decreased considerably (Table V).

TABLE V.

The contact angle between house-fly tarsi and droplets of 5 per cent DDT solution in deobase at intervals of 1 to 7 days after application on glass panels. Results based on five or more replications.

Age of spray droplets in days.	Advancing contact angle (degrees).
0	0-11
1	12
2	23
3	90
4	Approximately 180°, highly viscous, semi-solid.
5	do
6	do
7	Crystals.

The lower toxic activity of the older supersaturated droplets is, therefore, very likely due to poor contact with the insect cuticle. It should be borne in mind that these data refer to DDT-deobase solution only and cannot be assumed for DDT in other solvents.

(b) *Crystals vs. crystals*.—In order to find out whether or not there was any correlation between type of DDT crystal habit and toxicity, glass panels were sprayed at the rate of 100 mg. of DDT per sq. ft. with 5 per cent DDT emulsion, 5 per cent DDT solution in deobase, in xylene, and in acetone, respectively; and with a 5 per cent DDT suspension from water wettable powder. The glass panels were exposed to a temperature of 100°F. for 1 hour and then were suddenly cooled in an ice box for 15–30 minutes to induce complete crystallization within 1 or 2 days after spraying. The emulsion produced dendritic crystals; acetone solution interspersed masses of both needles and prisms; and water-wettable powder small fragments of crystals. The panels were tested for their toxicity against house-flies. In all cases more than 90 per cent kill was obtained (Table VI); it is therefore not possible to draw any conclusion.

TABLE VI.

The time (minutes) required for 50 per cent knockdown and the 24-hour mortality (per cent) of house-flies after 15-minute exposures to deposits of 100 mg. DDT per sq. ft. having different crystal habits. All formulations contained 5 per cent DDT and were applied on glass panels. Results are averages of five replicates.

Crystal habits.	Formulation.	Time for 50 per cent knockdown (minutes).	24-HOUR MORTALITY (PER CENT).	
			Males.	Females.
Dendritic ...	Xylene emulsion ...	20	100.0	99.9
Flat, fibrous needle-like ...	Deobase solution ...	20	99.9	99.8
Spherulitic ...	Xylene solution ...	20	100.0	99.9
Interspersed mass of both needles and prisms.	Acetone solution ...	25	99.1	87.7
Fragments of crystals ...	Water-wettable powder suspension.	25	100.0	91.1

Hadaway and Barlow (*loc. cit.*) have stated that the inverse relation between particle size and effectiveness is not necessary for crystals that grow on surface treated with DDT solutions. Crystals of DDT grown from solvents on surfaces vary in their orientation with regard to the plane of the surface as well as their size and degree of adhesion.

Further studies were made to determine whether there was any difference in the residual effectiveness of different crystal habits with repeated exposure to test insects. These studies were also designed to obtain information on the effect of normal insect activities on different types of DDT crystals.

Panels sprayed in a manner similar to that described above were divided into four sets. One set of panels was exposed to house-flies only once, a second set was exposed three times, the third six times, and the fourth set sixteen times. In all cases, 50 per cent knockdown time was recorded and careful study made of the effect of normal insect activity on crystal structure. DDT deposits from the xylene emulsion (flat horizontal dendritic crystals) did not deteriorate as fast as DDT deposits from xylene or acetone solutions (spherulitic or crystal fragments Table VII). This may be due to the ease with which the latter types of crystals can be rubbed off. It seems that from the standpoint of persistence and effectiveness, the flat dendritic DDT residual deposits from emulsions are the most desirable on glass surfaces.

The effect of insect activity on crystals may be briefly summed up as follows:—

1-2 Exposures : The majority of supersaturated droplets crystallized due to the mechanical agitation caused by the activities of test insects. Crystals were intact at the end of the second exposure.

3-6 Exposures : The upright crystals were badly damaged and were broken down into small fragments, but the identity of each crystal base was well marked.

Subsequent exposures : The crystals were broken down into very small fragments and empty places appeared on the glass plate. The insecticidal deposits were covered by the excreta and body films of the test insects.

The glass panels, which had been exposed to house-flies for sixteen times and had ceased to give satisfactory knockdown, were resprayed with acetone at the rate of 6 c.c. per sq. ft. There was a slight reactivation of the surface deposit as measured by the knockdown time, but the deposit did not approach freshly sprayed panels in knockdown effectiveness.

DISCUSSION.

Relatively few papers have dealt with the factors governing the formation of an effective surface deposit of DDT, although experience with insecticides in general has shown that its physical state may greatly influence its biological effectiveness. For example, the reluctance of DDT deposits to crystallize for a prolonged period of time seems to be an important factor governing the formation of an effective surface deposit.

TABLE VII.

The average exposure time (minutes) for 50 per cent knockdown of house-flies with DDT deposits showing different types of crystal habits. All formulations contained 5 per cent DDT and were applied at the rate of 100 mg. DDT per sq. ft.

Crystal habitus.	Solvent.	Exposure time (minutes).																						
		Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17*	18*	19*			
Spherulitic	..	Xylene	..	20	20	25	25	35	45	60	45	60	120	120	120	120	more than 240	more than 240	more than 240	more than 240	more than 240			
Flat fibrous needle-like.	..	Decolase	..	20	20	25	25	25	45	120	120	180	more than 240 minutes									more than 240		
Fracture	..	Dust	..	20	20	20	25	45	45	60	120	120	120	240	240	more than 240				more than 240				
Dendritic	..	Xylene emulsion.	..	20	25	25	25	35	45	45	45	45	45	45	45	45	45	45	60	120	120			
Interpenetrated masses of both needles and prisms.	..	Benzene	..	20	25	25	25	35	45	60	60	60	60	60	60	60	60	60	60	120	more than 240			

*Test panses sprayed with pure acetone at the rate of 6 c.c. per sq. ft.

DDT-deobase solution is very effective immediately after spraying but after evaporation of the volatile constituents of the spray, the DDT in the supersaturated droplets left on the treated surface is not as effective. But if these droplets are induced to crystallize, the effectiveness of the surface deposit increases again though not to same degree as freshly sprayed surfaces. The process of crystallization in droplets can be induced by mechanical agitation, such as stirring with a needle, or the disturbance caused by the normal activities of test insects, and also by sudden changes in temperature conditions.

Parkin and Green (*loc. cit.*) were the first to call attention to the above phenomenon. But the biological ineffectiveness of the DDT in supersaturated droplets presented a difficult problem to interpret in the light of known facts of insect physiology. Hoskins (1948) asked, 'How a crystal can be more effective than a supersaturated solution? One might imagine that a liquid would make contact with a larger area of the insect surface and hence penetrate in larger amounts.'

As the supersaturated droplets appeared to be highly viscous, it was believed that poor contact and poor wetting was the reason for their low activity, and observation of the angle of contact of these droplets to the tarsi of house-flies confirmed such an assumption.

Low activity of older supersaturated droplets is, therefore, very likely due to poor contact. However, when DDT in these droplets is induced to crystallize, the insects have improved contact with the insecticide, and very often some of the crystal fragments adhere to the bodies of insects and may, therefore, become effective.

The surface deposit with different crystal habits, however, did not seem to deteriorate at the same rate or in the same manner on repeated exposure to insects. Deterioration depended upon the ease or difficulty with which the crystals can be broken, dislodged, or rubbed off. It seems that, from the standpoint of persistence of effectiveness, the horizontal dendritic crystals from DDT-xylene emulsions are more desirable on glass surfaces.

The effect of crystal size and shape on the biological efficacy of insecticides is especially important in malaria control when mosquitoes have to pick up and carry away a toxic dose of insecticide after a relatively short period of contact with the treated surface. Moreover, the physical state of deposits from various formulations of insecticides has to be considered in relation to the nature of the treated surfaces as the amount of insecticide available to any insect will largely depend on this factor. On absorptive surfaces, for example, oil is absorbed initially in the surface layers and is not readily available to an insect for contact on the surface. Subsequently crystallization occurs and a greater quantity of insecticide is available to and detachable by insects and it is thus progressive crystallization that increases the biological effectiveness of the deposits. Emulsions behave similarly as oil solutions except for their better adherence to the surface owing to the presence of stickers and emulsifiers. Hadaway and Barlow (*loc. cit.*) and others have shown that when aqueous suspensions of insecticides are sprayed on to absorptive surfaces, such as mud plaster, the wetting agent solution penetrates into the material and leaves the solid particles

of insecticides freely available on the surface, and therefore aqueous suspensions of insecticides are more toxic on absorptive surfaces than oil solutions or emulsions. On non-absorptive surfaces on the other hand the wetting agents used in dispensing suspension of insecticides may stick down the particles and render them less available to the insects than on absorptive materials, hence aqueous suspensions are less effective on glass than on plaster. Crystals of DDT grown from solvents vary in their orientation with regard to the plane of the surface as well as their size and degree of adhesion. By using dispersible powders, however, desired crystal size can be obtained as the crystals can be ground to the required size to start with.

SUMMARY.

The relationship between the physical state and insecticidal action of DDT when applied as a residual spray has been discussed. It has been shown that the physical state of an insecticide may greatly influence its biological effectiveness, particularly when the deposit is available in the form of supersaturated droplets. DDT seems to undergo deterioration at different rates when applied in different solvents, which may be due to both physical and chemical factors.

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RAPID LOSS OF BIOLOGICAL EFFECTIVENESS OF D.D.T. APPLIED TO MUD SURFACES.

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INTRODUCTION.

WORKERS from different parts of the world have reported rapid loss of biological effectiveness of D.D.T. deposits in contact with mud surfaces. This is a serious practical problem and may have far-reaching repercussions on the application of D.D.T. in malaria control, particularly in countries where houses are generally constructed of mud.

Clapp *et al.* (1947) using panels made from an alluvial clay, Sundararaman and Pefly (1949) using panels made from clay-cowdung mixtures, Kruse and Konchady (1950) using plain clay with a surface coating of starch, cowdung, cowdung and carbon, and Hadaway and Barlow (1951) using mud blocks made from 'Murram' (a lateritic ironstone), have noted that D.D.T. rapidly loses its effectiveness on such surfaces.

Muirhead-Thomson (1947) in Africa, Downs *et al.* (1948) in Mexico, Maier *et al.* (1948) in Venezuela, Gaud *et al.* (1948) in Morocco, Puri (1947) Viswanathan and Gadre (1950) and Jaswant Singh *et al.* (1951) in India have observed loss of D.D.T. activity in a period of few weeks under field conditions.

The cause or causes responsible for inactivation of D.D.T. deposits applied on mud surfaces still remain to be investigated. One possible explanation has been offered by Hadaway and Barlow (1951; 1952) who suggest that the solid particles of insecticides are sorbed into inner layers of mud surfaces and are not available to insects on the surface.

Downs *et al.* (1951) are of the opinion that D.D.T. undergoes chemical deterioration due to the presence of various metallic salts in mud, such as

anhydrous ferric oxide, anhydrous ferric aluminium and chromium chloride and alkaline earth materials such as calcium carbonates.*

Bordas *et al.* (1952) point out a positive correlation between the iron oxide content of the soil and its capacity to inactivate D.D.T. deposits.† Soils with higher iron oxide content showed the highest rate of inactivation.

Hadaway and Barlow (*loc. cit.*), however, maintain that 'sorption and not catalytic decomposition is responsible for the rapid loss in effectiveness of insecticides at room temperature as is shown by the recovery of chemically unchanged D.D.T. below the surface and by the persistent fumigant effect of gamma B.H.C. long after the disappearance of surface deposits. The reaction of catalytic decomposition of D.D.T. at 130°C. does, however, provide a rapid method of foretelling the duration of effectiveness on a particular soil sample.'

In order to prevent such rapid sorption, tests have been made by applying a preliminary coating of other materials, for example, whitewash (Maier *et al.*, *loc. cit.*, and Hadjinicolaou and Busvine, 1948); whitewash and salt (Clapp *et al.*, *loc. cit.*) and urea formaldehyde resins (Bracey, as quoted by Symes 1951), and normal solution of phosphate (Macs, 1952) on the surface. The problem of rapid loss of biological effectiveness of D.D.T. demands the development of practical methods to prevent sorption and subsequent decomposition.

Under field conditions, rapid loss of biological effectiveness of D.D.T. may take place due to a variety of other factors, i.e. mechanical removal (peeling off), volatilization, catalytic action of sunlight, temperature and moisture‡, coverage by dust, smoke, etc. The problem is thus not simple and requires careful investigation.

The present paper reports studies on the biological inactivation of D.D.T. deposits in contact with mud surfaces in different parts of India and Afghanistan. The iron and carbonate contents of the scrapings from sprayed surfaces have also been determined.

INACTIVATION OF D.D.T. DEPOSITS.

First indication of rapid loss of biological effectiveness of D.D.T. deposits came from the laboratory observations. House-flies were exposed to mud panels sprayed with D.D.T. suspension at the rate of 50, 100 and 200 mg. D.D.T./sq. ft., by the technique described by Pal (1951). The insects were exposed to the treated panels for 15 minutes and then kept under observation for 24 hours after which the mortality was recorded. It was observed that mortality amongst the test insects was 50 per cent or more for a period of 3, 5 and 9 days respectively when the amount of D.D.T. deposited was 50, 100 and 200 mg. D.D.T. per sq. ft. The rate of loss of biological effectiveness was found to be proportionate to the dosage applied (Table I).

*Fleck and Haller (1944) showed earlier that Fe and Ca catalyse the decomposition of D.D.T.

†This catalytic decomposition according to Bordas *et al.* (*loc. cit.*) however, is arrested at some stage when technical grade D. D. T. is used instead of pure parapara isomer. They hold the view that this is probably due to the production of substances which block the catalytic action of iron. This observation, however, has not been confirmed (Coyne, 1952).

‡According to Hadaway and Barlow (*loc. cit.*) not only the process of sorption is more rapid when the atmosphere is dry, the decomposition of D. D. T. is also rapid.

Biological results were further confirmed by chemical tests. Scrapings from a measured portion ($25 \times 25 \times 1.3$ mm.) of the treated surfaces were chemically analysed by the Alessandrini method (Alessandrini, 1948) for the amount of D.D.T. present in them (Table II).

Analysis of the data collected has shown that after 11 weeks of application, no D.D.T. could be detected even in the scrapings removed from surfaces treated with 200 mg./sq. ft. Deposits obtained from emulsions appeared to deteriorate more rapidly than deposits obtained from suspensions. It was also found that D.D.T. at massive dosages (200 mg. D.D.T. emulsion) deteriorated on an average of 69.2 and 76.8 per cent during the 1st and 2nd weeks respectively after application, while with smaller dosages during the corresponding period, deterioration was comparatively less (45.8 and 56.7 per cent).

FIELD OBSERVATIONS.

Similar experiments were repeated in the field. A number of villages were sprayed with D.D.T. emulsion and suspension at the rate of 50 mg./sq. ft. Both biological and chemical assaying of deposits on the wall surfaces were undertaken. The former was carried out in the field by exposing standard laboratory bred ♀♀ *Culex fatigans* to the treated surfaces, by 'Wall Cage Technique' (Simmons *et al.*, 1945), and also by 'making total collections' of mosquitoes from the sprayed rooms at regular intervals. Simultaneously chemical assaying was carried out at regular intervals by testing scrapings from sprayed wall surfaces by the Alessandrini method to determine their D.D.T. content.

(a) *Biological tests.*—The insects were exposed to the treated surface for a period of 15 minutes and were then kept under observation for 24 hours after which the mortality was recorded. Twenty-four hours' mortality amongst the test insects was observed to be less than 50 per cent after four weeks of application of D.D.T. (Table III). The density of mosquitoes also showed a gradual rise after 4 weeks' period (Table IV), which corroborated the wall cage tests. It appears that under field conditions D.D.T. deposits are effective for about 4 weeks only in the area under observation.

(b) *Chemical tests.*—The results of chemical estimation of D.D.T. were, however, erratic. Minute quantities of D.D.T. were detected 26-32 weeks after application. Moreover, in consecutive weekly estimations, there were considerable variations, and at times after negative findings, positive reactions were obtained*.

*The Alessandrini method of chemical estimation of D. D. T. has been stated to give erratic results particularly when the samples are contaminated by straw and cowdung. Moreover, the reproducibility of the tests depends upon the quality of the reagents employed, particularly fuming nitric acid. It was observed that best results with this method were obtained when the amount of D. D. T. ranged from 0.8 to 2.0 mg. The maximum amount of D. D. T. recovered was about 70 per cent and minimum about 30 per cent. by this method. Thus there may be considerable variations in the estimations, particularly under field conditions. Known quantities of D. D. T. were mixed with mud scrapings and were tested for the amount of D. D. T. present in them. The results are summarised in the foot-note on page 254.

CHEMICAL ANALYSIS OF SCRAPINGS FROM MADHYA PRADESH, ORISSA AND AFGHANISTAN.

Scrapings received from Madhya Pradesh and Orissa were also analysed (Tables VI and VII). In samples from Madhya Pradesh (Central India), D.D.T. was detected up to 13 weeks, but after four weeks it was less than 10 mg./sq. ft. In samples from Cuttack, Orissa (South East India), D.D.T. was detected for 20 weeks or more, however, in this case the amount of D.D.T. deposited was 100 mg./sq. ft.

In Afghanistan, in some localities D.D.T. was detected only for about five weeks whereas in other places it was detected for 20 weeks or more (Table VIII).

CHEMICAL ANALYSIS OF SCRAPINGS FOR IRON OXIDE.

The scrapings from experimental villages around Delhi were also analysed for iron oxide, alkali carbonate and alkaline earth carbonate (Table IX) and in practically all cases iron oxide contents were more than 4 per cent. According to Bordas *et al.* (*loc. cit.*) the expected duration of activity of D.D.T. in such surfaces should be less than three months. Possibly the total quantities of iron oxide in soils as well as the manner in which it is physically dispersed are important factors in the inactivation of D.D.T.

Amount of technical D.D.T. actually taken.	Amount of technical D.D.T. estimated by Alessandrini method (average of 5 samples).	Percentage of recovery.
mg.	mg.	
0.1	0.03	30.0
0.2	0.11	55.0
0.4	0.15	37.5
0.6	0.35	58.3
0.8*	0.55	68.5
1.0*	0.65	65.0
2.0*	1.4	70.0
3.0	1.7	56.6
4.0	2.3	57.5
5.0	2.5	50.0

* Best results within range of 0.8 to 2.0.

DISCUSSION.

Contradictory results have been reported from different parts of the world regarding the duration of residual effectiveness of D.D.T. when applied on wall surfaces. This may be attributed to a variety of factors pertaining to the habits of the vector species, the nature of treated surfaces, environmental conditions and the formulations and dosages used. The present discussion is, however, confined only to the inactivation of D.D.T. in contact with mud surfaces.

The present paper provides further evidence of rapid loss of D.D.T. activity when applied to mud walls. It happens irrespective of the formulation or dosage employed. The evidence is based on both biological and chemical tests. Biological tests indicate whether or not the insecticide is available on the surface and the chemical tests indicate whether or not chemical decomposition takes place in the inner surface layers when sorption has taken place. For chemical estimation of D.D.T., scrapings are removed from treated surfaces up to a depth of 3 mm. This being the case, it should be theoretically possible to recover the full amount of D.D.T. applied on the surface soon after application. The results of analysis of scrapings, however, show that at no time more than 50 to 60 per cent of the D.D.T. was recovered even on the date of application (Day 0). A portion of initial loss of the order of 40 to 50 per cent may be due to the fact that part of the theoretical dosage sprayed did not reach or adhere to the walls. The results of analysis of scrapings, further show (Tables I and II) that biological effectiveness of a deposit of 50 mg. D.D.T. per sq. ft. suspension falls steadily from day 1 to day 7 and is very low on the 7th day but the change in the percentage of D.D.T. recovered is only from 55 to 43 per cent by the 13th day (in Table V, 50.2 per cent is recoverable on the 12th day). These results indicate that D.D.T. still present in the scrapings on the 13th day is not biologically active against mosquitoes on the surface.

Moreover, Downs *et al.* (*loc. cit.*) have shown that D.D.T. does not undergo decomposition at room temperature but only at elevated temperature of 130°C.* Any decomposition would be slow at room temperature and would not account for the rapid loss within a few days of surface deposits of D.D.T. suspension. In addition, decomposition may be preceded by sorption which accounts for the loss of biological effectiveness on mud surfaces.

That decomposition of D.D.T. also takes place, would be obvious from the data presented (Tables II, V, VI, VII, VIII) but the rate of decomposition seems to vary in different localities:

Delhi	..	..	11 weeks or more.
Madhya Pradesh (Central India)	..	..	13 weeks.
Cuttack (Orissa, S.E. India)	..	..	20 weeks.
Afghanistan: in some localities	..	..	5 weeks
in other localities	..	..	20 weeks or more.

Except for Delhi, analysis of soils for iron oxide contents are not available. However, as Bordas *et al.* (*loc. cit.*) have stated since there is a fairly close correlation between iron oxide contents of the soil and its capacity to inactivate D.D.T. deposits, the 'catalytic decomposition test' may be used in routine survey of soils.

*Fleck and Haller test (*loc. cit.*)

It seems to be clear that two factors are at play in the inactivation of D.D.T. on mud surfaces; one is the sorption of D.D.T. into the surface layers of mud, and second is the chemical decomposition of D.D.T. due to the presence of iron oxide or other metallic salts in the soil. Adequate data are not yet available to determine whether these take place simultaneously or successively, nor is there adequate data to establish the correlation between the iron oxide contents of the soil and the sorption of D.D.T. Moreover, what physical or chemical factors in the mud determine the rate and degree of sorption, is not clear.

The practical implication in malaria control, therefore, seems to be to prevent the sorption of D.D.T. into sprayed surfaces, particularly those of mud. This may perhaps be achieved by either giving a preliminary coating of other material on such surfaces or by incorporating such materials in the spray itself. This presents a different aspect of the problem, which forms the subject of a further communication.

SUMMARY.

The paper provides further evidence of the rapid loss of biological effectiveness of D.D.T. deposits in contact with mud surfaces. The rapid loss of biological effectiveness of D.D.T. is very marked but there does not appear to be sufficient evidence that this loss is due to decomposition of D.D.T. alone. Moreover, decomposition would have to be preceded by sorption which is probably the main cause of the loss of biological effectiveness. The results of chemical analysis of residual deposits have been compared with the iron and carbonate contents of soils.

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TABLE I.

2½ hours' mortality percentage in flies after 15 minutes' exposure to mud panels treated with D.D.T. suspension at different dosages.

Age in days.	24 HOURS' PERCENTAGE MORTALITY.		
	50 mg. D.D.T./sq. ft.	100 mg. D.D.T./sq. ft.	200 mg. D.D.T./sq. ft.
1	91.0	99.0	99.0
2	62.4	80.0	91.2
3	60.8	72.0	89.6
4	35.2	65.0	85.6
5	28.8	61.2	75.6
6	24.0	22.4	72.2
7	9.6	19.2	82.4
8	6.4	8.4	67.2
9	10.8	22.4	51.8

TABLE II.
Chemical estimation of D.D.T. (mg./sq. ft.) in scrapings removed from mud
surfaces treated with D.D.T.

Age in days.	50 mg. D.D.T./sq. ft. (suspension).	Recovery percentage.	Age in days.	200 mg. D.D.T./sq. ft. (suspension).	Recovery percentage.	Age in days.	50 mg. D.D.T./sq. ft. (emulsion).	Recovery percentage.	Age in days.	200 mg. D.D.T./sq. ft. (emulsion).	Recovery percentage.
0	27.5	55.0	0	127.5	63.7	0	26.6	53.2	0	133.2	66.6
5	20.1	40.2	7	49.6	24.8	7	14.4	28.8	7	41.0	20.5
13	21.6	43.2	14	47.5	23.7	14	11.5	23.0	12	30.9	15.4
19	11.5	23.0	17	34.5	17.2	21	0.0	0.0	19	30.9	15.4
26	10.8	21.6	23	33.8	16.9	28	0.0	0.0	25	29.5	14.7
33	7.2	14.4	30	32.4	16.2	35	0.0	0.0	32	6.5	3.2
	Resprayed						Resprayed				
0	28.5	57.0	37	25.2	12.6	0	25.2	50.4	40	4.3	2.1
6	28.0	56.0	43	13.6	6.8	7	16.5	33.0	46	3.6	1.8
13	25.0	50.0	50	7.2	3.6	14	7.9	15.8	53	0.0	0.0
18	14.4	28.8	57	7.2	3.6	19	0.0	0.0	58	0.0	0.0
24	12.9	25.8	60	0.0	0.0	25	0.0	0.0	64	0.0	0.0
33	3.6	7.2	67	0.0	0.0	34	0.0	0.0	73	0.0	0.0
38	0.0	0.0	72	0.0	0.0	39	0.0	0.0	78	0.0	0.0

TABLE III.

24 hours' mortality percentage in Culex fatigans after 15 minutes' exposure to wall surfaces sprayed with different formulations of D.D.T.

Age in Weeks.	D.D.T. 50 mg./sq. ft. suspension.	D.D.T. 50 mg./sq. ft. emulsion.
1	75	95
2	100	75
3	100	65
4	50	80
5	30	25
6	35	15
7	25	20
8	10	...

TABLE IV.

Weekly percentage density of mosquitoes as compared to the density at the start of the experiment in the case of villages treated with D.D.T. suspension and emulsion 50 mg./sq. ft.

Weeks.	Percentage mosquito density. (Suspension)	Percentage mosquito density. (Emulsion)
0	100.0	100.0
1	7.1	0.0
2	6.1	2.09
3	4.2	7.09
4	7.7	8.7
5	24.6	14.6
6	50.0	13.06
7	20.6*	37.0

* Natural decline in the mosquito population.

TABLE V.
Field Trials—1951-52.

Chemical estimation of D.D.T. in scrapings removed from mud walls treated with D.D.T.

50 mg. D.D.T./sq. ft. (suspension).			50 mg. D.D.T./sq. ft. (emulsion).		
Age in days.	D.D.T. recovery.	Recovery percentage.	Age in days.	D.D.T. recovery.	Recovery percentage.
0	36.0	72.0	0	25.9	51.8
12	25.1	50.2	11	28.7	57.4
23	19.2	38.4	22	30.6	61.2
30	14.4	28.8	45	10.0	20.0
43	7.2	14.4	51	12.9	25.8
56	10.8	39.6	63	23.7	47.4
79	5.1	10.2	72	6.4	12.8
87	15.8	31.6	79	8.6	17.2
97	0.7/18.7	1.4/37.4	86	17.2	34.4
105	10.7	21.4	104	17.9	35.8
112	18.0	36.0	109	18.7	37.4
125	17.3	34.6	118	14.4	28.8
130	11.5	23.0	127	8.4	16.8
139	14.4	28.8	133	5.7	11.4
148	20.1	40.2	140	0.0	0.0
156	14.4	28.8	146	10.8	21.6
163	5.1	10.2	153	4.3	8.6
169	9.2	18.4	159	5.7	11.4
176	7.9	15.8	166	10.2	20.4
183	5.0	10.0	173	3.6	7.2
190	10.9	21.0	181	0.0	0.0
197	5.9	11.8	187	4.5	9.0
205	14.4	28.8	193	0.0	0.0
211	10.1	20.2	200	0.0	0.0
218	17.3	34.6	207	1.4	2.8
225	2.9	5.8	222	0.0	0.0
232	4.3	8.6			
247	0.0	0.0			

TABLE VI.

Field Trials—Madhya Pradesh (Jalgaon)—1951-52.

Chemical estimation of D.D.T. (mg./sq. ft.) in scrapings removed from mud walls treated with D.D.T. (50 mg. D.D.T. suspension/sq. ft.)

Age in days.	D.D.T. recovery percentage.
1	2.9
7	11.5
13	6.5
19	14.4
26	10.8
33	3.6
52	6.5
54	6.5
61	1.4
73	3.6
75	3.6
81	2.9

TABLE VII.

Field Trials—Cuttack—1951-52.

Chemical estimation of D.D.T. (mg./sq. ft.) in scrapings removed from mud surfaces treated with D.D.T. (100 mg. emulsion/sq. ft.)

JAIPUR.		CHANDANPUR.		TALPATNA.		ARMEDPUR.	
Age in days.	D.D.T. recovery percentage.	Age in days.	D.D.T. recovery percentage.	Age in days.	D.D.T. recovery percentage.	Age in days.	D.D.T. recovery percentage.
36	24.5	29	17.8	9	21.6	11	26.1
43	23.0	38	27.7	16	27.4	20	15.8
50	24.5	45	17.3	23	30.2	26	22.2
57	14.4	52	15.8	37	13.0	34	18.7
64	15.4	59	...	44	20.2	46	...
71	13.0	83	18.5	51	18.7	65	8.1
78	30.0	90	...	58	24.4	72	3.6
92	10.1	94	20.2	65	23.0	80	27.3
99	8.6	105	17.6	73	...	87	13.4
113	0.0	108	15.9	79	23.0	94	12.9

TABLE VIII.
Field Trials—Afghanistan—1950-51.
Chemical estimation of D.D.T. (mg./sq. ft.) in scrapings removed from mud walls
treated with D.D.T.

UTMENZAI.	KUNDUZ.	NAKHJUN	CHARTOOR.	KHANABAD.	TALUKAN	SHORATTU.	KARGAZ.
D.D.T. re- covered. Age in covered. days.	D.D.T. re- covered. Age in covered. days.	D.D.T. re- covered. Age in covered. days.	D.D.T. re- covered. Age in covered. days.	D.D.T. re- covered. Age in covered. days.	D.D.T. re- covered. Age in covered. days.	D.D.T. re- covered. Age in covered. days.	D.D.T. re- covered. Age in covered. days.
D.D.T. 1320 de- posited. meter.	889 mg/sq. meter.	1530 mg/sq. meter.	830 mg/sq. meter.	2100 mg/sq. meter.	1900 mg/sq. meter.	2100 mg/sq. meter.	2100 mg/sq. meter.
15 573	14 171	13 726	39 615	16 792	69 315	58 587	38 418
29 488	17 26	36 436	45 378	21 814	96 362	85 422	66 244
(average of 6)	18 40	51 257	(average of 18 samples)	29 568	144 249	123 422	113 1012
	29 79	(average of 6 samples)		36 627	(average of 3 samples)	(average of 3 samples)	(average of 3 samples)
	34 0			44 576			
	(average of 3-6 samples)			52 502			
				59 448			
				61 669			
				89 402			
				76 402			
				(average of 6-9 samples)			

TABLE IX.
Chemical analysis of scrapings from mud walls.

Village.	Fe ₂ O ₃ .	Alkaline earth carbonate (as CaCO ₃ .)
Sarai ...	5.28	3.86
Khatola ...	5.03	0.75
Ashanpur ...	4.97	3.87
Ratipur ...	4.84	2.75
Raipar ...	5.28	0.83
Dhundra ...	0.21	0.34
Softa ...	4.79	1.26
Allahpur ...	5.38	1.12
Meghpur ...	4.36	3.49
Kakrali ...	3.92	0.77
Behraula ...	5.34	4.90
Atohan ...	4.87	3.32
Miranpur ...	6.51	4.46
Khusropur ...	6.32	3.90
Kushipur ...	3.73	0.41
Dholagarh ...	5.77	4.73

No alkali carbonate was detected in all cases.

ANALYSIS OF RESIDUAL DEPOSITS OF D.D.T. ON SPRAYED SURFACES

BY

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[July 10, 1952.]

INTRODUCTION.

As part of the routine activities of the Malaria Organization in Bombay State, several samples of scrapings from sprayed surfaces in the different areas, where malaria control with the use of D.D.T. as an indoor residual spray is in progress, were analysed in the laboratory, employing Alessandrini's (1948) method, slightly modified by Gadre as recorded by Viswanathan and Gadre (1950). This paper records the results of such analysis.

RESULTS OF ANALYSIS OF RESIDUAL DEPOSITS.

Tables I and II show the results of analysis of scrapings from surfaces treated with D.D.T. emulsions and wettable powders in approximate dosages of 56 mg./sq. ft., 112 mg./sq. ft. and 168 mg./sq. ft. in either formulation. Emulsions were applied in a concentration of 5 per cent and wettable powders in 2.5 per cent.

As a routine field practice in this organization, one gallon of a 5 per cent emulsion is sprayed over 4,000 sq. feet to secure a deposit of 56 mg./sq. ft. and this is repeated twice or thrice over if dosages of 112 and 168 mg./sq. ft. respectively have to be applied. In the case of wettable powders the suspension has generally a concentration of 2.5 per cent D.D.T. In this case, therefore, one gallon is sprayed over 2,000 sq. ft. to secure 56 mg./sq. ft. deposit and the spraying is repeated twice to secure 112 mg./sq. ft. and thrice for 168 mg./sq. ft. This procedure gives the best approximation between the dosage practically achieved and theoretically contemplated. By this procedure the speed with which the labourer walks and operates the pumps is kept constant.

TABLE I.

Analysis of scrapings from surfaces in successive weeks after initial application of 5 per cent D.D.T. emulsion in different dosages.

Number of weeks after initial application.	INITIAL DOSAGE.		
	56 mg./sq. ft.	112 mg./sq. ft.	168 mg./sq. ft.
1	45.1 (63)	71.8 (45)	102.6 (27)
2	33.6 (72)	49.2 (109)	69.2 (27)
3	27.5 (45)	39.2 (27)	53.8 (27)
4	18.1 (90)	29.9 (171)	40.2 (27)
5	19.8 (45)	20.5 (54)	28.5 (27)
6	11.8 (99)	19.0 (117)	24.5 (27)
7	10.4 (45)	16.8 (27)	21.7 (27)
8	8.3 (90)	14.9 (53)	19.1 (27)
9	7.3 (45)	14.1 (27)	17.4 (27)
10	5.2 (90)	13.7 (90)	16.6 (27)
11	6.2 (45)	11.8 (27)	14.2 (27)
12	4.6 (36)	10.5 (81)	13.1 (27)
13	4.6 (27)	10.0 (27)	11.7 (27)
14	2.9 (81)	7.2 (81)	10.9 (27)
15	4.0 (18)	8.1 (27)	9.8 (27)
16	...	6.6 (54)	10.4 (18)
17	...	7.4 (18)	9.6 (18)
18	...	5.6 (27)	8.6 (18)
19	...	6.4 (18)	7.8 (18)
20	...	4.1 (27)	7.0 (18)
21	...	4.6 (18)	7.2 (18)
22	...	4.8 (18)	6.4 (18)
23	...	3.8 (18)	5.6 (18)
24	...	3.8 (18)	4.6 (18)

Note :—Figures in brackets represent the number of samples analysed and those preceding them the average values expressed as milligrammes D. D. T. per sq. ft.

TABLE II.

Analysis of scrapings from surfaces in successive weeks after initial spray with 2.5 per cent D.D.T.-wetable powders in different dosages.

Period after initial appli- cation in weeks.	INITIAL DOSAGE.		
	56 mg./sq. ft.	112 mg./sq. ft.	168 mg./sq. ft.
1	41.6	67.2	104.0
2	24.8	45.6	64.0
3	24.0	32.8	51.2
4	17.6	25.6	38.4
5	11.2	18.4	29.6
6	8.8	16.8	22.4
7	8.0	14.4	19.2
8	6.4	13.6	17.2
9	5.6	11.6	15.6
10	5.2	11.2	13.2
11	4.4	9.2	12.4
12	4.0	8.8	11.0
13	3.2	8.8	9.2
14	...	7.8	8.0
15	...	6.4	7.2

Note :—The above figures represent the average of 9 samples analysed on each occasion.

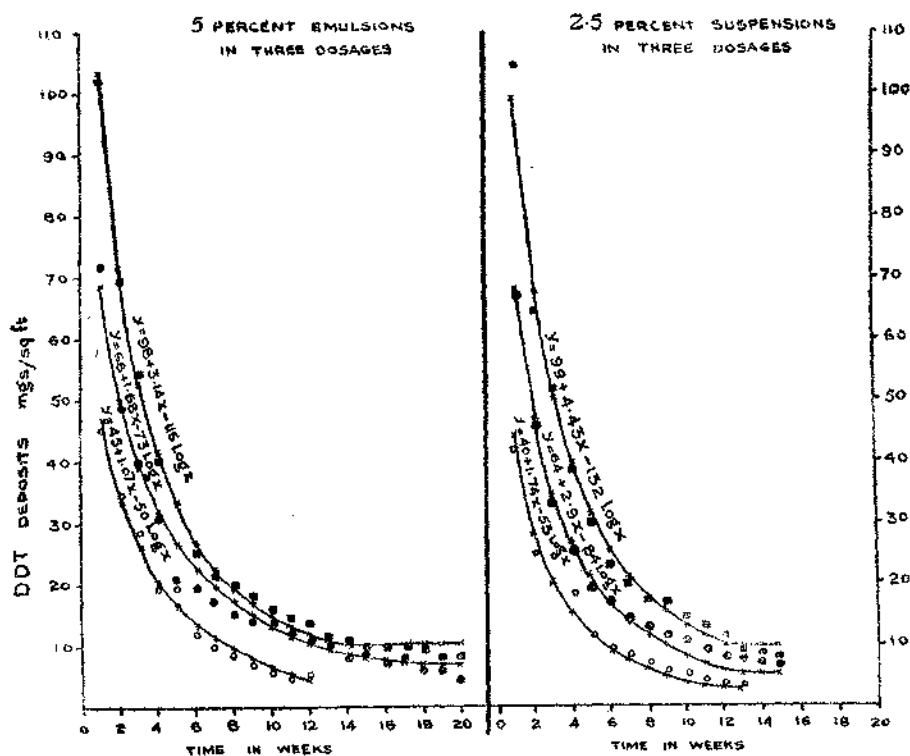
The data presented in this paper refer to 2,926 samples of scrapings from surfaces sprayed with D.D.T. emulsions and 387 samples of scrapings from surfaces treated with wettable powders. The samples were collected from distant districts like Ahmedabad, West Khandesh and Thana. The uniformity of the results of analysis within the limits of sampling variations suggests that, in practice, this organization presumably secures a fair degree of approximation between intended dosages and the dosages actually applied assuming that Alessandrini's test is properly applied. The author is, however, aware of inconstant results obtained by some workers in India.

The data presented in Tables I and II are also shown graphically. A straight line cannot obviously fit the data. A little scrutiny would show that a

quadratic parabola would not fit the data either. Apart from the greater of deterioration in the earlier weeks there is a tendency for the curve to flatten out later. On account of these two characteristics a curve in accordance with the formula $y = a + bx - c \log x$ is fitted to the above data. In each of the six series of observations, (Chart 1) such a curve shows a close fit to the observed

CHART 1
DETERIORATION OF DDT
FROM SPRAYED SURFACES

x CALCULATED VALUES
o OBSERVED VALUES 56 mgs/sq ft
● ----- DO ----- 112 " "
■ ----- DO ----- 168 " "



data. The formulae ($y = a + bx - c \log x$) determining the residual deposits of D.D.T. are solved below separately for D.D.T. emulsions and wettable powders applied in different dosages:

Initial dosage.	D.D.T. emulsions.	D.D.T. Wettable powders.
56 mg./sq. ft.	$y = 45 + 1.07x - 50 \log x$;	$49 + 1.74x - 53 \log x$
112 mg./sq. ft.	$y = 68 + 1.68x - 73 \log x$;	$64 + 2.9x - 84 \log x$
168 mg./sq. ft.	$y = 98 + 3.14x - 116 \log x$;	$99 + 4.43x - 132 \log x$

Note:—In each case $a + b$ represents the residual D.D.T. one week after the application of the specified dose.

The most conspicuous feature of all the curves which run more or less parallel is that the rate of deterioration is proportional to the mass of D.D.T. present. In every series of observations the coefficient b is significantly different from 0 and from each other in the case of the larger dosages, the values for b being 1.07 ± 0.45 , 1.68 ± 0.11 and 3.14 ± 0.07 in the case of emulsions and 1.74 ± 0.25 , 2.89 ± 0.11 and 4.4 ± 0.14 in the case of wettable powders for each of the 3 doses applied in either formulation. Similar values for c are 50 ± 4.9 and 53 ± 3.1 for a dosage of 56 mg./sq. ft. of emulsion and wettable powder respectively, 73 ± 5.8 and 84 ± 4.6 in the case of 112 mg./sq. ft. and 116 ± 3.7 and 132 ± 5.8 in the case of 168 mg./sq. ft. Neither the coefficient c nor the factor c/a is significantly different for a specified dosage of either formulation. The consistent and significantly higher values of b (which is positive) in the case of wettable powders than in emulsions in the case of the two larger dosages would seem to betoken a slightly higher residual deposit of D.D.T. in the case of wettable powders with larger dosages. But the recorded observations actually show a slight bias in favour of emulsions especially in the smaller dosage. While, in the experience here recorded, there is no definite evidence of the superiority of either formulation, the general pattern of the equations for the residual deposit is not inconsistent with a slight superiority of wettable powders especially with the larger dosages.

The analysed data further show that, whatever the formulation, larger dosages of 168 mg./sq. ft. yield comparable residual deposits for 4 weeks more, and 112 mg./sq. ft. for two weeks more than 56 mg./sq. ft. This difference is not adequate to justify the adoption of the larger doses with our relative costs of insecticide and labour.

Viswanathan and Gadre (*loc. cit.*) showed that in Ahmedabad District in Bombay State there was no evidence that a single massive application of 168 mg./sq. ft. of D.D.T. would be attended with adequate malaria control judging from the well-known indices of malariometry such as spleen rates, parasite rates among infants, morbidity figures, etc. In a limited experience (unpublished) in West Khandesh District the author found that a single application of such a large dosage was successful in some talukas in one year but not in neighbouring talukas where a second application of the insecticide was found necessary. On the whole, three rounds of application of 56 mg./sq. ft. were found quite adequate to effectively control malaria transmitted by *A. fluviatilis* or *A. culicifacies*, the extent of control of the much higher degree of endemic malaria transmitted by the former species being far more spectacular and immediate than in the latter case. In some regions and in some years when the monsoon terminates earlier than normal, two rounds of 56 mg./sq. ft. have been adequate to establish full control in areas where *culicifacies* is the vector. The author assumed, on the evidence of limited simultaneous records of Anopheles

densities, that differences in the degree of malaria control when a single large dosage was applied were primarily related to differences in the duration of the prevalence of vector species above the critical densities. It was found such differences were of no moment if three rounds of application of 56 mg./sq. ft. were made. This practice has accordingly been consistently advocated and adopted throughout Bombay State and as a measure of economy the third round is suspended during a year in which the monsoon ends earlier than usual. In West Khandesh District, however, experiments are being carried out again with a single large dosage of 168 mg./sq. ft. Though in 1951, the spray had to be repeated a second time two months later due to the reappearance of *culicifacies* in large numbers in certain areas the experiment is again being repeated in July-August 1952 with a view to watch results during a second season more especially because of the reported efficacy in the neighbouring State of Mysore of a single dosage of 200 mg./sq. ft. for as long as a year (Rao, 1952).

Hadaway and Barlow (1951) offer an interesting explanation for the conflicting results reported in field trials against malarial vectors in different parts of the world. They attribute these discrepancies mainly to differences in the nature of the sprayed surfaces. They report that crystals of all insecticides rapidly disappear from the surface of mud blocks made of 'murrum' at 25°C., crystals of D.D.T. being visible only for a few days. The larger the particle size the longer the deposits persist and in any given size range, D.D.T. crystals disappear more rapidly than gamma B.H.C. Along with the disappearance of the crystals from the surface, the mud blocks lose their toxicity to mosquitoes. But in the case of gamma B.H.C. the blocks were effective for a considerable time after disappearance of crystals, which they attribute to the fumigant effect of the volatile insecticide inside the block. They thus describe a process of sorption in the first instance and of desorption which occurs in the case of B.H.C. but not in the case of D.D.T. In their view under dry conditions the sorbed D.D.T. was further catalytically decomposed to the ethylene derivative when the mud used has a high iron content. The process of sorption according to them is more rapid when the atmosphere is dry.

Downs, Bordas and Navarro (1951) have also shown that iron in the soil plays a much more important rôle than aluminium in catalysing any decomposition of D.D.T. The same authors (1952) have observed that different types of adobe and mud surfaces retained the activity of D.D.T. for different periods of time. In a further note they accept the conclusions of Hadaway and Barlow with regard to the phenomenon of sorption. In their chemical tests they were able to establish a correlation between the iron oxide content of the soils and their capacity to inactivate the D.D.T. deposits. This catalytic decomposition, however, is arrested at some stage when technical grade D.D.T. is used instead of the pure para para isomer. They hold that such an arrest is probably due to the production of a substance that blocks the catalytic action of iron. They have devised elaborate chemical tests with standard samples of technical grade D.D.T. and standard samples of soils by which one can predict safely the expected period of residual efficacy of D.D.T. on a sprayed surface of a given composition.

In the light of the work of the authors quoted above, the first question that one should address himself is whether the phenomenon of sorption and subsequent catalytic decomposition of D.D.T. takes place in Bombay State and if it does

whether it exhibits variations in different surfaces. The uniformity of the results, earlier recorded, of the analysis of scrapings made through a depth of 3 mm., whatever the initial dosages or formulations may be, would seem to indicate that there is no material variation in the degree of D.D.T. lost through the process of sorption up to a depth of 3 mm. All the D.D.T. residual deposit in a depth of 3 mm. may not, doubtless, be available for insecticidal action on the surface. Hence, while there is no data to show whether or not the phenomenon of sorption occurs, it cannot be assumed that all the D.D.T. that is determined as residual deposit in the analysis has effective insecticidal action on the surface. But, if, subsequent to sorption, catalytic decomposition takes place within a depth of 3 mm., the results show either that there is no such catalytic decomposition or that the degree of such decomposition is more or less uniform in the different districts in which samples were collected for analysis. This is also consistent with the fairly uniform results of malaria control achieved in the different districts with the application of three doses of 56 mg./sq. ft.

The second question for consideration is whether the variation noticed in mosquito densities and in the degree of malaria reduction obtained after a single large application of 168 mg./sq. ft. is due to variations in the phenomenon of sorption and subsequent catalytic decomposition of D.D.T. Downs *et al.* (*loc. cit.*) claim that catalytic decomposition is arrested in the case of D.D.T. technical either on account of impurities or on account of some substance which is produced during the process of decomposition. Is it possible that only the large dosages are attended with widely varying catalytic decomposition in different surfaces but with smaller dosages the degree of such catalytic decomposition is small and hence variations in different surfaces are masked?

The third question is if there is no variation in the degree of catalytic decomposition of D.D.T. in various parts of Bombay State how can one explain the differences in the residual effects of a single large application in different parts of Bombay State? It was previously assumed that such variations betoken a natural difference in the maintenance of critical densities of vector species during the malaria season. Hadaway and Barlow (*loc. cit.*) offer a further explanation that some of the good results obtained with the application of D.D.T. on mud surfaces may be due solely to the efficacy of D.D.T. sprayed on the roofs. In the course of the present series of observations the author found after the application of a single large dosage of 168 mg./sq. ft. many of the reappearing mosquitoes rested on the walls but comparatively a few on the roofs. While the author was then unaware of the phenomenon of sorption since described it was concluded that the reappearance of mosquitoes on walls and their comparative scarcity on the roofs was due to the practice of washing the walls with mud plaster subsequent to the insecticidal application. Thus, in one series of observations where the walls were plastered with mud after insecticidal application the density of *culicifacies* was 98 on the walls and 1 on the roof per 10 man-hours. In neighbouring houses where the walls were not plastered after the insecticidal application the density was 30 on the walls and 22 on the roof. These collections were made at different periods after the insecticidal application and are not, therefore, strictly comparable. It was, however, concluded that it would be extremely difficult to change the age-long habits of the people with respect to renovation of walls, etc., and it would, therefore, be more

expedient to resort to repeated application of smaller dosages during the malaria season rather than to depend upon a single large application. It is possible that the uniformly successful results by way of malaria control obtained by the author in different parts in Bombay State are due solely to the efficacy of the insecticide remaining on the roofs as stated by Hadaway and Barlow. In that case it is not understood why a single large application, which will persist on the roofs unaffected either by the phenomenon of sorption or the process of coverage with mud plaster which occurs on the walls, should not also give uniform results in different districts?

Deterioration of D.D.T. from sprayed surfaces may be due to absorption of material through the depth of sprayed surfaces along with solvent when a solution or an emulsion is used, sorption and subsequent catalytic decomposition whatever formulations are used, mechanical removal by insects, wind action, etc., coverage by dust, smoke, etc., "weathering" or degradation due to atmospheric action such as temperature, radiation, etc. The majority of houses in Bombay State consist of mud walls though the making of bricks out of mud in the first instance is not resorted to in many cases. Yet in the authors' experience no indisputable evidence to show the superiority of wettable powders over emulsions was ever found. It must, however, be added that the wettable powders used by the authors were formulated in this country and, while in recent times there have been considerable improvements, they do not yet come up to the standard of the formulations made abroad with regard to their suspensibility and range of particle size. It had, therefore, been concluded that either there is a very little loss by absorption into the depth of the sprayed walls when an emulsion is applied or that such loss is no worse than the inherent disability in the use of locally available wettable powders with their inconsistent physical properties as regards suspensibility and uniformity of particle size. A more valid consideration with the author, however, has been the relative cheapness in cost of an emulsion made in bulk as compared to a wettable powder purchased in the market. While still further improvements in the formulation of a wettable powder within the country might conceivably make its use more acceptable there is still no evidence to show that a single large dose of either formulation should be preferred to two or three applications of a smaller dose.

SUMMARY.

The results of analysis of 3,311 samples of scrapings from surfaces sprayed with D.D.T. emulsions and wettable powders in three different dosages of 56, 112 and 168 mg./sq. ft. are presented. The rate of deterioration of D.D.T. is shown to be proportional to the mass of D.D.T. present whether it was applied initially or whether it represents the residue of an earlier larger application. The difference in the period of persistence of comparable deposits is longer by 4 weeks between 168 mg./sq. ft. and 56 mg./sq. ft. and by 2 weeks between 112 mg./sq. ft. and 56 mg./sq. ft. There is no material difference in the rate of deterioration with emulsions or wettable powders. The results of analysis of residual deposits are discussed in the light of the recent work on the phenomenon of sorption and subsequent catalytic decomposition of D.D.T. in the sprayed surfaces.

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MICROBIOASSAY METHOD FOR D.D.T.

BY

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AND

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[August 30, 1952.]

INTRODUCTION.

In malaria control programmes besides entomological and epidemiological data, periodic determination of the amount of D.D.T. present on sprayed surfaces provides a reliable index of the duration of residual effectiveness of insecticides and for drawing up of schedules for reapplication of insecticides. Of all the chemical methods employed for D.D.T. estimation, the Alessandrini method (Alessandrini, 1948) has proved useful under field conditions at the hands of public health workers with limited experience in chemical analytical techniques. Results of this method, however, can only be considered in a qualitative rather than quantitative way because the test is not meant for precise determination of D.D.T. Furthermore, the method gives erratic results particularly when the scrapings obtained from sprayed surfaces are contaminated with organic matter such as straw and cow dung commonly used for plastering of walls in rural dwellings in India.

Laug (1946 : 1948) carried out estimations of D.D.T. and B.H.C. gamma isomer by bioassay methods. He compared the mortality in the housefly *M. domestica* exposed to tissue extracts contaminated with insecticides to a set of known standards. Furman and Hoskins (1948), Dahm and Pankaskie (1949), Hoskins and Messenger (1950) and Sun and Sun (1952) adopted similar methods for determination of insecticides by using houseflies. Schiavi and Proenca (1948) employed mosquito larvae to detect small traces of D.D.T. dusted off treated walls by cotton wool plugs. Nolan and Wilcoxon (1950) and Bushland (1951) used *A. aegypti* larvae for estimation of parathion in plants and for testing the insecticidal residues in animal products respectively.

Studies were undertaken to assess the scope and limitations of bioassay technique for D.D.T. determination in scrapings obtained from sprayed surfaces. It was hoped that bioassay method might be substituted for chemical tests.

CHOICE OF TEST INSECTS.

Preliminary experiments were carried out on anopheline, culicine and *Aedes* larvae. *A. aegypti* larvae gave consistent results throughout; whereas results

with *C. fatigans* and *A. stephensi* were not satisfactory. *A. aegypti* larvæ were, therefore, chosen as suitable test insects.

STANDARD DOSAGE MORTALITY CURVE.

The mortality in mosquito larvæ with known D.D.T. concentrations in water was determined in the first instance. The quantities of D.D.T. tested were 0.0025, 0.005, 0.0075, 0.01, 0.0125 and 0.015 mg. D.D.T. per 100 c.c. of water, because preliminary tests had shown that the larval mortality at these concentrations of D.D.T. was less than 100 per cent. D.D.T. dilutions were prepared by mixing an alcoholic solution of D.D.T. (0.01 per cent) with water. Comparative tests were also carried out by making serial dilutions of absolute alcohol without D.D.T. in water. Ten early 4th instar larvæ were used for each test. Five tests were set up simultaneously. The mortality amongst the larvæ was recorded after 24 hours. Larvæ which pupated during the period of observation were not considered in calculating the mortality rate. Ten such replicate experiments were performed and a standard dosage mortality curve was drawn (Table I, Chart J). There was practically no larval mortality in alcoholic dilutions. Larval mortality at dosages of 0.0025 to 0.01 mg. D.D.T. per 100 c.c. of water progressively increased with higher dosages, but at concentrations varying from 0.01 to 0.015 mg. D.D.T. per 100 c.c. of water there was no appreciable difference in larval mortality.

TABLE I.

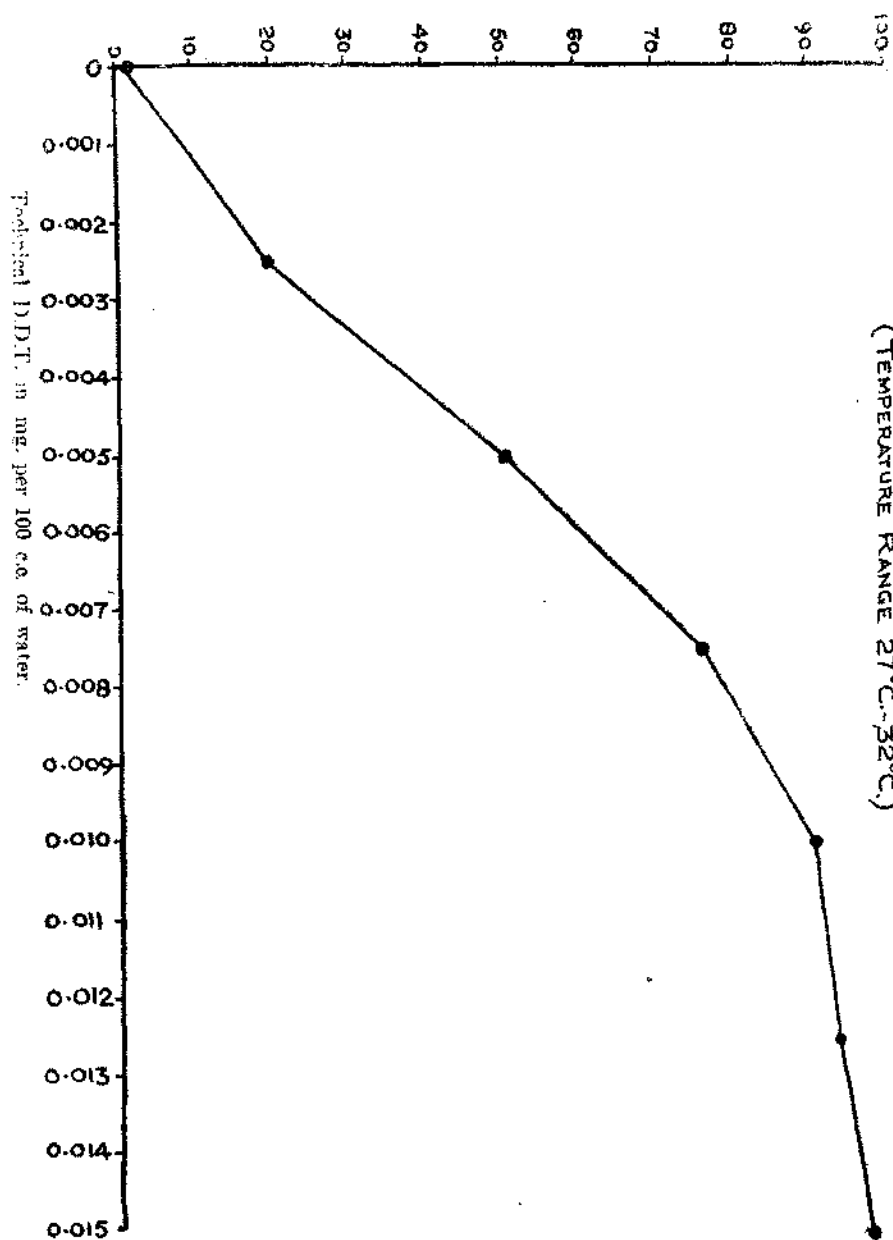
Percentage larval mortality with different concentrations of D.D.T.

D.D.T. technical mg./per 100 c.c. of water.	Percentage larval mortality.										Average.
	1	2	3	4	5	6	7	8	9	10	
0.0025	6.2	18.4	18.0	34.8	20.0	8.5	17.0	19.1	38.0	14.5	19.4 ± 19.3
0.005	50.0	51.1	56.0	64.2	53.0	27.5	52.0	64.0	38.9	56.2	51.3 ± 20.9
0.0075	78.7	82.9	92.0	86.6	66.0	76.5	64.5	80.0	66.6	80.0	77.3 ± 18.8
0.01	97.9	97.8	98.0	91.3	88.0	95.7	79.5	97.5	87.5	90.0	92.3 ± 12.6
0.0125	100.0	97.9	98.0	94.0	88.0	96.0	95.9	100.0	99.0	100.0	96.0 ± 7.0
0.015	100.0	100.0	100.0	100.0	100.0	100.0	98.0	100.0	100.0	100.0	99.8 ± 3.8

N. B.—In the standard dosage mortality curve dosages above 0.015, and below 0.0025 mg. per 100 c.c. of water have been omitted because the larval mortality is either 100 per cent or negligible (0-5 per cent).

ESTIMATION OF D.D.T. IN SCRAPINGS.

In order to assess the accuracy of bioassay technique, samples of scrapings were obtained from measured untreated portions (25×25×1-3. mm.) of well

24 hours percentage mortality in *Aedes aegypti* larvae.

surfaces and known quantities of D.D.T. varying from 0.25 to 1.0 mg.* were mixed with the scrapings.

The amount of D.D.T. present in the scrapings was estimated both by the Alessandrini method and by the bioassay technique.

D.D.T. from the scrapings was extracted with absolute alcohol. Each scraping was thoroughly washed with alcohol at least 3 times during 24 hours. The final volume of the extract was made up to 10 c.c. in each case.

Serial dilutions of D.D.T. alcoholic extract were prepared by adding 0.025, 0.05, 0.1 and 0.2 c.c. per 100 c.c. of water to give D.D.T. concentrations varying from 0.000625 to 0.02 mg. per 100 c.c. of water (Table II). Comparative studies

TABLE II.

Different serial dilutions of D.D.T. used for bioassay tests.

Maximum amount of D.D.T. deposit per sq. ft. (mg.)	Amount of D.D.T. per scraping (25 × 25 × 1-3 mm.)	D.D.T. mixed with scrapings, and extracted with 10 c.c. of alcohol (mg.)	Alcoholic extract per 100 c.c. of water (c.c.)	D.D.T. per 100 c.c. of water (mg.)
1	2	3	4	5
50	1/36 × 50 mg. = 1.38 mg.	1.0	0.025	0.0025
			0.05	0.005
			0.1	0.01
			0.2	0.02
		0.50	0.025	0.00125
			0.05	0.0025
			0.1	0.005
			0.2	0.01
		0.25	0.025	0.000625
			0.05	0.00125
			0.1	0.0025
			0.2	0.005

For calculating the amount of D.D.T. per scraping, the set of figures in Column 5 are multiplied by 400, 200, 100 and 50 respectively depending upon the amount of D.D.T. alcoholic extract used for serial dilutions.

*The amounts of D.D.T. were calculated on the assumption that the maximum amount of D.D.T. which may be present in a scraping obtained from sprayed surfaces in the field would be about 1 mg. per scraping. In India the dosage of D.D.T. applied for malaria control is 50 mg. per sq. ft. and is repeated after an interval of about six weeks. As the scrapings are obtained from measured area equivalent to 1/36th of a sq. ft., the amount of D.D.T. in the scraping may be about 50/36 mg. or 1.3 mg.

were carried out by making serial dilutions of absolute alcohol. Ten early 4th instar larvæ of *A. aegypti* were introduced in each case and the larval mortality was noted after 24 hours.

The amount of D.D.T. present in the scrapings was determined by interpolating the larval mortality thus obtained in the standard curve.

The estimations of D.D.T. by bioassay and the Alessandrini methods are given in Table III. It would be evident that D.D.T. estimations by bioassay

TABLE III.

Actual amounts of D.D.T. present in scrapings and the amount of D.D.T. as estimated by bioassay method from the standard dosage mortality curve, and by the Alessandrini method.

Serial number.	D.D.T. actually present in scraping (mg.).	D.D.T. estimated by bioassay method (average of 5 scrapings.) (mg.).	Difference between (2) and (3).	D.D.T. estimation by Alessandrini method (average of 5 scrapings.)	Difference between (2) and (5).
1	2	3	4	5	6
1	0.25	0.24	- 0.01	0.10	- 0.15
	0.5	1.33	+ 0.83	0.16	- 0.34
	1.0	1.84	+ 0.84	0.5	- 0.5
2	0.25	0.46	+ 0.21	0.12	- 0.13
	0.5	0.64	+ 0.14	0.18	- 0.32
	1.0	1.84	+ 0.84	0.48	- 0.52
3	0.25	0.48	+ 0.23	0.18	- 0.07
	0.5	1.19	+ 0.69	0.22	- 0.28
	1.0	1.71	+ 0.71	0.62	- 0.38
4	0.25	0.32	+ 0.07	0.2	- 0.05
	0.5	0.64	+ 0.14	0.24	- 0.26
	1.0	1.12	+ 0.12	0.6	- 0.4
5	0.25	0.41	+ 0.16	0.12	- 0.13
	0.5	1.01	+ 0.51	0.3	- 0.2
	1.0	1.66	+ 0.66	0.6	- 0.4

method were in excess of the amount of D.D.T. actually present; on the other hand with the Alessandrini method, the amount of D.D.T. was less than the actual amounts. Both these methods do not give satisfactory results if precise quantitative estimation of D.D.T. is required but may be used as routine methods for determination of D.D.T. in the field.

In order to eliminate any error which may be resulting due to biological or other environmental factors, tests on known amounts of D.D.T. may be repeated simultaneously each day and a standard dosage mortality curve drawn for comparison. The bioassay technique appears to be comparatively more satisfactory than the chemical tests, because in the latter slight variations in the reagents may give divergent results.

SUMMARY.

A micro-bioassay method for the estimation of D.D.T. in scrapings obtained from sprayed surfaces has been described.

A. aegypti larvæ were used for these tests.

The method compares favourably with the Alessandrini chemical method for estimation of D.D.T.

Both the tests are suitable as routine methods for the estimation of D.D.T. in the field.

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BEHAVIOUR OF MOSQUITOES IN RELATION TO INSECTICIDAL APPLICATIONS.

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INTRODUCTION.

CONSIDERABLE studies on the behaviour of mosquitoes have been conducted in different parts of the world from time to time. Bates has admirably summarized the results in his book 'Natural history of mosquitoes' (1949). The object behind all these investigations was to discover efficient and economical methods for the control of mosquitoes and mosquito-borne diseases. Some of the aspects which have engaged attention are whether the attack should be directed at larval or adult stage, and the place and time of attack for achieving a maximum effect with a minimum expenditure. The control efforts so far have been confined to engineering methods, extermination or abatement of breeding through biological or naturalistic methods or through insecticides by the use of chemicals to kill the aquatic stages of mosquitoes or their adults.

Systematic spray-killing of adult mosquitoes with insecticides has revolutionized the control methods. Not only has it proved the most effective and practical method but also economical for use in rural areas, with the result that the residual insecticides such as D.D.T. and B.H.C, completely dominate the field of malaria control, although use of pyrethrum originally played an important rôle in the development of the present method of attack.

In the past, control measures were mainly directed against anopheline larvæ, necessitating attention on larval breeding habits and limiting observations on infectivity rate, etc., of adults. The target of attack during the subsequent years having, however, shifted to adult mosquitoes, detailed studies were undertaken to determine the behaviour of mosquitoes in relation to insecticidal applications, particularly pyrethrum sprays. The results have been most valuable in interpreting present-day work on the residual insecticides, but the present-

day knowledge of the behaviour of mosquitoes in relation to control by residual insecticides is still elementary.

Muirhead-Thomson, has not only stressed the importance of such a study, but has provided a comprehensive review of the work on this subject in his book 'Mosquito behaviour' (1951).

Of late, some malariologists have held the view that if application of residual insecticides is sufficiently thorough and widespread, it is bound to control malaria in any locality irrespective of the species of Anopheles concerned or their behaviour. They feel, to quote Muirhead-Thomson, 'That there is no point in wasting time on the preliminaries or in allowing treatment to be withheld pending the accumulation of more exact studies on the vector mosquitoes' . . . 'That this attitude has been justified in some instances is evident from the success which has followed widespread treatment in some countries such as that against *darlingi* in the coastlands of British Guiana, as well as in Amazonia. On the other hand, there are parts of the tropics where large-scale treatment of houses has produced disappointing results in the reduction of malaria'.

It is on the basis of such observations that Muirhead-Thomson and others have been led to conclude, not without justification, that mere absence or reduction in *resting* mosquitoes in houses does not by itself constitute a sufficient proof that the mosquitoes which enter treated houses are killed, and that the probability is that despite the treatment, the mosquitoes may be obtaining a blood meal and disseminating malaria in the houses. In the circumstances it is highly imperative that the behaviour of mosquitoes in relation to control by *residual* insecticides should be studied more closely to establish control schemes on solid foundations. It is natural for malaria control organizations before embarking on any large-scale control scheme to seek advice regarding the insecticide and formulation to be used, the dosage to be applied and the frequency of treatment. In order to answer these questions preliminary tests have to be carried out in the laboratory and in the field, for which exact appraisal of the mosquito behaviour in houses is absolutely essential. An attempt has been made in this paper to investigate some of the basic aspects of problems concerned in governing the interaction of mosquitoes and dry deposits of insecticides on wall surfaces.

THEORETICAL CONSIDERATIONS.

Any attempt to interpret the behaviour of mosquitoes in relation to the application of insecticides presupposes knowledge of the mosquito behaviour in general. It may be found helpful to carry out simultaneous observations in an identical locality where no insecticide has been applied.

In theory, the application of residual insecticides should be completely effective against anophelines which rest indoors during the day time, but only partially so against those frequently human habitations to obtain a blood meal.

The contact between the resting or house frequenting mosquitoes and the treated surface may be disturbed in the case of insecticides likely to exert an 'excito-repellent' effect on the mosquitoes, or in the case of unusually sensitive species.

Even those anophelines which feed indoors but do not rest there by day spend quite a time in contact with the indoor treated surfaces during the hours of darkness before and/or after feeding. In such circumstances their resting places are not restricted as is the case with some of the domestic mosquitoes; for example, *Anopheles culicifacies*, one of the principal vector species in India, on which the present observations are based, shows marked preference for hanging clothes, umbrellas, furniture, firewood, etc., while *A. minimus* usually rests low down in the rooms up to a height of 2 to 3 ft. In order to achieve successful control, it is not only important to know whether a species rests outdoors or indoors but also the exact nature of its daytime resting places if it is a domestic species. Differences in reaction among different domestic species may be explained by a knowledge regarding these details.

NORMAL BEHAVIOUR.

The following activities may be listed during a gonotrophic cycle of an individual, keeping in mind the longevity of the mosquitoes and density of the mosquito population.

Adult mosquito may emerge during the day or at night, and on emergence rest outdoors for a short while and then the females enter a house for a blood meal. It may be assumed that it obtains a blood meal late at night and rests indoors throughout the following day. Assuming again that the gonotrophic cycle is completed in about 48 hours, it would be ready to oviposit the following night and would move out at dusk to lay eggs. After oviposition it may return and obtain another blood meal the same night and repeat this performance for about 2 weeks which may be regarded as the normal expectancy of life of a mosquito under favourable conditions (Fig. 1.). In other words the movements of domestic species are restricted to dusk when ovipositing female mosquitoes leave the houses to deposit eggs and before dawn when the mosquitoes return for feeding and resting purposes.

How such behaviour is affected by application of residual insecticides indoors has been investigated in respect of *A. culicifacies* and other mosquitoes. Experiments were carried out in a group of villages near Palwal, District Gurgaon, Punjab (India), about 36 miles south of Delhi. This area is known to be highly malarious and epidemiology of disease in this district is similar to the rural areas of Delhi State where *A. culicifacies* is the vector. In Delhi State, D.D.T. indoor residual spray at a dosage of 50 mg./sq. ft. has been found efficacious for a minimum period of 6 weeks in keeping down the density of *A. culicifacies* and in intercepting the transmission of malaria. No antimalaria measures had been taken for combating the disease in this district previously and the spleen rates ranged from 34 to 54 per cent.

Majority of the houses in the experimental villages are poorly built and 'kutchha' with mud plastered walls and thatched roofs. The ceiling is generally about 10 ft. high and floors plastered in the same manner as walls. Most of the houses are without windows but may contain one or two entrances with or without doors. The number of houses, total population and the insecticide applied in each village is shown in Table I. All structures, whether human

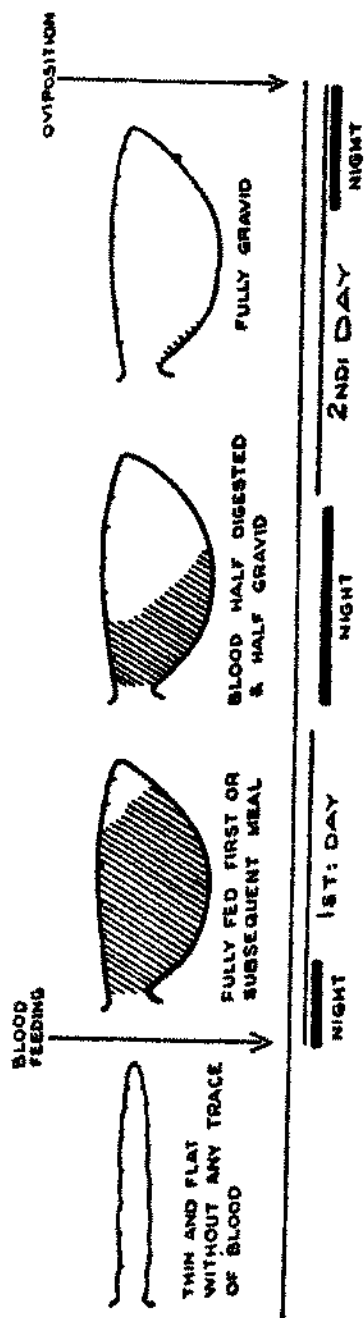


Fig. 1.—Showing the gonotrophic cycle and abdominal stages.

TABLE I.

Particulars of the experimental villages in which routine night catches, total mosquito catch and window trap mosquito collections were made.

Village.	Number of houses.	Population.	Total area sprayed, sq. ft.	Insecticide and formulation used.	Dosage mg./sq. ft.
Kharvapur	102	511	Comparison village.		
Mirampur	49	313	58,988	2.5 per cent D.D.T. emulsion	50
Kakrali	34	204	36,006	do. do.	200
Meghpur	24	120	18,575	2.5 per cent D.D.T. suspension	50
Dholagari	26	101	12,492	do. do.	200
Bahrana	78	429	55,071	0.5 per cent B.H.C. gamma isomer suspension.	10

dwellings or cattlesheds, were treated. Inner surfaces of walls and as much of the low roof as could be easily reached, were sprayed.

DATA COLLECTED.

In each village a number of catching stations, both sprayed and unsprayed, were established. The unsprayed catching stations served as comparison; besides, an entire village (unsprayed) was kept under observation as control.

Observations were started 2 to 3 weeks before insecticidal applications. The following data were recorded:

(a) *Density of mosquitoes per week*.—(i) By hand catch for 15 minutes and (ii) By making a total catch of mosquitoes after spraying a weak solution of pyrethrum and collecting the dead mosquitoes on a sheet spread on the floor.

(b) *Weekly night catches from 6 p.m. to 6 a.m. after every 2 hours*.—Mosquito catches were recorded as:

(a) *A. culicifacies*.

(b) Other anophelines.

A record was also kept as to whether or not blood meal had been taken, the condition of the ovaries and age of mosquitoes.

The classification adopted was as follows:—

UF	Unfed.	Abdomen thin without trace of blood, freshly hatched or those returning after ovipositing for a subsequent blood feed.
FF	Fully fed.	Abdomen distended with fresh blood irrespective of the ovarian development.
HG.	Blood half digested. Half gravid.	Abdomen showing blood and developing eggs, each roughly occupying half the space.
G	Blood completely digested. Gravid.	Fully gravid, abdomen distended with mature ovaries.

The observations regarding time of entry and feeding of mosquitoes, and their crepuscular movements, discussed in this paper pertain only to the comparison and the (D.D.T.) treated village (Miranpur).

FINDINGS.

(A) ALL SPECIES ANOPHELINE MOSQUITOES.

(i) *Density of mosquitoes.*—The houses treated with D.D.T. invariably showed a striking reduction both in the daytime and night catches of anophelines. This was, as it should be, as the entire village was systematically treated with D.D.T. Majority of the adult mosquitoes were killed which greatly reduced breeding and emergence of new broods of adult mosquitoes. The fall in the number of mosquitoes caught in treated huts may also be due to the repellent, excito-repellent or lethal effect of the treatment.

(ii) *Time of entry.*—The most striking point which emerges from this study is that the proportion of mosquitoes caught from the comparison and treated huts at different hours of the night is more or less similar. The data furnished in Table II and Appendices I, II and III show that about 41 per cent of mosquitoes entered the huts before midnight and 53 per cent after midnight in both the cases. The proportion that entered at 6 p.m. was the highest. Collections made at 6 a.m. closely followed 6 p.m. collections. From 8 p.m. to 4 a.m. the mosquito catches were more or less evenly distributed. The treatment did not seem to affect the entry of mosquitoes in the huts nor did it appear to affect their movement or behaviour.

TABLE II.

Percentage mosquitoes caught in comparison and treated huts at different hours of the night.

Huts.		6 p.m.	8 p.m.	10 p.m.	12 midnight.	2 a.m.	4 a.m.	6 a.m.
Comparison	...	27.5	11.0	7.9	5.6	5.5	9.4	32.7
Treated hut	...	25.8	5.2	6.5	10.0	8.4	8.7	35.1

(iii) *Time of feeding and crepuscular movement.*—Catches of adult mosquitoes were further analysed (Table III). Highest number of unfed mosquitoes were caught at 6 p.m. in both the untreated and treated houses. Very high proportion of fed mosquitoes were encountered after 8 p.m. and the residual treatment did not appear to affect the feeding activity of mosquitoes. Probably the feeding activity commences after 8 p.m. and continues throughout the night. Comparatively higher proportion of half gravid and gravid mosquitoes were found during the early part of the night (6 p.m.).

TABLE III.

Percentage unfed, fully fed, half gravid and gravid mosquitoes caught in comparison and treated huts at different hours of the night.

Huts.		6 p.m.	8 p.m.	10 p.m.	12 midnight.	2 a.m.	4 a.m.	6 a.m.
Comparison	UF	25.2	13.7	4.4	5.0	1.9	3.5	12.7
	FF	8.1	50.2	73.2	73.1	64.9	31.0	57.2
	HG	37.0	25.9	16.8	18.4	20.3	15.5	16.8
	G	28.6	10.1	3.3	3.3	12.7	0.0	13.1
Treated hut	UF	19.4	11.1	0.0	0.0	0.0	8.8	6.5
	FF	10.3	77.7	84.0	84.6	76.0	82.3	77.0
	HG	10.2	0.0	0.0	0.3	23.0	8.8	11.1
	G	20.8	11.1	0.0	0.0	0.0	0.0	4.9

UF = Unfed.

FF = Fully fed.

HG = Half gravid.

G = Gravid.

(B) *A. CULICIFACIES*.

In Tables IV and V and Appendices IV, V and VI, data on *A. culicifacies* alone has been analysed. The number of *A. culicifacies* caught was comparatively very low and formed about 10 to 14 per cent of the catches of all species of anopheline mosquitoes. The other prevalent anopheline species were *A. subpictus*, *A. annularis* and *A. stephensi*. The behaviour of mosquitoes in the comparison and treated huts appears to be identical except 8 p.m. and 6 a.m. catches. In the comparison village, 22.5 per cent of mosquitoes entered the hut at 8 p.m., whereas only 7.6 per cent entered the treated hut. At 6 a.m., the percentage of mosquitoes caught in the comparison village was 27.7 and in the treated huts 58.9. The results are based on comparatively very small samples of *A. culicifacies* caught and it is not justified to draw any conclusions.

TABLE IV.

Percentage A. culicifacies caught in comparison and treated huts at different hours of the night.

Huts.		6 p.m.	8 p.m.	10 p.m.	12 midnight.	2 a.m.	4 a.m.	6 a.m.
Comparison	...	17.0	22.5	13.6	5.7	4.6	7.5	27.7
Treated hut	...	15.1	7.6	0.0	2.5	2.5	12.5	58.9

Unfortunately the number of *A. culicifacies* was so small that the data regarding the proportion of unfed, fully fed, half gravid and gravid mosquitoes does not give any clear idea of the feeding activity and movements of this species of mosquitoes.

TABLE V.

Percentage unfed, fully fed, half gravid and gravid mosquitoes caught in comparison and treated huts at different hours of the night.

Huts.		6 p.m.	8 p.m.	10 p.m.	12 midnight.	2 a.m.	4 a.m.	6 a.m.
Comparison	UF	3.7	15.3	8.6	0.0	0.0	0.0	0.0
	FF	18.5	33.8	69.5	66.6	37.6	76.9	76.1
	HG	66.6	50.6	21.7	33.3	62.5	23.0	23.8
	G	11.7	1.1	0.0	0.0	0.0	0.0	0.0
Treated hut	UF	0.0	0.0	0.0	0.0	0.0	0.0	5.0
	FF	0.0	100.0	0.0	0.0	100.0	50.0	80.0
	HG	10.0	0.0	0.0	100.0	0.0	0.0	15.0
	G	50.0	0.0	0.0	0.0	0.0	50.0	0.0

UF = Unfed.

FF = Fully fed.

HG = Half gravid.

G = Gravid.

Field studies of Hocking (1947) on *A. gambiae* and *A. funestus* also showed that there was no interference with the normal entry of mosquitoes into the D.D.T.-treated huts or with their resting habits. The scientific study of these problems is, however, still at preliminary stages but more critical study may help to throw some further light on the problem. Releasing mosquitoes dusted with printers' dust or fluorescent powder and watching their behaviour and movements in and out of huts may possibly yield more comprehensive data.

EXCITO REPELLENT EFFECT OF D.D.T. AND B.H.C. ON MOSQUITOES.

Data on the excito-repellent effect of D.D.T. and B.H.C. on mosquitoes was also collected. The villages were sprayed with D.D.T.-aromex-soap emulsion, D.D.T. suspension in water* and with B.H.C. suspension** (Table I). The dosage employed was 50 mg. D.D.T. and 10 mg. gamma isomer per sq. ft. In

*The emulsion was prepared at the Institute according to the formula described by Puri and Pal (1947) and the suspension from 'Geigy malaria spray' 50 per cent water dispersible powder.

**The B.H.C. suspension was prepared from Imperial Chemical Industries' 'Gammaxene' p. 520 water dispersible powder.

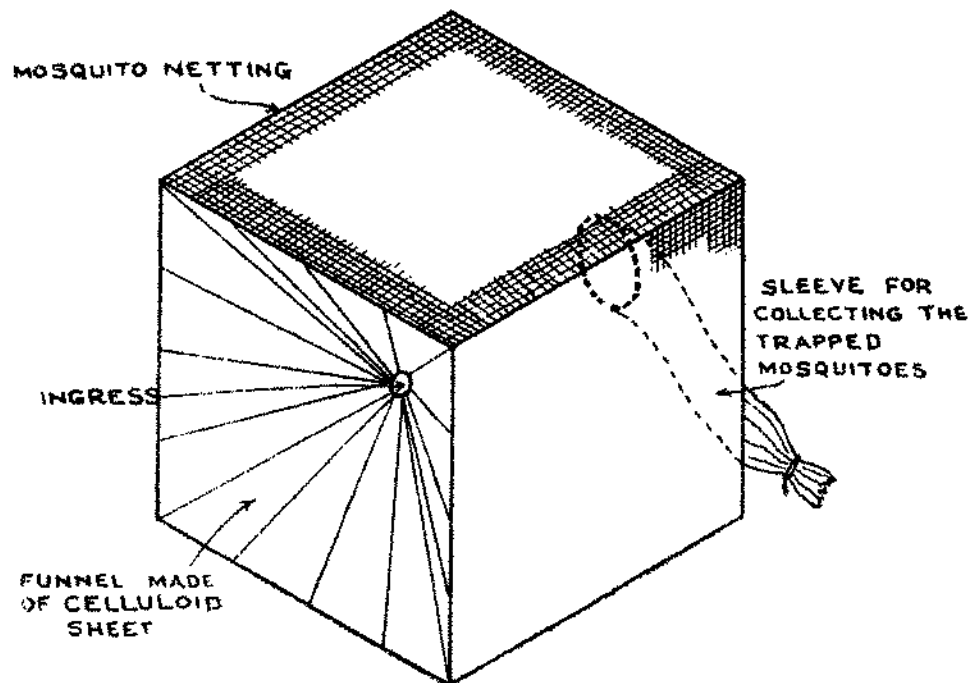
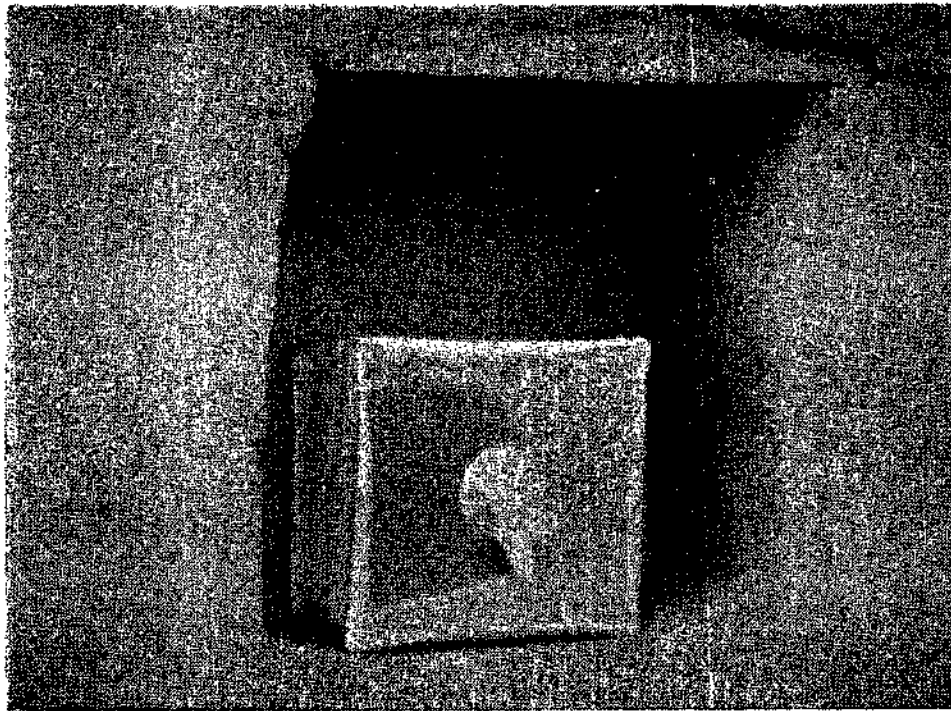


FIG. 2.—Showing the modified type of window trap cage: (top) cage fixed to a window of a catching station, (bottom) design of a cage showing the celluloid funnel.

each of the treated villages, and in the comparison village, specially constructed window traps were fixed. For the first few days, window traps of the design used by Muirhead-Thomson (1948) were employed, but it was found that mosquitoes would not enter such traps readily; this necessitated slight modification in the construction of traps. The traps are made from ordinary mosquito breeding cages of 1 ft. cube, made of light wire framework and white mosquito netting. On one side of the cage, a funnel made out of a celluloid sheet is stitched. The sides of this celluloid front piece converge to a small central opening about $\frac{1}{2}$ inch in diameter. The window trap is fitted in such a way that the celluloid side opens into the room and the opposite side, which is provided with a sleeve for the removal of mosquitoes, opens on the outside. The window trap is securely stitched to a sheet of sacking which covers the rest of the window. The celluloid front piece being smooth, offers minimum obstruction to the entry of mosquitoes (Fig. 2 and Jaswant Singh *et al.*, 1951).

Each morning, mosquitoes were collected for a period of 15 minutes from the rooms by experienced insect collectors. Mosquitoes from window traps were also simultaneously collected and brought to the field laboratory at Palwal in Barraud cages where they were kept under observation for the following 24 hours after which the mortality was recorded.

ENTOMOLOGICAL DATA.

The window trap catches have been expressed as percentage of room catches (Table VI and Chart I). The percentage figures for the whole period of six weeks indicate high repellency in the case of D.D.T. suspension. D.D.T. emulsion and B.H.C. suspension treatments exhibit a comparatively lesser degree of repellency. The comparison village also showed a high percentage of window trap catch during the second week. On investigation it was found that due to smoke from fire being lit in the room, mosquitoes were driven to the window trap. Steps were from then on taken to prevent such extraneous factors interfering with the behaviour of mosquitoes.

The survival rates of mosquitoes collected both from sprayed rooms and window traps are given in Table VII. With D.D.T. suspension, D.D.T. emulsion, and B.H.C. suspension, there was no survival for 24 hours among mosquitoes collected up to 2, 3 and 4 weeks respectively after application.

From the results it would be evident that D.D.T. does not deter mosquitoes from frequenting rooms, but exerts an irritant effect after they have been in contact with a treated surface for a short time. Gahan *et al.* (1945) in their laboratory studies on *A. quadrimaculatus* and *Aedes aegypti* found that D.D.T. irritated mosquitoes that rested on treated surfaces and caused them to fly away but not before most of them had obtained a lethal dose. On the other hand, Kennedy (1947) in his laboratory studies on *A. maculipennis* var. *atroparvus* and *Aedes aegypti* found that there were few deaths after 24 hours following exposures to D.D.T. The exposures were long enough to cause excitation but not death. Similarly, field studies of Muirhead-Thomson (1947a, 1947b, 1949, 1950) have shown the repellent effect of D.D.T. deposits towards *A. gambiae* and *A. melas*. These mosquitoes were repelled from the treated surfaces before picking up a lethal dose. He concluded that D.D.T. for all practical purpose was non-toxic whereas B.H.C. was 100 per cent effective. Field studies of Hocking (*loc. cit.*) on *A. gambiae* and *A. funestus* showed that there was no

TABLE VI
Anopheles mosquito collections from rooms (15 minutes hand catches and window traps)

Weeks	50 mg. D.D.T. (sq. ft. (SUSPENSION))			10 mg. D.D.T. (sq. ft. (EMULSION))			10 mg. B.H.C. GAMMA ISOMER (sq. ft. (SUSPENSION))			COMPARISON		
	Room catch (daily average)	Window trap collection (daily average)	Per cent window trap collection compared to room catch	Room catch (daily average)	Window trap collection (daily average)	Per cent window trap collection compared to room catch	Room catch (daily average)	Window trap collection (daily average)	Per cent window trap collection compared to room catch	Room catch (daily average)	Window trap collection (daily average)	Per cent window trap collection compared to room catch
1	9.2	0.0	0.0	23.8	0.0	0.0	0.7	0.0	0.0	116.1	0.0	0.0
2	22.8	12.2	53.5	19.4	1.4	7.2	1.5	0.4	26.6	47.0	7.5	15.9
3	10.5	11.8	112.3	33.8	6.1	18.0	0.3	0.1	33.3	79.0	4.5	5.7
4	17.8	11.7	65.7	32.5	4.1	12.6	2.2	0.3	13.6	80.2	4.1	5.1
5	17.7	18.7	105.6	28.0	4.4	15.7	1.0	0.0	0.0	75.7	3.8	5.0
6	33.4	13.4	40.1	16.2	4.2	25.9	3.8	0.3	7.9	36.5	0.7	1.9

CHART 1.

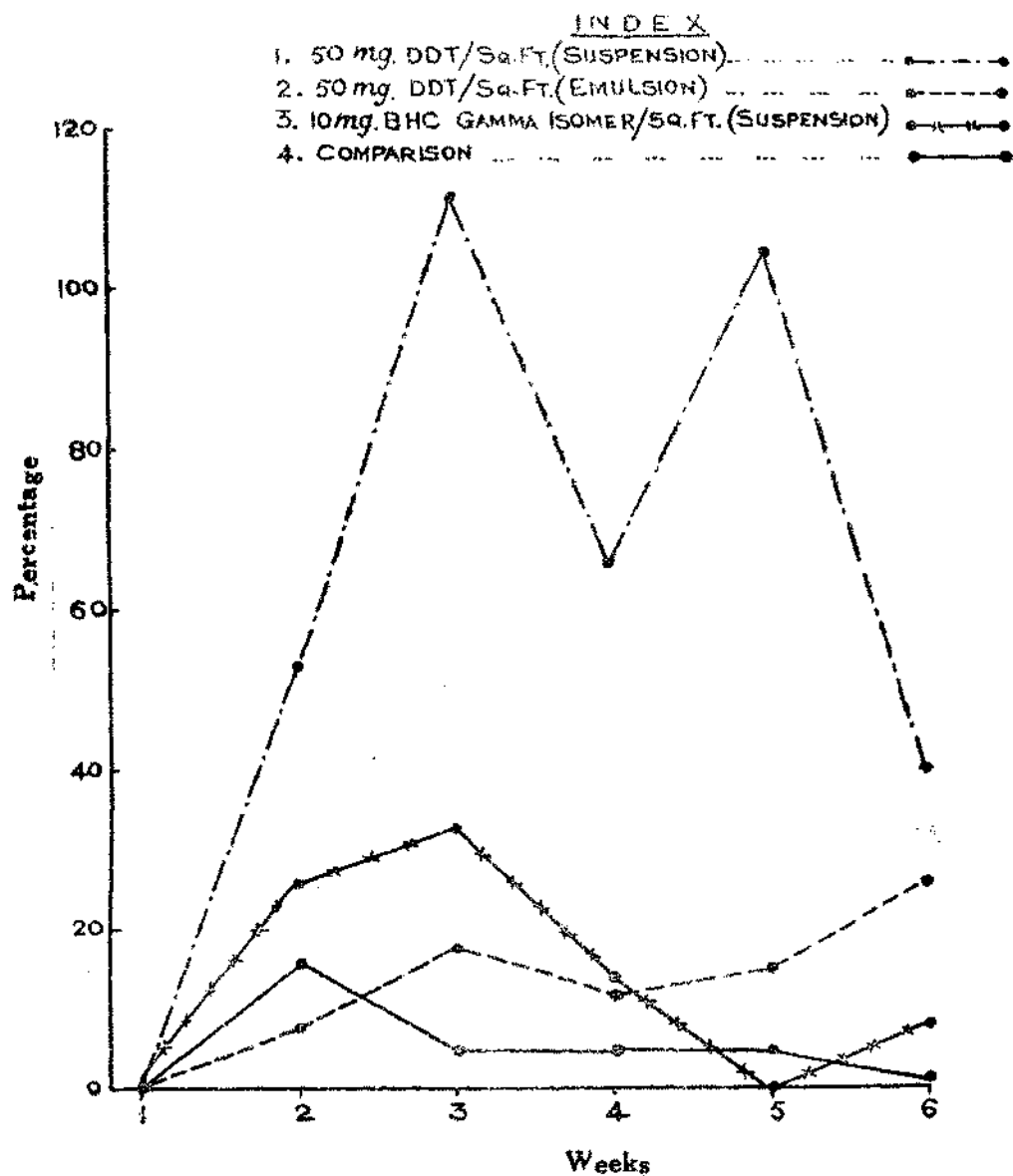
REPELLENCY OF TREATMENTS AS MEASURED BY
PERCENTAGE OF WINDOW TRAP CATCHES TO ROOM CATCHES

TABLE VII.

24 hours' survival rate amongst the mosquitoes collected from rooms and window traps.

Weeks.	Comparison.	D.D.T. Suspension.	D.D.T. Emulsion.	B.H.C.
1	37.5	0.0	0.0	0.0
2	28.7	0.0	0.0	0.0
3	29.2	34.2	0.0	0.0
4	45.2	48.8	0.3	0.0
5	31.7	23.4	34.1	0.0
6	43.0	20.0	17.5	2.8

interference with the normal entry of mosquitoes into the D.D.T.-treated huts or with their biting habits. The mosquitoes tried to leave the building after being in contact with D.D.T., and that the mortality amongst fed mosquitoes was very high and closely approached 100 per cent. Where there was no source of blood or where there were alternative untreated surfaces on which the mosquitoes could rest, a number of them escaped. Metcalf *et al.* (1945) recorded in the case of *A. quadrimaculatus* that at least 95 per cent and probably hundred per cent received a lethal dose before leaving houses and the chain of malaria was thereby effectively broken. Swellengrebel and Lodens (1949) in the case of *A. aconitus* found a considerable number of dead and dying mosquitoes in the houses, and they concluded that the repellent effect was not strong enough to protect them from the lethal effects of D.D.T. Wallace (1950) considering the effect of insecticides on *A. maculatus* observed that almost all mosquitoes that came into contact with surfaces sprayed with D.D.T. formulations died in less than 30 hours. Mosquitoes not exposed to any insecticide lived almost twice as long. They further observed that 'Gammexane' dispersible powder (P. 520*) killed in a much shorter time than D.D.T. and, therefore, holds out a greater hope of success in controlling *A. maculatus* malaria. Nair (1951) noticed some repellent effect of D.D.T. on *A. letifer* but he recorded that these mosquitoes could still be controlled by the use of D.D.T.

Bertram (1950) described a similar reaction with *A. minimus* in Upper Assam. When a low dose (45 mg. sq. ft.) was applied, mosquitoes were merely driven out of huts into window traps; but at a dosage of 215 mg. per sq. ft. a large number of mosquitoes inside the hut were killed though many mosquitoes escaped unharmed. B.H.C. was found to be more effective.

This, however, has been refuted by Macdonald (1950)

* Imperial Chemical Industries Ltd.

who reporting the work of Gilroy in Assam stated that D.D.T. was effective against *A. minimus* even at a lower dose. In the present studies B.H.C. application, strictly speaking, also exerted repellent effect on the mosquitoes. There is no evidence that contact with gamma B.H.C. is irritating to mosquitoes though it may act as a repellent at a distance when freshly applied (Wharton, 1950). This repellency of B.H.C. is, however, of little significance because mosquitoes pick up a lethal dose after contact with sprayed surface and eventually die.

A true assessment of any insecticidal treatment should always be made on epidemiological data which measures the incidence of malaria in a community. In Table VIII are shown the malaria morbidity rates (per 1,000 of population) in the experimental villages. The weekly distribution of malaria morbidity is comparatively a more significant index than the rate itself, as a village with a low population even with a single case would show a very high per 1,000 rate. It was significant that in the comparison village malaria cases were encountered throughout the period of observation but in treated villages malaria cases were sporadic and were probably relapse cases. From the morbidity rate alone, it is not possible to say which of the three insecticidal treatments, *i.e.* D.D.T. as suspension, D.D.T. as emulsion or B.H.C. as suspension, was better. One thing, however, is clear that D.D.T. both as emulsion and suspension applied at the rate of 50 mg./sq. ft. was effective to control malaria in spite of the excite-repellent effect exhibited by the mosquitoes.

SUMMARY.

Importance of the study of the behaviour of mosquitoes in relation to insecticidal application has been stressed. The entry and feeding activity of mosquitoes and their movements in comparison and treated huts have been studied. It appears that the insecticidal application does not alter the normal behaviour of the species studied. Behaviour of *A. culicifacies* has been discussed in particular.

Excite-repellent effect was observed in mosquitoes after contact with surfaces treated with D.D.T. suspension and emulsion at the rate of 50 mg. D.D.T./sq. ft. B.H.C. deposits at the rate of 10 mg. gamma isomer/sq. ft. also exerted repellent effect on mosquitoes but this is of little significance as regards the control of mosquitoes is concerned because in both cases the mosquitoes pick up a lethal dose and eventually die.

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TABLE VIII

Malaria morbidity rate per 1,000 of population from August to November, 1950.

Village.	Insecticide used	Weeks.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Miranpur	50 mg. D.D.T./sq. ft. (emulsion)	...	...	...	...	...	...	...	...	3	...	...	...	3	...	...	...
Meghpur	50 mg. D.D.T./sq. ft. (suspension)	...	3	3	3	...	...	...	...	3	...	...	...	25	...	...	...
Bahraula	10 mg. B.H.C. gamma isomer/sq. ft. (suspension)	...	5	...	...	12	...	...	...	2	...	...	...	...	...	...	...
Khusrupur (comparison)		...	6	4	6	4	...	4	4	4	...	8	8	...	...	...	...

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APPENDIX I.

All species of anopheline mosquitoes caught in catching stations at different hours of night and the density of mosquitoes (routine morning catch).

Weeks.	COMPARISON VILLAGE.								Total morning catch after pyrethrum spray.	VILLAGE TREATED WITH 50 MG. D.D.T. (SQ. FT. SUSPENSION-TREATED CATCHING STATIONS.)								Total morning catch after pyrethrum spray.
	P.M.				A.M.					P.M.				A.M.				
	6	8	10	12	2	4	6			6	8	10	12	2	4	6		
1	...	57	13	8	32	24	47	147	...	29	3	5	12	4	11	12	...	
2	...	57	51	31	16	13	74	61	...	14	1	8	4	3	2	38	...	
3	...	103	33	24	0	0	6	83	...	9	11	4	2	14	10	7	...	
4	...	148	38	32	21	9	9	148	...	29	2	4	16	5	7	41	...	
5	...	103	38	23	13	28	20	121	5,889	7	2	1	2	4	2	12	146	
6	...	26	13	12	8	19	24	51	5,743	6	0	2	0	1	1	7	145	
7	...	35	34	18	11	8	20	30	6,279	1	0	0	0	1	0	8	229	
8	...	8	6	2	1	0	0	2	3,135	2	1	0	2	0	0	3	9	
9	...	101	39	36	30	29	20	126	1,057	1	0	1	0	0	0	5	...	

APPENDIX

Feeding activity of anopheline mosquitoes

Weeks.	6 P.M.				8 P.M.				10 P.M.				12 mid-night			
	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
1	0	0	20	30	0	0	11	2	0	5	2	1	1	10	6	4
2	6	0	27	13	8	35	5	0	0	23	3	1	2	12	1	0
3	15	3	51	9	0	9	29	3	5	10	9	0	0	0	0	0
4	25	11	17	36	2	26	5	4	3	29	0	0	3	16	2	0
5	25	4	17	32	4	13	9	12	0	23	0	0	0	13	0	0
6	6	0	16	3	0	12	1	0	0	7	5	0	0	4	0	0
7	9	5	20	0	0	26	6	0	0	15	1	0	0	7	4	0
8	0	0	2	5	0	1	3	2	0	2	0	0	0	1	0	0
9	35	16	12	9	20	2	4	2	0	20	10	4	0	15	0	0

UF = Unfed. FF = Fully fed. HG = Half gravid. G = Gravid.

APPENDIX

Feeding activity of anopheline mosquitoes

Weeks.		6 P.M.				8 P.M.				10 P.M.				12 mid-night			
		UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
1	...	0	0	12	9	0	2	0	0	0	3	2	0	0	7	4	0
2	...	3	0	0	3	1	0	0	0	0	8	0	0	0	6	0	0
3	...	0	0	3	2	0	10	0	0	0	4	0	0	0	2	0	0
4	...	3	6	12	0	1	0	0	1	0	3	1	0	0	14	2	0
5	...	0	2	3	2	0	1	0	1	0	1	0	0	0	2	0	0
6	...	0	0	0	6	0	0	0	0	0	2	0	0	0	0	0	0
7	...	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
8	...	1	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0
9	...	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0

UF = Unfed. FF = Fully fed. HG = Half gravid. G = Gravid.

H.*at different hours of night--Comparison catching station.*

2 A.M.				4 A.M.				6 A.M.				TOTAL MORNING CATCH AFTER PYRETHRUM SPRAY.			
UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
0	41	7	3	0	38	4	0	0	103	18	11	...	...	...	...
0	10	3	0	6	51	8	0	8	34	8	6	...	...	...	...
0	0	0	0	0	2	4	0	14	39	10	4	...	...	...	...
2	7	9	0	0	7	0	0	16	35	17	24	...	...	...	...
0	15	5	8	0	20	0	0	19	35	17	16	0	1,001	3,639	533
1	8	6	4	0	20	4	0	2	31	9	5	35	1,662	3,229	121
0	3	5	0	0	9	8	0	0	19	4	0	...	...	...	...
0	0	0	0	0	0	0	0	0	0	2	0	...	...	...	...
0	18	6	5	1	15	3	0	17	44	15	12	43	652	325	34

III.*at different hours of night--Treated catching station.*

2 A.M.				4 A.M.				6 A.M.				TOTAL MORNING CATCH AFTER PYRETHRUM SPRAY.			
UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
0	3	1	0	0	7	2	0	0	10	0	0	...	...	...	...
0	2	1	0	0	2	0	0	0	15	0	0	...	...	...	...
0	6	2	0	3	10	1	0	5	23	2	3	...	...	...	...
0	5	0	0	0	7	0	0	2	23	0	3	0	40	63	9
0	3	1	0	0	1	0	0	1	14	2	0	0	46	63	8
0	0	1	0	0	1	0	0	0	3	4	0	24	43	87	41
0	1	0	0	0	0	0	0	0	2	4	0	0	2	5	1
0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0
0	0	0	0	0	0	0	0	0	3	0	0	5	9	20	3

APPENDIX IV.

A. culicifacies caught in catching stations at different hours of night and the density of *A. culicifacies* (routine morning catch).

Weeks.	COMPARISON VILLAGE.							Total morning catch after pyrethrum spray.	VILLAGE TREATED WITH 50 MG. D.D.T./SQ. FT. (SUSPENSION-TREATED CATCHING STATIONS.)							Total morning catch after pyrethrum spray.
	P.M.								A.M.							
	6	8	10	12	2	4	6		6	8	10	12	2	4	6	
1	1	3	0	2	3	2	4	...	1	0	0	1	0	1	0	...
2	4	6	3	2	0	3	2	...	0	0	0	0	0	0	0	...
3	8	9	6	0	0	2	7	...	0	2	0	0	0	1	8	...
4	6	6	3	1	0	1	11	...	3	0	0	0	1	1	7	...
5	3	7	3	1	2	1	6	23	1	1	0	0	0	1	3	2
6	1	0	2	2	0	2	6	17	0	0	0	0	0	1	3	10
7	2	2	3	1	1	2	5	65	0	0	0	0	0	0	0	20
8	2	0	0	0	0	0	0	51	0	0	0	0	0	0	0	0
9	4	1	4	1	2	0	7	40	1	0	0	0	0	0	2	0

APPENDIX*Feeding activity of A. culicifacies*

Weeks.	6 P.M.				8 P.M.				10 P.M.				12 MIDNIGHT.			
	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
1	0	0	1	0	6	2	0	0	0	0	0	0	0	1	0	0
2	0	0	3	1	0	5	1	0	0	2	0	0	0	2	0	0
3	0	3	5	0	0	2	7	0	2	1	3	0	...	...	...	...
4	1	0	2	1	0	6	0	0	0	3	0	0	0	1	0	0
5	0	0	1	0	0	3	2	2	0	3	0	0	0	1	0	0
6	0	0	1	0	0	0	0	0	0	0	2	0	0	0	2	0
7	0	0	2	0	0	2	0	0	0	3	0	0	0	0	1	0
8	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	2	1	1	0	1	0	0	0	4	0	0	0	1	0	0

UF = Unfed. FF = Fully fed. HG = Half gravid. G = Gravid.

APPENDIX*Feeding activity of A. culicifacies*

Weeks.	6 P.M.				8 P.M.				10 P.M.				12 MIDNIGHT			
	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
4	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

UF = Unfed. FF = fully fed. HG = Half gravid. G = Gravid.

V.*at different hours of night—Comparison catching station.*

2 A.M.				4 A.M.				6 A.M.				TOTAL MORNING CATCH AFTER PYRETHRUM SPRAY.			
UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
0	1	2	0	0	2	0	0	0	1	2	0	0	9	7	0
0	0	0	0	0	3	0	0	0	2	0	0	0	10	3	2
...	...	...	...	0	0	2	0	0	5	2	0	4	15	21	10
0	0	0	0	0	1	0	0	0	0	2	0	0	8	20	16
0	0	2	0	0	1	0	0	0	3	2	0	0	28	9	0
0	0	0	0	0	2	0	0	0	5	1	0	5	19	16	4
0	0	1	0	0	1	1	0	0	5	0	0	0	7	10	0
0	0	0	0	0	0	0	0	0	0	0	0	2	1	8	0
0	2	0	0	0	0	0	0	5	1	0	0	0	2	5	1

VI.*at different hours of night—Comparison catching station.*

2 A.M.				4 A.M.				6 A.M.				TOTAL MORNING CATCH AFTER PYRETHRUM SPRAY.			
UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G	UF	FF	HG	G
0	0	0	0	0	1	0	0	0	0	0	0	...	...	...	...
0	0	0	0	0	0	0	0	0	0	0	0	...	...	...	...
0	0	0	0	0	1	0	0	1	4	1	0	...	...	...	...
0	1	0	0	0	1	0	0	0	7	0	0	0	2	0	0
0	0	0	0	0	1	0	0	0	3	0	0	0	7	2	0
0	0	0	0	0	1	0	0	0	1	2	0	0	6	8	4
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	2	1	0

STUDIES ON THE DEVELOPMENT OF RESISTANT STRAINS OF HOUSE-FLIES AND MOSQUITOES.

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IN recent years, development of resistant strains of insects to insecticides, particularly chlorinated hydrocarbons, have assumed great importance because the appearance of resistant strains is a potential danger to the successful implementation of insect control in many parts of the world. Development of resistance to insecticides by certain species of insects is not new, but the problem did not assume general concern to entomologists until sometimes after D.D.T. had come into widespread use followed by the appearance of resistance in housefly *Musca domestica* L. The entire subject of insect resistance was thus brought into limelight. The following may be recorded as landmarks :

1914	..	Scale insects developed resistance to lime sulphur (Melander, 1914).
1916	..	Red scale insects to hydrogen cyanide (Quayle, 1938).
1928	..	Codling moth to lead arsenate (Hough, 1928).
1946	..	Houseflies to D.D.T. (Sacca, 1947; Wiesmann, 1947).
1947	..	Blue tick to arsenical dip (Whitnall and Bradford 1947).
1947	..	Culex mosquitoes to D.D.T. (Missiroli, 1947; Mosna, 1947).
1949	..	Salt marsh mosquitoes to D.D.T. (Deonier and Gilbert, 1950; Deonier <i>et al.</i> , 1950).
1951-52	..	<i>Anopheles albimanus</i> (Trapido, 1952) and <i>Anopheles sacharovi</i> (Georgopoulos, 1951; Livadas, 1952) to D.D.T.

A few noteworthy reviews on the resistance of insects to insecticides have been recently published by Babers (1949), Babers and Pratt (1951), Harrison (1952) and Hess (1952). Mention may also be made of the symposia arranged on this subject by the Entomological Society of Ontario, 1950, IXth International Congress in Entomology, 1951, National Research Council, U.S.A., 1952, and the Royal Society of Tropical Medicine and Hygiene, 1952.

This paper does not attempt at any exhaustive review of the problem; it rather presents the salient features of the present state of knowledge on this subject. It also includes studies on the resistance of *Musca nebulosa* F., *Culex fatigans* Wiedemann and local anopheline species viz. *A. culicifacies* Giles, and *A. stephensi* Liston to D.D.T. which have been in progress in the Malaria Institute of India since 1950.

HOUSEFLIES.

Development of resistant strain of houseflies has been well established in many countries, e.g. Italy (Sacca, *loc. cit.*), Sweden (Wiesmann, *loc. cit.*), Denmark (Kieding and Van Deurs, 1949), U.S.A. (number of workers—Hess, *loc. cit.*), Middle East (Gaban and Weir, 1950).

It has been found that flies are not only resistant to D.D.T. but also to some of the other chlorinated compounds and even to pyrethrum.

Different strains of houseflies have been reported, some of which are highly resistant to knockdown and the others are as rapidly affected as normal flies, but unlike normal flies they subsequently recover (Busvine, 1951; Pal and Sharma, 1951).

The degree of resistance of different strains also varies, which may be due to natural variations in susceptibility of strains from different countries. Busvine (*loc. cit.*) has also pointed out that the degree of resistance as determined by various workers may be misleading since the type of D.D.T. solvent used greatly affects the results.

The rate at which houseflies develop resistance to insecticides in the field in different countries also shows considerable variations which may be due to various factors such as genetic differences, climate and the intensity and frequency of insecticidal treatment, etc.

There is yet no satisfactory explanation of the mechanism of development of resistance and it may be attributed to a variety of factors such as genetical, morphological or physiological. Of course morphological and physiological characters have their foundation in genetic factors.

Wiesmann (*loc. cit.*) and D'Alessandro *et al.* (1949) reported morphological differences between resistant and non-resistant strains.

Bettini (1949) and March and Metcalf (1950), however, showed that even when D.D.T. solution is injected into the body of flies, the flies of the resistant

strain are comparatively more resistant to D.D.T. than the normal flies. They concluded, therefore, that resistance is very likely due to physiological mechanism than to morphological differences.

There is yet no satisfactory explanation of the physiological mechanism of D.D.T. resistance in houseflies.

A number of workers have shown that the resistance of flies was not due to their inability to absorb insecticide but due to difference in metabolic processes.

March and Metcalf (*loc. cit.*) found that both resistant and susceptible flies could metabolize D.D.T. to D.D.E., but resistant flies metabolized at a greater rate. While Stenborg *et al.* (1950) found that resistant flies could metabolize D.D.T. into non-toxic metabolite D.D.E. and D.D.A., susceptible flies do not possess this ability. Normal flies are capable of metabolizing small quantities of D.D.T. to some unknown product.

Winteringham *et al.* (1951) and Perry and Hoskins (1951) suggested that dehydrochlorination of D.D.T. is affected by enzymes and the difference between resistant and normal flies is quantitative rather than qualitative.

It is still more difficult to understand that insects do not suffer lethal injury in the first few hours prior to detoxification of D.D.T.; in certain cases the amount of D.D.T. present even after detoxification in resistant flies is enough to kill the normal flies. As Busvine (*loc. cit.*) has pointed out 'Dehydrochlorination mechanism does not fully account for the D.D.T. resistance and one is driven to postulate some additional defence mechanism'. Some of the other workers are, however, of the opinion that dehydrochlorination hypothesis is as good a physiological explanation as any, despite the difficulties pointed out by Busvine. It may be that the difference in cytochrome oxidase content has something to do with it (Chadwick, 1952).

Presence of synergists seems to inhibit the metabolic processes. Search has been made for the compounds which would improve the effectiveness of D.D.T. against resistant flies. The synergists are detoxified in preference to D.D.T. which is available to insects without decomposition. U.S.A. workers have screened nearly 2400 compounds out of which D.M.C. (1-1-bis-p-chloro-phenyl-ethanol) and 17 other chemicals have been demonstrated to have marked action (Summerford *et al.*, 1951).

There have also been many speculations regarding the inheritance of resistance to D.D.T. in houseflies. Bruce and Decker (1950) reported that it was a multiple gene character carried by both male and female flies. Harrison (1950) on the other hand reported that D.D.T. resistance is controlled by a single pair of allelomorphs. Harrison's evidence for a single pair of alleles is with reference to knockdown, not necessarily to kill. Decline in the resistance, when resistant strains are bred without D.D.T. treatment, has been reported by Barber and Schmitt (1949), Kieding *et al.* (*loc. cit.*), Harrison (*loc. cit.*), Pimentel *et al.* (1950), Missiroli (1951), March and Metcalf (*loc. cit.*) and Upholt (1950). Brown (1950) has pointed out that 'if the factors of resistance are genetic as opposed to cytoplasmic factors or characters acquired from the environment, the level of resistance should axiomatically be maintained in the

stock'. He suggests that presumably strains when collected in the field did not have homozygosity for the genes responsible for their resistance and hence the reversion of resistant strains; or rather, as Harrison (1951) has pointed out that the field populations are not homogeneous with respect to the alleles which they have in homozygous form*.

The development of resistant strains in the field has been explained by the fact that in any large community of flies, there would be some individual specimens which are less susceptible to D.D.T., may be either due to biochemical or other reasons; such individuals would survive D.D.T. treatment, and when bred, will reproduce in their own kind of resistant strain. In spite of the overwhelming will reproduce in their own kind of resistant strain. It may, however, be pointed out that D.D.T. resistant flies have not so far been reported from Ceylon (Bavine, 1949); India (Pal, 1951); Korea, Japan etc. (Hess, 1952). Even efforts to raise a strain of housefly *M. nebulosa* F. (the common housefly in India) resistant to D.D.T. by means of selection in the laboratory have not been successful in developing resistance to the same degree as in *M. domestica* L. (Pal and Sharma, *loc. cit.*). Negative findings are often a little inconclusive but species differences may be a factor in the failure of *M. nebulosa* F. to develop marked D.D.T. resistance.

The question of the control of resistant flies has also been studied and it has been suggested that either mixtures of insecticides† may be used or insecticidal treatments may be alternated every year. The procedure adopted in routine application of insecticides seems to affect the development of resistance. Indiscriminate use and faulty application of insecticides appear to promote the development of resistant strain. Formulation and dosages which are used may also have a bearing upon the rate and degree of development of resistance. Behaviouristic differences between resistant and normal strains have been reported. Resistant flies have been stated to be more vigorous and appear in large numbers (Missiroli, *loc. cit.*).

Majority of the workers are of the opinion that at the present time the prevention of breeding by hygienic measures should be recommended for the control of resistant insects. Brown (*loc. cit.*) has admirably summed up the situation. He states, 'The rapid rise of D.D.T. resistant strain of houseflies has thus focussed attention on the many-sidedness of the problem of insect resistance in response to insecticides. On the physiological side it may be due to differences in habits, cuticle, enzymes or general vigour or to combination of any of these factors. On the genetical side, single, few or multiple genes may be involved. Moreover, the same species may well respond to the same insecticide in different ways in different areas. The overall problem is challenging to the breadth and depth of the entomologist's training and ability as an investigator'.

* The last sentence has been added at the suggestion of Professor A. W. A. Brown who was very kind to go through the manuscript.

† It has been pointed out by Coyne (1951) that application of mixtures of insecticides may result in the development of strains resistant to both insecticides simultaneously.

RESISTANT STRAIN OF *MUSCA NEBULO* F.

Regular spraying of houses with D.D.T. as an antimalaria measure was started in Delhi and other parts of India during 1945 but enquiries in 1949 revealed no definite indication of increased resistance of houseflies to D.D.T. (Pal, *loc. cit.*). It may be pointed out that D.D.T. resistant strain of flies so far reported has been mostly from temperate zones where *M. domestica* L. is the common housefly. Moreover, in India D.D.T. spraying is carried out only during the malaria transmission season which does not last more than 3 to 6 months. It seems very likely that if some resistance is developed during the malaria transmission season it is lost during the rest of the year. Similarly in Ceylon after 2 years of D.D.T. spraying, Busvine (*loc. cit.*) found that *M. vicina* Macq., the dominant housefly in that area, had not developed any resistance to D.D.T. All the available evidence lends support to the conclusion that flies in this part of the world have not yet developed any significant resistance to D.D.T. in the field.

Efforts were then directed to raise, if at all possible, a strain of housefly resistant to D.D.T. by means of selection in the laboratory. It was found that the flies developed a slight degree of resistance to D.D.T. during the first three generations but during the subsequent generations no further increase in resistance was observed (Pal and Sharma, *loc. cit.*). Pal and Sharma (*loc. cit.*) also showed that resistance among houseflies may be of two kinds—those highly resistant to knockdown and those which are affected as rapidly as normal flies, but unlike the normal flies, recover subsequently. The Indian strain of *M. nebulosa* F. is not resistant to knockdown, but is resistant to mortality.

It would be seen from Table I and Charts 1 and 2 that if the knockdown time alone is considered, the resistant strain was found to be 2.0 times more resistant than the normal strain. If, however, 24 hours' percentage survival of completely knocked down flies is considered as a criterion for measuring the resistance developed, then the resistant strain was found to be 13 to 39 times more resistant than the normal flies. It has been emphasized, therefore, that resistance of flies should be specifically mentioned in terms of 'knockdown resistance' and 'mortality resistance' because wide variations exist between the two; and until the technique is standardized, erroneous conclusions may be reached.

Considerable amount of variations which have been observed both in respect of knockdown time and 24 hours' mortality amongst the flies of different generations cannot be explained, except for the fact that in these studies the weight of flies was not given serious consideration. This problem is, however, under investigation.

Reversion or loss of resistance in successive generations of flies without exposure to D.D.T. was also studied. Batches of resistant strain of 35th and 41st generations were allowed to breed without exposure to D.D.T. and the offspring were tested for resistance to D.D.T. It was observed that these flies lost resistance fairly rapidly and were as susceptible to D.D.T. as normal flies (Table II).

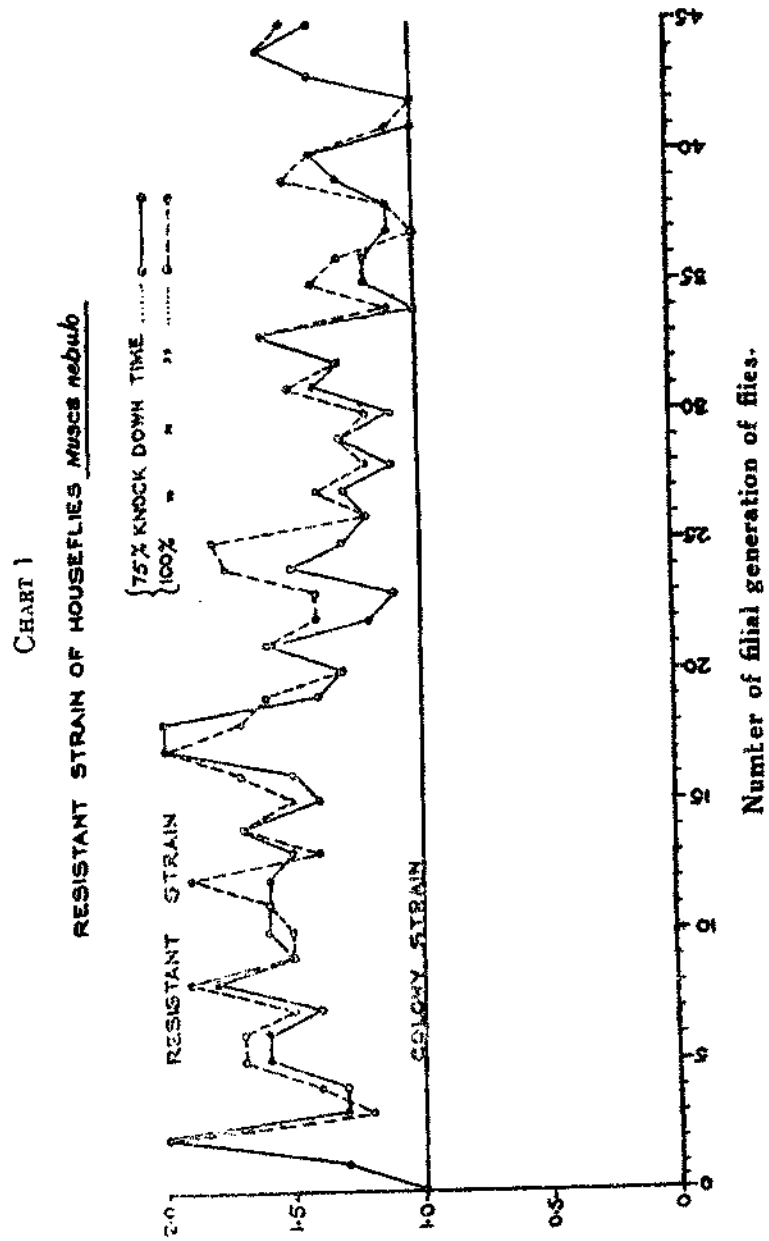
TABLE I.

Time in minutes to obtain 75 and 100 per cent knockdown of resistant and normal fly strains and 24 hours' percentage survival of knocked down flies.

Number of filial generations.	RESISTANT STRAIN KNOCKDOWN TIME IN MINUTES.		NORMAL COLONY STRAIN KNOCKDOWN TIME IN MINUTES.		24 HOURS' PERCENTAGE SURVIVAL.	
	75 per cent knockdown.	100 per cent knockdown.	75 per cent knockdown.	100 per cent knockdown.	Resistant strain.	Colony strain.
0	30	40	30	40	...	...
1	39	46	29	37	...	...
2	55	74	32	39	...	...
3	51	62	41	53	...	...
4	44	53	32	39	...	...
5	50	59	31	36	...	...
6	47	55	31	36	...	...
7	62	73	45	59	...	...
8	42	54	23	28	...	...
9	52	70	36	46	...	...
10	52	61	34	42	...	...
11	46	55	23	35	...	...
12	41	57	25	30	...	...
13	48	63	32	41	27	0
14	45	64	27	37	16	0
15	44	52	31	36	38	0
16	34	47	24	29	39	0
17	50	62	25	30	28	0
18	43	53	24	32	23	0
19	40	57	31	33	25	0
20	37	41	29	34	33	0
21	32	40	20	25	28	0
22	27	42	27	30	17	0

TABLE I.—*concd.*

Number of filial generations	RESISTANT STRAIN KNOCK- DOWN TIME IN MINUTES.		NORMAL COLONY STRAIN KNOCKDOWN TIME IN MINUTES.		24 HOURS' PERCENT- AGE SURVIVAL.	
	75 per cent knockdown.	100 per cent knockdown.	75 per cent knockdown.	100 per cent knockdown.	Resistant strain.	Colony strain.
23	41	60	40	45	19	0
24	40	50	28	30	18	0
25	34	54	25	30	23	0
26	41	54	33	43	18	0
27	41	50	30	35	18	0
28	28	36	25	30	20	0
29	34	40	25	30	20	0
30	23	30	20	25	14	0
31	35	43	25	28	28	0
32	20	27	15	20	18	0
33	38	47	23	28	18	0
34	36	46	35	40	20	0
35	16	21	13	15	18	0
36	18	24	14	18	13	0
37	19	23	17	23	15	0
38	26	36	23	31	15	0
39	29	55	30	35	13	0
40	35	42	25	30	18	0
41	51	61	50	65	18	0
42	41	47	38	43	23	0
43	70	82	50	55	31	0
44	64	76	40	45	23	0
45	36	45	25	30	19	0



RATIO INCREASE IN RESISTANCE AS COMPARED TO NORMAL

RATIO INCREASE IN RESISTANCE AS COMPARED TO NORMAL.

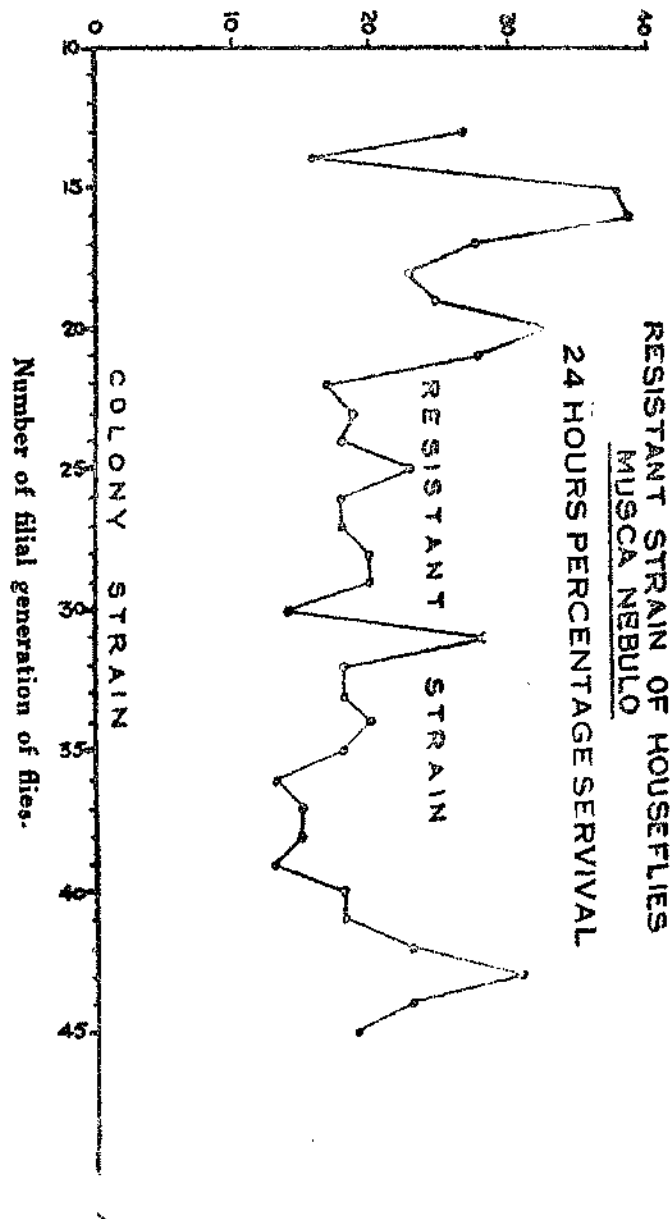


TABLE II.

75 and 100 per cent knockdown time and 24 hours' percentage survival of knocked down flies of the offspring of 35th and 41st generations of the resistant strain withheld from D.D.T. treatment and the normal colony strain.

Number of filial generations.	RESISTANT STRAIN EXPOSED TO D.D.T. BEFORE EGG LAYING, KNOCKDOWN TIME IN MINUTES.		NORMAL COLONY STRAIN KNOCKDOWN TIME IN MINUTES.		24 HOURS' PER- CENTAGE SURVIVAL	
	75 per cent knockdown.	100 per cent knockdown.	75 per cent knockdown.	100 per cent knockdown.	Resistant strain.	Colony strain.
35 { 1st generation ...	22	29	28	35	0	0
	20	24	25	32	0	0
41 { 1st generation ...	20	26	20	35	0	0
	19	26	30	45	0	0

MOSQUITOES.

The first report of D.D.T. resistant strain of mosquitoes came from Italy where Missiroli (*loc. cit.*) and Mosna (*loc. cit.*) presented information on the occurrence of D.D.T. resistant strain of *Culex pipiens*. In Florida, both adult and larvæ of salt marsh mosquitoes *Aedes taeniorhynchus* and *Aedes sollicitans* developed resistance to D.D.T. after the area had been sprayed for 5 consecutive years with D.D.T. (Deonier and Gilbert, *loc. cit.*; Deonier *et al.*, *loc. cit.*; King, 1950). Knipling (1952) carried out studies in California and found that several species of *Culex* had developed resistance in localized areas. In parts of California treated with D.D.T. larvæ of *A. nigromaculis* were found to be more resistant to D.D.T. than those from untreated areas (Bohart and Murray, 1950). Fay *et al.* (1949) found that exposure of adults of *A. quadrimaculatus* Say through several generations to residues of D.D.T. produced only slight resistance to insecticide. Trapido (*loc. cit.*) has reported evidence of the development of resistance in *Anopheles albimanus*, and Georgopoulos (*loc. cit.*) and Livadas (*loc. cit.*) in *anopheles sacharovi*.

With this evidence available, the possibility of local mosquitoes developing resistance to D.D.T. was realized and studies were undertaken both in the laboratory and in the field.

Anopheles culicifacies Giles.—Adults of *Anopheles culicifacies* Giles, an important vector species in India, were collected from the rural areas in Delhi, which has been sprayed with D.D.T. since 1945. Offspring of these were obtained in the laboratory and tested for their susceptibility to D.D.T. The technique employed was similar to that used in the case of houseflies. (Pal and Sharma *loc. cit.*) The results gave no indication of increased resistance

in *Anopheles culicifacies* Giles to D.D.T. in any of the localities around Delhi. Gahan *et al.* (1949) from Mexico, Ludvix *et al.* (1951) from U.S.A. and Trapido (1951) from Italy have also reported that *Anopheles pseudopunctipennis*, *Anopheles quadrimaculatus* and *Anopheles claviger* respectively have not developed resistance to D.D.T. although D.D.T. has been used in these areas for a number of years.

Anopheles stephensi Liston.—Efforts were made to raise a resistant strain of *Anopheles stephensi* Liston* by exposing the adults to sublethal dosages of D.D.T. (12.5 mg. sq. ft.) for varying periods. Preliminary trials failed to produce a resistant strain. It was, however, observed that when mosquitoes were exposed to D.D.T. for more than 10 minutes, they were not able to take a blood feed afterwards. But an exposure to D.D.T. for 10 minutes inhibited the development of eggs. Fully fed mosquitoes exposed to D.D.T. for 5 minutes and afterwards kept under normal conditions, also failed to lay eggs, whereas completely gravid mosquitoes laid viable eggs after exposure to D.D.T. deposits for 5 minutes. Adults raised from these eggs were found to be as susceptible to D.D.T. as those of the normal strain.

These preliminary studies indicate that the local anopheline mosquitoes do not seem to develop resistance as readily as houseflies.

Culex fatigans Wiedemann.—*Culex* mosquitoes were captured from a sprayed village namely Khuraiji Khas (Delhi State) which had been regularly sprayed with D.D.T. during the past six years, and from an unsprayed village Ram Garhi (U.P.) 10 miles apart. These two strains were reared for one generation simultaneously under identical conditions. The progeny of the strains was tested for resistance to D.D.T.

The results indicated increased resistance of mosquitoes to D.D.T. 100 per cent knockdown of mosquitoes collected from unsprayed village was obtained within five hours, whereas only 66 per cent (females) and 79 per cent (males) mosquitoes from sprayed village were knocked down during that period. Even after 24 hours contact, only 78 per cent (females) and 94 per cent (males) mosquitoes were knocked down (Table III).

In subsequent tests the mosquitoes were exposed for half an hour to glass panels sprayed with D.D.T. at the rate of 50 mg./sq. ft. The exposed insects were kept under observation for 48 hours and the mortality was recorded after

* Colony of *A. stephensi* can be easily established in the laboratory, as compared to *A. culicifacies*. Hence laboratory studies were carried out on this species.

24 and 48 hours. Twenty experiments were carried out (Table III). Mortality amongst the mosquitoes from sprayed villages was 9 per cent (females) and 31 per cent (males) after 48 hours, and the corresponding figures for the mosquitoes from unsprayed village were 90 and 93 per cent respectively.

TABLE III.

Percentage mosquito knockdown per hour and percentage mortality after 24 and 48 hours (average of 20 replicate tests).

	Percentage knockdown (when exposed to 300 mg. D.D.T. per sq. ft. for 1-24 hours).							Percentage mortality after $\frac{1}{2}$ hour exposure to 50 mg. D. D. T. / sq. ft.	
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	24 hours	24 hours	48 hours
Sprayed ($\varnothing \varnothing$) ..	6	24	37	50	66	74	78	7	9
do. ($\delta \delta$)	21	42	51	66	79	81	94	28	31
Unsprayed ($\varnothing \varnothing$)	28	66	88	95	100	..	..	48	90
do. ($\delta \delta$)	47	88	96	96	100	..	..	84	93

SUMMARY.

This paper outlines the present state of knowledge of the resistance of houseflies and mosquitoes to D.D.T. and some of the other organic chlorinated insecticides. The existence of resistant strain of *M. domestica* L. in different countries has been well established although the questions relating to the development of resistant strain, degree of resistance and mechanism of the development of resistance, are to be investigated more thoroughly. Among culicine mosquitoes there are clear cases of D.D.T. resistance comparable with those of houseflies, whereas in the case of anopheline only a few cases of resistance have been reported.

In India where the development of resistance in houseflies and mosquitoes has been studied in the field, no definite resistant strain of either of the insects has been observed so far except in the case of *Culex fatigans*. *Culex fatigans* from a D.D.T. sprayed village appear to be less susceptible to D.D.T. than those from an unsprayed village.

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COMPARATIVE FIELD STUDIES ON THE RESIDUAL
EFFECTIVENESS OF D.D.T., B.H.C., D.D.T. AND
B.H.C. COMBINED SPRAY, AND DIELDRIN
AGAINST MOSQUITOES

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INTRODUCTION

FIELD trials on the comparative efficacy of D.D.T. and B.H.C. against mosquitoes when applied separately and in combination and on dieldrin were carried out during 1950 in a group of villages near Palwal, District Gurgaon, Punjab, India. Jaswant Singh *et al.* (1951) reported that irrespective of the dosage employed (50 to 200 mg. D.D.T. per sq. ft.) D.D.T. suspension was more effective than emulsion on mud-plastered walls and that massive doses of D.D.T. when applied on similar surfaces whether as suspension or emulsion do not seem to show proportional increase in the duration of residual effect. After 12 weeks of application, no D.D.T. could be detected chemically even in the scrapings removed from the houses treated with 200 mg. D.D.T. per sq. ft. They have also recorded that under local conditions there was little to choose between D.D.T. 50 mg. and B.H.C. 10 mg. gamma isomer per sq. ft. Both gave protection for about six weeks.

The synergistic action of D.D.T. and B.H.C. in combined spray (25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer per sq. ft.) was also reported by them. They observed that D.D.T. and B.H.C. combined spray was not only effective for a longer period, but was also more efficacious in keeping down the density of mosquito population than either of them applied separately. Dicke and Paul (1951) and Van Tiel (1952) have also found synergism between D.D.T. and B.H.C.

Pal *et al.* (1951) found dieldrin to be effective for about 7 weeks against mosquitoes when applied at the rate of 50 mg. per sq. ft.

During 1951, similar field trials were undertaken in Palwal area and the results obtained are briefly summarised in this publication.

Palwal area is known to be malarious and epidemiology of the disease is similar to the rural areas of Delhi where *A. culicifacies* Giles is the vector. A group of seven villages, with as far as possible similar epidemiological conditions, were selected for these trials. Majority of the houses in these villages are poorly built and are *kutchas* with mud-plastered walls and thatched roofs. The number of houses and total population in each village are shown in Table I.

INSECTICIDAL FORMULATIONS TESTED.

D.D.T.* and B.H.C.† emulsions were prepared from technical D.D.T. and 'Gammexane' LG110‡ (containing 10 per cent B.H.C gamma isomer) respectively with Lissapol NX‡ as an emulsifier. Suspensions were prepared from 'Geigy malaria spray'§, 50 per cent D.D.T. water dispersible powder and 'Gammexane' P-520 containing 6.5 per cent B.H.C. gamma isomer. D.D.T. was sprayed at 2.5 per cent and B.H.C. at 0.5 per cent gamma isomer concentrations. D.D.T. and B.H.C. combined sprays were prepared by mixing in equal parts 2.5 per cent D.D.T. and 0.5 per cent B.H.C. gamma isomer formulations. The dosages of insecticides employed were 50 mg. D.D.T./sq. ft., 10 mg. B.H.C. gamma isomer/sq. ft and 25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer/sq. ft. 2.5 per cent dieldrin emulsion was prepared from 24 per cent dieldrin emulsifiable concentrate** by mixing 1 part of emulsifiable concentrate with 8.6 parts of water. It was applied at the rate of 2 c.c. per sq. ft. so as to give a deposit of 50 mg. dieldrin per sq. ft.

Surfaces of all houses in the villages were measured and the quantity of formulation required for each house or group of houses was sprayed in particular areas. The spraying equipment consisted of double barrel stirrup pump fitted with an oil resistance hose and a brass lance. The nozzle used had an orifice of 3/64 inch diameter with an output of about 600 c.c. per minute in a cone-shaped spray. All structures whether human dwellings or cattlesheds were treated. Inner surfaces of walls and as much of the ceilings as could be easily reached, were sprayed.

ENTOMOLOGICAL DATA

In each of the experimental villages, four suitable mosquito catching stations were established in the treated villages, two of these catching stations were not

* Aromex ...	... 32 gls.
D.D.T. ...	... 100 lb.
Lissapol NX ...	... 2 gls.

40 gls. of 25 per cent D.D.T. emulsifiable concentrate.

† 'Gammexane' LG110 ...	1 gallon.
Lissapol NX ...	½ lb.

Diluted with water to give 20 gls. of 0.5 per cent B.H.C. gamma isomer emulsion.

‡ Supplied by Imperial Chemical Industries (India) Ltd., Calcutta.

§ Supplied by Geigy Insecticides, Ltd., Bombay.

**Supplied by Julius Hyman & Co., Denver, Colorado, U.S.A.

TABLE I.
Particulars of experimental villages : Formulations and dosages of insecticides employed.

Name of the village.	Number of houses.	Population.	Total area sprayed sq.ft.	Insecticides and formulations used.	Dosage mg./sq.ft.	Quantity sprayed, c.c./sq.ft.
Sasai ...	94	516	68,739	2.5 per cent D.D.T. suspension	50 mg. D.D.T.	2
Khatola	67	476	67,321	0.5 per cent B.H.C. gamma isomer suspension.	10 mg. B.H.C. gamma isomer	2
Ahaspur	86	481	45,958	D.D.T. (1.25 per cent) and B.H.C. (0.25 per cent gamma isomer) combined suspension.	25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer	2
Dhunda	44	292	38,368	2.5 per cent D.D.T. emulsion	50 mg. D.D.T.	2
Baipur	40	200	42,167	0.5 per cent B.H.C. gamma isomer emulsion.	10 mg. B.H.C. gamma isomer	2
Ratipur	64	356	54,584	D.D.T. (1.25 per cent) and B.H.C. (0.25 per cent gamma isomer) combined emulsion.	25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer	2
Meghaur	20	106	20,503	2.5 per cent dieldrin emulsion.	50 mg. dieldrin.	2
Alahpur	115	575	..	C o m p a r i s o n		

sprayed for purposes of comparison. Total mosquitoes caught were made every week from the catching stations by spraying the village with D.D.T. and B.H.C. combined sprays respectively. In the case of emulsions the results are not conclusive as there was decline in the mosquito population of experimental villages due to natural causes also (Table III).

1. Weekly percentage mosquito density as compared to pre-spraying density.
2. Ratio of mosquitoes caught from sprayed and unsprayed catching stations (S/US).

RESULTS.

It would be evident from Table II (suspension) that the increase in mosquito density to 50 per cent of the pre-spraying density was noticeable in the 6th, 7th and 8th week after spraying in the case of D.D.T., B.H.C. and D.D.T. and B.H.C. combined sprays respectively. In the case of emulsions the results are not conclusive as there was decline in the mosquito population of experimental villages due to natural causes also (Table III).

In the village sprayed with dieldrin there was a gradual rise in the density of mosquitoes after spray and during the fifth week the density was as high as 77.4 per cent of the pre-spraying density (Table IV).

The duration of residual effect as indicated by mosquito density and the ratio of mosquitoes caught from sprayed and unsprayed catching stations (S/US) is given in Table V.

The combined D.D.T. and B.H.C. spray was effective for 1—2 weeks more than either B.H.C. or D.D.T. applied separately. As suspension, B.H.C. was effective for a slightly longer period than D.D.T., whereas reversed was the case with emulsions. Dieldrin emulsion at the rate of 50mg./sq. ft. was found to be effective for about 4 weeks.

The apparent discrepancy between the last year and this year's results in the case of dieldrin may be explained by the fact, that last year, dieldrin was applied in the form of suspension. Many workers have pointed out the superiority of suspension over emulsion when applied on mud-plastered surfaces.

SUMMARY AND CONCLUSIONS.

1. Field trials on comparative effectiveness against mosquitoes of D.D.T. and B.H.C. both as suspensions and emulsions when applied separately and in combination and of dieldrin are discussed. Studies were conducted in villages near Palwal, District Gurgaon, Punjab (India).

2. The dosages employed were 50 mg. D.D.T./sq. ft., 10 mg. B.H.C. gamma isomer/sq. ft., 25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer/sq. ft. and 50 mg. dieldrin/sq. ft.

3. D.D.T. and B.H.C. combined spray (both as suspension and emulsion) was effective for a longer period (1 to 2 weeks) than either D.D.T. or B.H.C. applied separately.

TABLE II.

Percentage mosquito density as compared to pre-spraying density (pre-spraying) and ratio of mosquitoes caught from sprayed and unsprayed catching stations (suspensions).

		50 mg. D.D.T. per sq.ft.	10 mg. B.H.C. gamma isomer/sq.ft.	25 mg. D.D.T. & 5 mg. B.H.C. isomer/sq.ft. gamma	Comparison			
Week.		Percentage mosquito density as compared to pre-spraying density (sprayed catching stations).	Ratio of mosquitoes caught from sprayed and unsprayed catching stations.	Percentage mosquito density as compared to pre-spraying density (sprayed catching stations).	Ratio of mosquitoes caught from sprayed and unsprayed catching stations.	Percentage mosquito density as compared to pre-spraying density (sprayed catching stations).	Ratio of mosquitoes caught from sprayed and unsprayed catching stations.	Percentage mosquito density as compared to pre-spraying density.
0	...	100.0	0.60	100.0	1.15	100.0	0.89	100.0
1	...	7.1	0.07	0.6	0.06	1.8	0.02	173.7
2	...	6.1	0.06	1.2	0.02	2.7	0.06	142.2
3	...	4.2	0.03	7.6	0.23	2.7	0.07	126.7
4	...	7.7	0.11	1.2	0.03	2.0	0.12	87.9
5	...	24.6	0.16	7.3	0.08	14.6	0.34	114.6
6	...	50.0	1.13	26.0	0.20	23.1	0.30	70.8
7	...	20.7	0.23	67.0	1.00	28.7	0.31	79.6
8	...	7.4	0.10	70.7	1.02	63.02	0.62	101.8
9	...	11.6	0.21	69.8	1.11	67.8	0.75	133.4
10	...	16.2	0.80	11.7	0.71	13.1	0.39	78.9

TABLE III.

Weekly percentage density of mosquitoes as compared to density at the start of the experiment (pre-spraying) and ratio of mosquitoes caught from sprayed and unsprayed catching stations (emulsions).

Week.	50 mg. D.D.T./sq. ft.		10 mg. B.H.C. gamma isomer/sq. ft.		25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer per sq. ft.		Companiser.
	Percentage mosquito density as compared to pre-spraying density (sprayed catching stations).	Ratio of mosquitoes caught from sprayed and unsprayed catching stations.	Percentage mosquito density as compared to pre-spraying density (sprayed catching stations).	Ratio of mosquitoes caught from sprayed and unsprayed catching stations.	Percentage mosquito density as compared to pre-spraying density (sprayed catching stations).	Ratio of mosquitoes caught from sprayed and unsprayed catching stations.	
0	100.0	0.91	100.0	1.19	100.0	1.13	100.0
1	0.0	0.00	0.6	0.04	0.2	0.01	86.5
2	2.0	0.04	8.5	0.39	1.1	0.14	82.0
3	7.0	0.05	17.1	0.86	0.5	0.16	88.6
4	8.7	0.15	22.2	1.35	0.3	0.07	116.0
5	14.6	0.22	12.2	0.43	0.3	0.10	68.6
6	13.0	0.27	10.2	0.51	2.3	1.43	78.6
7	37.0	1.38	10.7	0.93	0.1	0.03	56.6
8	8.9	3.97	6.8	0.57	0.2	0.12	50.6

TABLE IV.

Weekly percentage density of mosquitoes as compared to density at the start of the experiment (pre-spraying), and ratio of mosquitoes caught from sprayed and unsprayed catching stations.

Weeks	Village sprayed with dieldrin 50 mg./sq. ft. Percentage mosquito density as compared to pre-spraying density (Sprayed catching stations.)	Ratio of mosquitoes caught from sprayed and unsprayed catching stations.	Comparison village (Not treated) Percentage mosquito density as compared to pre-spraying density.
0	100.0	0.50	100.0
1	3.5	0.09	173.7
2	2.1	0.09	142.2
3	7.4	0.09	126.7
4	11.8	0.16	87.9
5	77.4	0.79	114.9
6	41.7	0.38	79.6
7	23.3	0.52	79.8
8	22.3	0.34	101.8

TABLE V.

Duration of residual effectiveness in weeks.

Treatment	Dosage mg. per sq. ft.	Mosquitoes approached 50 per cent pre-treatment level (weeks).	Abrupt rise in sprayed/unsprayed house mosquito rates.
D.D.T. suspension.	50	6	6
γ B.H.C.	10	7	7
D.D.T. and γ B.H.C.	25 } 5 }	8	8
Dieldrin	50	12.8 per cent in the second week after which there was natural drop in the mosquito population.	7
D.D.T. emulsion	50	37 per cent in the seventh week after which there was natural drop in the mosquito population.	7
γ B.H.C.	10	Natural drop in the mosquito population after the fourth week.	4
D.D.T. and γ B.H.C.	25 } 5 }	Mosquito density remained low throughout the period of observation.	6
Dieldrin	50	5	5

4. As suspension B.H.C. was effective for a slightly longer period than D.D.T., whereas reverse was the case with emulsions.

5. It was found that under conditions prevalent in such villages, dieldrin emulsion at 50 mg./sq. ft. was effective for about four weeks against mosquitoes. Previous year's results indicated that dieldrin suspension applied at the same rate was effective for about seven weeks.

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DIELDRIN FOR MALARIA CONTROL

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INTRODUCTION.

SINCE the discovery of D.D.T., several new chlorinated hydrocarbon insecticides supposed to be more effective than D.D.T. have appeared on the market after several years of developmental work. Of these, special mention may be made of chlordane, aldrin and dieldrin.

Dieldrin has proved to be the most toxic of any of this class of insecticides to mosquitoes, flies and cockroaches (King, 1951). Dieldrin has been used extensively during the recent years for the control of a variety of agricultural and forest pests. Its use against insects of public health importance is, however, still in the developmental stage as dieldrin is comparatively new in this field.

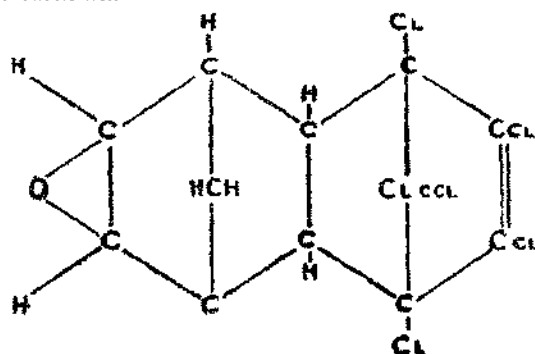
Kearns *et al.* (1949) studied the relative toxicity of dieldrin against ten species of insects including flies and cockroaches and found that dieldrin was most effective. According to these authors, the residual effectiveness of dieldrin seems to fall in order dieldrin, D.D.T., aldrin, heptachlor and B.H.C. Dieldrin has been found quite effective against sandflies (Stutz and Heidt, 1951) and houseflies (Simmons, 1950; Schoof *et al.*, 1951). Very few reports are, however, available at present about its utility in the control of mosquito larvae. Dieldrin has also been tried as an aerial spray against adult salt marsh mosquitoes resistant to D.D.T., but was found inferior to lindane (Deonier *et al.*, 1950). Pal *et al.* (1951) found it comparatively more effective than D.D.T. against *Anopheles stephensi* and *Culex fatigans* larvae and adults as a result of field studies in the villages around Delhi in India.

'It seems likely that dieldrin will have usefulness as mosquito larvicide and may be helpful addition to the list of mosquito residual sprays' (Russell, 1952).

CHEMICAL AND PHYSICAL PROPERTIES.

Dieldrin is the official name for an insecticidal product containing not less

than 85 per cent of 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a octahydro-1, 4, 5, 8-dimethano-naphthalene and not more than 15 per cent of insecticidally active related compounds. The principal constituent of this material is a white crystalline solid having the molecular formula $C_{12}H_4Cl_6O$ and the structural formula.



Molecular weight = 380.94

It is a white almost odourless crystalline solid, with a melting point of 114–116°C. The technical grade material may have a slight tinge of brown or rust colour. It was formerly known as compound '497' and its trade name is octalox.

It is moderately soluble in the usual organic solvents except aliphatic petroleum solvents and methanol in which it is sparingly soluble. The solubility of dieldrin in various solvents is given in Table I.

TABLE I.
Solubility of dieldrin in various solvents gm./100 c.c. at 25–30° C.

Acetone*	...	54
Aromex	...	25.6
Benzene*	...	75
Carbondisulfide	...	80
Chloroform	...	80
Iso propyl alcohol	...	3.4
Malariaol	...	4.6
Methyl alcohol*	...	4.9
Sovacide	...	26.8
Xylene	...	56

* After Brown (1951).

Dieldrin is insoluble in water, although decomposed by strong acids, it is completely stable to weak acids and to alkalies. It is only very slightly volatile but because of its very high toxicity to insects, it does have some fumigant properties in an enclosed space (Upholt, 1950). It is perhaps more stable than D.D.T. and as such may have a longer residual toxicity.

Dieldrin is readily formulated as a wettable powder, as an emulsifiable concentrate, as a xylene solution, and as low percentage dusts. The high melting point of this material makes it easy to formulate as a dust or a dust concentrate in any grinding equipment.

ESTIMATION OF DIELDRIN.

Sun and Sun (1952) have described a micro bioassay method for determining insecticidal residues with adult flies on dry deposits for both aldrin and dieldrin. Chemical method has been described by Julius Hyman and Company which is a modification of Swern's (Swern *et al.* 1947) analytical procedure for oxirane ring compounds.

TOXICITY TO MOSQUITOES.

(a) *Mosquito larvæ*.—For larval control, dieldrin has been found to be effective against many species, at concentrations ranging from 1 part in 100–300 million parts of water. For the control of mosquito larvæ under field conditions, dosages ranging from one half to one fourth of those normally recommended for D.D.T. have been found to be effective. 'In limited tests in the vicinity of Savannah, Ga., U.S.A., an emulsion of dieldrin applied at the rate of 1 lb. of dieldrin per acre has given an effective control for from 1 to 2 years of *Aedes*, *Culex* and *Anopheles* mosquito breeding in the ponds. This was conveniently applied by preparing a xylene concentrate containing 1 lb. of dieldrin and 2 per cent emulsifier (Triton X-155, X-100 or equal) per gallon of concentrate; diluting this concentrate with an equal volume of water and applying the finished spray at the rate of 2 gallons per acre, using the equipment and spraying technique normally used in larvicidal operations. This treatment is totally destructive to fish and most other aquatic organisms present at the time of treatment and retains its toxicity to some forms for many months. It should be used only in such places as temporary pond areas where there is no hazard to fish and other aquatic wild life' (Operational Memorandum: Technical Development Branch, Communicable Diseases Centre, Savannah, Ga., U.S.A.).

Deonier *et al.* (1950) have recorded comparative data for dieldrin and other insecticides in controlling *A. crucians* and found it very effective.

Whitehead (1951) used dieldrin pellets of bentonite containing 5 per cent dieldrin in xylene and applied in such a manner as to obtain 0.1, 0.05 and 0.02 lbs. of dieldrin per acre. He obtained 100 per cent kill of the larvæ with all the above dosages. Dieldrin was found to be more toxic and retained its toxicity better when exposed to weathering conditions than other insecticides tested.

Keller *et al.* (1951) carried out field tests against the larvæ of *A. crucians* and *A. quadrimaculatus*. Dieldrin was found to be the most toxic of all the compounds tested both as an oil solution and as an emulsion.

Reid and Wharton (1952) carried out experiments with dieldrin against anopheline larvæ in flowing water and concluded that dieldrin at 1 oz. per acre as an emulsion was only slightly inferior to D.D.T. emulsion at 4 oz. against *A. maculatus*.

Pal *et al.* (*loc. cit.*) tested dieldrin against *A. stephensi* larvæ and found that at dosages of 0.5, 1.0, 10, 20, 40 mg., per sq. ft., dieldrin was effective for 3, 3, 15, 33 and 56 days respectively. At dosages of 0.5 mg. and 1 mg. per sq. ft. of water surface it was effective against *Culex fatigans* larvæ on the day of application only; and at dosages of 10, 20 and 40 mg. per sq. ft. of water surface it was effective for 8, 24 and 47 days respectively.

(b) *Adult mosquitoes*.—For the control of adult mosquitoes, dieldrin has been found highly effective when used at the rate of 8 lb. of 25 per cent water dispersible powder or 1 gallon of 24 per cent emulsifiable concentrate per 100 gallons of ready-to-use spray formulation. Such a formulation should be applied to the wall surface to the point of run off; which will give a deposit of 10-20 mg. per sq. ft. Residual deposits applied in this manner have been found to remain effective from 4 to 12 weeks following application.

Dieldrin gave, good to excellent, reduction of both species of adult mosquitoes *A. sollicitans* and *A. taeniorhynchus* (Stutz and Heidt, 1951).

Pal *et al.* (*loc. cit.*) found as a result of laboratory and field experiments that chlordane, aldrin and dieldrin were effective for 7, 7 and 9 weeks respectively against mosquitoes both culicine and anopheline.

Davidson (1952) as a result of field studies on dieldrin as a residual insecticide against adult mosquitoes (*A. gambiae*, *A. funestus*, and *Culex fatigans*) in East Africa concluded that dieldrin was the most efficient insecticide tested.

TOXICITY TO FLIES.

(a) *As a fly larvicide*.—As a fly larvicide, dieldrin has been found to be very effective. Dilutions of less than 1 part per million in larval breeding media have been reported to completely prevent the emergence of adult flies (although some maggots may pupate).

Tests with chlordane and dieldrin used as a fly larvicide in dairies where D.D.T. resistant strains of flies are present, showed that satisfactory control could be achieved and maintained at least in some situations by this method (Simmon, 1950).

(b) *Adult flies*.—As in the case of adult mosquitoes, for the control of adult flies dieldrin has been found highly effective when used at the rate of 10-20 mg. per sq. ft.

A number of insecticides were investigated by Hansens (1950) as residual sprays for fly control both in the laboratory and in dairy barns; dieldrin gave

about a month of satisfactory fly control under field conditions when applied either at 8 or 20 lbs. of 25 per cent wettable powder per 100 gallons (the area sprayed has not been indicated). In laboratory tests, this material has given much better results. Poor results in the field are probably due to slow killing action of the insecticide.

Schoof *et al* (1951) used dieldrin for the citywide residual treatment for fly control in four municipalities with excellent results. However, a high degree of resistance in a field strain of house-flies to dieldrin has been detected approximately six months after the initial application of dieldrin.

TOXICITY HAZARDS TO MAN AND DOMESTIC ANIMALS.

Dieldrin administered in edible oil to albino rats demonstrates an acute LD-50 toxicity of 50-55 mg./kg. Other tests have shown an L.D. 50 of 87 mg./kg. (Lehman, 1951). This is roughly five times that of D.D.T. (Wharton, 1950). Chronic toxicity of dieldrin has also been determined. Rats are considerably more resistant than rabbits. Young calves are most susceptible.

Precautions.—Investigations indicate that, weight for weight, dieldrin is more toxic to laboratory animals and presumably, also to man, than D.D.T. It is, however, used at much lower concentrations (0.625 per cent dieldrin) and is probably as safe to handle as the biologically equivalent concentration of D.D.T. (5 per cent). Millions of acres have been treated in the U.S.A. with dieldrin and there is no record of any death or really serious illness. In the U.S.A., the following precautions are actually recommended:—Users should at all times use proper care in handling dieldrin. Waterproof and xylene resistant gloves and other protective clothing, together with a simple respirator, should be worn when handling technical dieldrin, or highly concentrated dusts or wettable powders, or emulsion concentrates. These precautions are particularly necessary when operators are exposed continuously to contamination during mixing, dusting or spraying operations. Every feasible precaution should be taken to avoid the inhalation of dieldrin dusts or sprays and to avoid skin contamination. Contaminated skin areas should be washed immediately with soap and water and contaminated clothing should be thoroughly cleaned or washed before being re-worn.

Dieldrin, like most other chlorinated hydrocarbon insecticides, must not be applied to dairy animals or to food containers nor used in the presence of exposed food.

SUMMARY.

The chemical, physical and insecticidal properties of dieldrin have been discussed. Its use in the control of mosquitoes and houseflies has been reviewed. Dieldrin is a promising insecticide for the control of house-flies and mosquitoes. It is comparatively more toxic than D.D.T., but its ready-to-use concentration (0.625 per cent) is probably as safe to handle as the biologically equivalent concentration of D.D.T. (5 per cent).

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TOXICITY OF SYNTHETIC AND NATURAL PYRETHRINS INCORPORATING SYNERGISTS AGAINST MOSQUITOES.

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INTRODUCTION.

Since the discovery of D.D.T., B.H.C., and other residual insecticides, the use of pyrethrum and other space sprays in malaria control has been relegated to the background because unlike residual insecticides, space sprays do not have prolonged persistent lethal effect. However, due to its quick knockdown and the rapidity with which mosquito population can be checked, this method still remains the only measure available which is immediately effective in controlling malaria epidemics.

The wartime shortage of pyrethrum in some countries stimulated work on synergists for pyrethrum and on the synthesis of its active principles.

These efforts have recently resulted in the discovery of a number of synergists (Haller *et al.*, 1942; McGovran and Fales, 1944; David and Bracy, 1944; Gersdroff and Gertler, 1944 and Wachs, 1947) and the synthesis by Schechter *et al.* (1949) of organic esters analogous to cinerin, one of the toxic constituents of pyrethrum.

The toxicity of synthetic and natural pyrethrins incorporating different synergists such as n-propyl isome, piperonyl butoxide and cashewnut shell oil has been investigated and the results obtained are presented in this publication.

SYNTHETIC PYRETHRINS.

One of these synthetic compounds is manufactured commercially under the trade name of 'Allethrin'. This synthetic pyrethrin-like compound has been variously named as allyl homologue of Cinerin, *Allyl Cinerin*, *Allethrin*, *pyresyn* and *Devinyll pyrethrin I* or *PV PY* (Russell, 1952)*. Allethrin has been

*Chemical name: dl-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one esterified with a mixture of Cis-and-trans-dl-chrysanthemum monocarboxylic acid.

evaluated against a wide variety of insects and it has proved more effective than natural pyrethrins against some species and less so against others (King, 1951). As an aerosol spray against mosquitoes, Allethrin gave satisfactory results (Fales *et al.*, 1951). Gersdroff *et al.* (1951) found that n-propyl isome, a synergist, increased its toxicity to houseflies. Allethrin is reported to be comparatively less liable to decomposition when exposed to light or on storage (Grannet *et al.*, 1951) than natural pyrethrins; it has, therefore, an appreciably longer residual action than natural pyrethrins.†

A formulation under the name of 'Allexeel 20' which contains 1 per cent Allethrin and 5 per cent synergist, n-propyl isome, was tested both as a space and residual spray against *Culex fatigans*.

Synergists.—Piperonyl butoxide** and cashewnut shell oil besides n-propyl isome were selected for testing their action as synergists in these investigations. Piperonyl butoxide has been known as an effective synergist for pyrethrum since 1947 (Wachs *loc. cit.*). Cashewnut shell oil is an indigenous product available in large quantities in India and has been found to be very toxic to mosquito larvae and pupæ (Wats and Bharucha, 1938).

AS SPACE SPRAY.

Technique.—The technique adopted was a modification of the Peet-Grady method (1928). Standard test insects are exposed for a fixed period to a known quantity of insecticidal spray atomised inside a test chamber. After a known exposure period they are collected and kept under observation for 24 hours after which mortality is recorded.

Test chamber.—A room 10×15×10 feet, specially fitted for the purpose, serves as a test chamber. The floor, walls and ceiling are all painted white after making sure that there are no cracks or crevices anywhere. The windows are provided with mosquito netting, and the glass panels of doors and windows pasted with tissue paper so that the lighting in the chamber is dim and uniform. The entrance to the room is provided with two sets of doors, one with mosquito-proof netting and the other of wood. The latter has a small doorlet 6×12 inches at a height of about 5½ feet through which the room is sprayed. This small doorlet has a circular opening (3 inches in diameter) which can be closed, through this opening mosquitoes are blown in the test chamber.

The room is provided with a 100-watt electric bulb, a wet and dry bulb thermometer, and an exhaust fan.

Procedure.—The room is ventilated by working the exhaust fan and keeping the doors and windows open for half an hour before commencing the tests. A white sheet is spread on the floor. This sheet is washed daily and is exposed to sunlight for at least an hour before each test. Before spraying the insecti-

† Operational memoranda on economic poisons: Communicable Disease Centre, Technical Development Service, Savannah, Ga., U.S.A.

* Marketed by Messrs. S. A. Pennick & Co., U.S.A.

** A sample of 'Pyrocon 20' containing 2 per cent pyrethrins and 20 per cent piperonyl butoxide marketed by Messrs. Bombay Chemicals Ltd., Bombay, and cashewnut shell oil pyrethrum spray prepared in the laboratory, were used for these tests.

side, the windows and doors are closed, the wet and dry bulb readings taken and the light inside the room switched off. The chamber is sprayed with 7.5 c.c. of the insecticidal formulation (5 c.c. per 1,000 c. ft.). The insecticide is sprayed into the chamber through the opening provided in the wooden door with an electric-driven sprayer fitted with DeVilbiss spray gun with No. 1 nozzle and specially designed funnel, to spray any measured quantity of the spray to the last drop (Fig. 1). Within 30 seconds of this, the test insects are blown into the chamber. The insects are introduced after atomising the spray into the room (unlike Peet-Grady method): this ensures that the insects do not receive a direct hit of the spray. This may appear unnatural but in order to detect minute differences between the efficacy of different space sprays, any uncontrollable factor, such as direct hit to mosquitoes, should be avoided.

The insects are exposed to the spray for 20 minutes. At the end of this period, the insect collector switches on the light and collects all the insects whether knocked down on the sheet or flying. The knockdown rate is noted.

The mosquitoes are kept in small cages under optimum conditions of temperature and humidity and are provided with cotton pads soaked in a solution of glucose as food supply. Mortality is recorded after 24 hours (Appendix I).

A comparison test is run simultaneously with standard 0.1 per cent pyrethrum spray in kerosene oil.

A blank test is also performed, 20 mosquitoes from the same batch as are used in the routine tests are kept under observation for 24 hours in holding

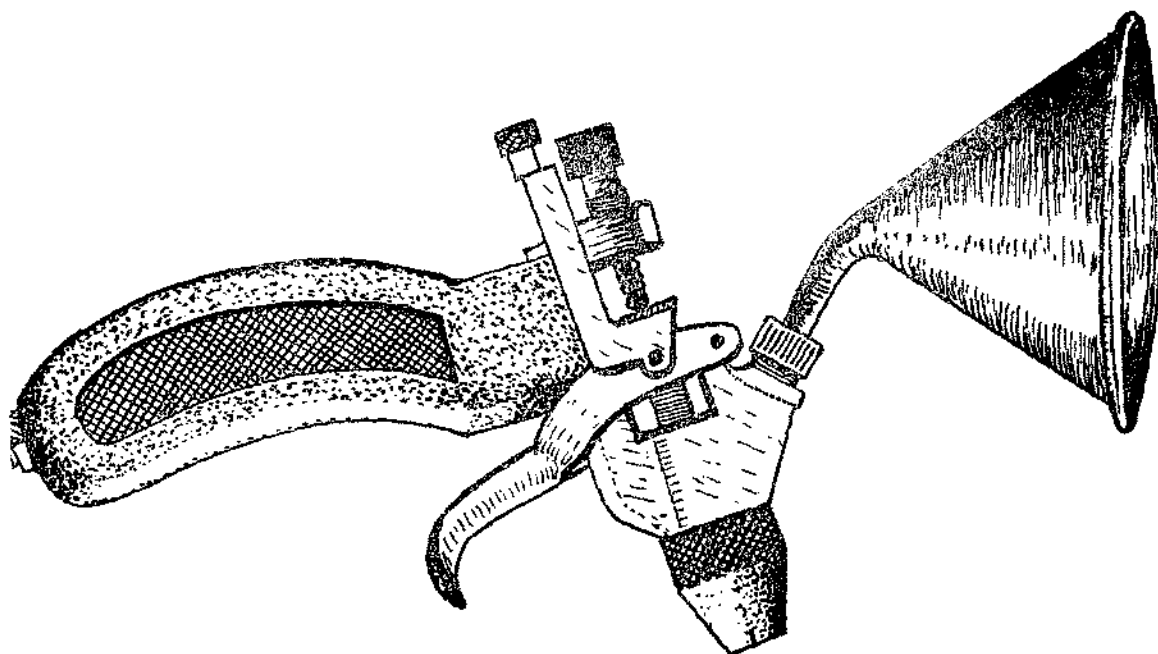


Fig. 1 DeVilbiss spray gun fitted with a specially designed funnel.

TABLE I.
Percentage knockdown and 24 hours mortality (*Culex fatigans* ♀♀)
after 20 minutes exposure to insecticidal sprays.

	Pyrethrum (0.1 per cent allethrin and 0.5 per cent n-propyl isome.)	Pyrethrum spray (0.1 per- cent pyre- thrins)	Pyrethrum and piperonyl butoxide spray (0.05 per cent pyrethrins and 0.5 percent pip- eronyl butoxi- de.)	Pyrethrum spray (0.1 per cent pyrethrins).	(a) PERCENTAGE KNOCKDOWN		Pyrethrum and cashewnut shell oil (0.07 per cent pyrethrins and 0.03 per cashewnut shell oil.)	Pyrethrum spray (0.1 per cent pyrethrins).
					Pyrethrum and cashewnut shell oil (0.03 per cent pyrethrins; and 0.02 per cent cashewnut shell oil.)	Pyrethrum spray (0.03 per cent pyrethrins)		
1	80.0	70.0	90.0	94.0	96.0	72.0	96.0	98.0
2	81.3	84.2	98.0	92.0	90.0	82.6	93.0	92.0
3	78.0	92.0	98.0	98.0	92.8	76.0	97.0	84.0
4	76.0	84.0	99.0	98.0	92.0	80.2	98.0	96.0
5	76.0	88.8	99.0	94.0	92.0	84.2	..	..
6	74.0	88.5	99.0	92.0	91.2	72.0	..	..
7	82.0	84.5	..	..	88.8	72.8	..	..
8	..	..	98.0	94.3	91.2	80.6	..	..
Average	78.3	84.5	98.0	94.3	91.75	77.55	97.55	93.0
(b) 24 HOURS PERCENTAGE MORTALITY.								
1	93.3	80.5	98.0	88.0	93.2	72.8	93.0	83.0
2	82.8	90.0	99.0	92.0	88.0	79.2	96.0	94.0
3	85.0	88.5	97.0	96.0	94.4	82.0	93.0	80.0
4	87.3	75.0	97.0	82.0	84.8	72.2	96.0	84.0
5	92.0	80.6	98.0	90.0	83.2	79.2	..	..
6	90.0	91.0	96.0	86.0	86.4	80.4	..	..
7	98.0	84.4	..	..	80.6	76.2	..	..
8	..	..	97.16	98.0	76.8	60.4	..	..
Average	80.7	84.2	97.16	98.0	85.92	75.3	91.5	83.5

cages. If mosquito mortality after 24 hours in the blank test is more than 25 per cent, the experiments are rejected.

Two days old standard laboratory-bred *Culex fatigans* mosquitoes were used as test insects in these trials (Appendix II).

'Allexel 20' was diluted with kerosene grade II in the proportion of 1:10 so as to contain 0.1 per cent allethrin and 0.5 per cent n-propyl isomer. 'Pyrecon 20' was diluted with kerosene in the ratio 1:40 so as to contain 0.03 per cent pyrethrins and 0.5 per cent piperonyl butoxide. Pyrethrum cashewnut shell oil sprays were prepared containing 0.03 per cent pyrethrins and 0.02 per cent cashewnut shell oil, and 0.07 per cent pyrethrins and 0.03 per cent cashewnut shell oil. 78.3 per cent of the mosquitoes were knocked down after 20 minutes exposure to 'Allexel 20' spray. 89.7 per cent of the mosquitoes were, however, eventually killed in 24 hours. Mosquito knockdown and mortality figures in the case of standard pyrethrum spray were found to be 84.5 and 84.2 per cent respectively (Table I). 'Allexel 20' compares favourably with natural pyrethrins when used as a space spray against *Culex* mosquitoes. When pyrethrum piperonyl butoxide spray was tested in a similar manner, 98.6 per cent of the mosquitoes were knocked down after 20 minutes exposure and 97.1 per cent were found dead after 24 hours.—Standard pyrethrum spray when tested simultaneously produced 94.3 per cent knockdown and 88 per cent 24 hours kill (Table I). Pyrethrum with half the dosage produced comparable kill when piperonyl butoxide was incorporated in the spray.

When pyrethrum cashewnut shell oil spray containing 0.03 per cent pyrethrins and 0.02 per cent cashewnut shell oil was tested in a similar manner, 91.7 per cent of the mosquitoes were knocked down after twenty minutes exposure and 85.9 per cent were found dead after 24 hours holding period. Pyrethrum spray (0.03 per cent pyrethrins) produced 77.5 per cent knockdown and 75.3 per cent mortality when tested simultaneously. Pyrethrum cashewnut shell oil spray containing 0.07 per cent pyrethrins and 0.03 per cent cashewnut shell oil produced 97.5 per cent knockdown and 94.5 per cent mortality. Standard pyrethrum spray (0.1 per cent pyrethrins) produced 93.0 per cent knockdown and 86.5 per cent mortality after 24 hours. Cashewnut shell oil effectively increases the toxicity of pyrethrins to *Culex* mosquitoes and may be used with advantage as a synergist for pyrethrum sprays (Table I).

AS RESIDUAL SPRAY.

Technique.—Laboratory technique for testing the biological effectiveness of insecticidal deposits described by Pal (1951) was adopted. Two days old, standard laboratory bred *Culex fatigans* were exposed to sprayed panels (glass, wood or mud). The test insects were kept in contact with the sprayed surface for 15 minutes and were then removed into holding cages. The mortality was recorded after 24 hours. Tests were repeated every week. When the mortality rate dropped to 50 per cent, it was considered that the residual effectiveness had worn off.

'Allexel 20' was applied at the rate of 2 c.c. per sq. ft. giving a deposit of 20 mg. allethrin and 100 mg. n-propyl isomer/sq. ft. Pyrethrum piperonyl butoxide (1.0 per cent pyrethrins and 5 per cent piperonyl butoxide) and

pyrethrum cashewnut shell oil (1.0 per cent pyrethrins and 0.5 per cent cashewnut shell oil) were also sprayed at the rate of 2 c.c. per sq. ft. giving deposits of 20 mg. pyrethrins with 200 mg. piperonyl butoxide and 20 mg. pyrethrins with 10 mg. cashewnut shell oil per sq. ft. respectively. Comparison tests were carried out with panels treated with a deposit of 20 mg. pyrethrins/sq. ft.

Wooden and mud panels treated with 'Allelcel 20' showed very little residual toxicity to mosquitoes even during the first week after spray, though the deposits on glass panels were slightly more effective (Table II).

TABLE II

24 hours' percentage of mortality (*C. fatigans* ♀♀) after 15 minutes' exposure to surfaces treated with insecticides.

Age of insecticidal deposit in weeks.	ALLELCEL 20 MG. and N-PROPYL ISOMER 100 MG./SQ. FT.			PYRETHRINS 20 MG./SQ. FT.		
	Glass surface.	Wooden surface.	Mud surface.	Glass surface.	Wooden surface.	Mud surface.
1	37.5	30.8	11.0	3.7	20.5	8.0
2	20.0	19.0	...	...	13.0	...

On glass panels pyrethrum-piperonyl butoxide combination was found to be effective for 5 weeks, pyrethrum cashewnut shell oil for 3 weeks and pyrethrum alone for about 1-2 weeks (Table III).

TABLE III.

24 hours percentage mortality (*Culex fatigans* ♀♀ mosquitoes) after 15 minutes exposure to glass surfaces treated with insecticides (average of 5 replicate tests).

Age of insecticidal deposit in weeks.	Percentage mortality in mosquitoes exposed to surfaces treated with 20 mg. pyrethrins and 200 mg. piperonyl butoxide per sq. ft.	Percentage mortality in mosquitoes exposed to surfaces treated with 20 mg. pyrethrins and 10 mg. cashewnut shell oil per sq. ft.	Percentage mortality in mosquitoes exposed to surfaces treated with 20 mg. pyrethrins per sq. ft.
1	95.0	75.0	90.0
2	85.0	65.0	87.5
3	75.0	67.5	30.0
4	65.0	45.0	30.0
5	60.0	32.5	25.0
6	42.5	0.0	0.0

There is a distinct advantage in incorporating these synergists to pyrethrum sprays. Yates and Lindquist (1950) and Chamberlain (1950) also found that piperonyl butoxide increased the residual toxicity of pyrethrum deposits.

Metcalf and Wilson (1945) tested the residual toxicity of pyrethrins against *A. quadrimaculatus* and suggested that further field trials should be carried out on the toxicity of pyrethrum deposits. Kruse and Konchady (1950) tried combined pyrethrum piperonyl butoxide spray on mud panels for residual toxicity against *A. quadrimaculatus* and concluded that their laboratory results justified further investigations on pyrethrum sprays incorporating synergists for use in residual house-spraying.

Field studies were, therefore, conducted in four villages near Palwal, District Gurgaon, Punjab (India). Three of the villages were treated with pyrethrum (20 mg. pyrethrins/sq. ft.), pyrethrum with cashewnut shell oil (20 mg. pyrethrins and 10 mg. cashewnut shell oil per sq. ft.) and pyrethrum with piperonyl butoxide (20 mg. pyrethrins and 200 mg. piperonyl butoxide per sq. ft.) respectively and the fourth unsprayed village was kept under observation for purposes of comparison (Table IV). The houses in these villages are 'kutcha' with mud-plastered walls and thatched roofs. *A. culicifacies* is the vector mosquito. Spraying was carried out by experienced workers and under the direct supervision of one of the authors. Stirrup pump fitted with an oil resisting hose and brass lance and a nozzle of 3/64 inch giving a cone-shaped spray was used. In each village, four suitable mosquito catching stations were established where routine collections were made to determine the density of mosquitoes. Total mosquito catches were made every week from these catching stations by spraying a weak solution of pyrethrum and collecting all the mosquitoes knocked down on a sheet spread on the floor.

TABLE IV.
Particulars of experimental villages, for formulation and dosages
of insecticides employed.

Name of village.	Number of houses.	Population.	Total area sprayed, sq. ft.	Insecticide and formulation used.	Dosage mg./sq. ft.	Quantity sprayed c.c./sq. ft.
Sohta ...	31	109	13,482	1 per cent pyrethrins in kerosene.	20 mg. pyrethrins	2
Kashipur ..	18	94	15,082	1 per cent pyrethrins and 0.5 per cent cashewnut shell oil in kerosene.	20 mg. pyrethrins and 10 mg. cashewnut shell oil.	2
Dholagarh...	25	105	14,974	1 per cent pyrethrins and 10 per cent piperonyl butoxide in kerosene.	20 mg. pyrethrins and 200 mg. piperonyl butoxide.	2
Allahpur ..	115	575	Comparison	Not treated.	...	...

In Table V are summarised the entomological data.

TABLE V.

Total mosquito catch, percentage mosquito density as compared to pre-spraying density, and ratio of mosquitoes caught from sprayed and unsprayed catching stations.

Number of weeks.	Village sprayed with pyrethrum and piperonyl butoxide spray (1.0% pyrethrins + 5.0% piperonyl butoxide).		Village sprayed with pyrethrum cashewnut shell oil spray (1.0 per cent pyrethrins and 0.5 per cent cashewnut shell oil).		Village sprayed with pyrethrum (1.0 per cent pyrethrins).		Comparison village. (Not treated)
	Percentage mosquito density as compared to pre-spraying density.	Ratio of mosquitoes collected from sprayed and unsprayed catching stations (S/US).	Percentage mosquito density as compared to pre-spraying density.	Ratio of mosquitoes collected from sprayed and unsprayed catching stations (S/US).	Percentage mosquito density as compared to pre-spraying density.	Ratio of mosquitoes collected from sprayed and unsprayed catching stations (S/US).	
0	100.0	1.00	100.0	0.9	100.0	1.27	100.0
1	10.4	1.05	9.9	0.1	0.0	0.0	173.7
2	4.6	0.16	134.0	0.51	13.0	0.23	142.3
3	48.0	0.86	41.4	0.83	24.7	0.69	126.7
4	32.0	0.44	46.4	1.13	25.9	1.25	87.9
5	42.0	0.24	233.7	1.2	60.4	1.0	114.0
Resprayed							
6	9.6	0.09	5.1	0.2	35.1	0.26	79.8
7	55.3	1.44	29.6	1.19	83.0	2.59	79.6
8	24.3	0.28	61.2	2.31	57.5	1.27	101.8
9	105.0	1.03	51.0	0.9	80.6	1.75	115.1

It would be seen from the data presented that there was marked reduction in the mosquito population during the first week after spray but the density gradually increased to above 50 per cent of the pre-spraying mosquito density in most cases.

It appears that pyrethrum formulations incorporating synergists do not exhibit any residual toxicity to insects on mud surfaces. It may perhaps be due to the fact that pyrethrins deteriorate fairly rapidly and piperonyl butoxide does not prevent deterioration of pyrethrins under field conditions. Cashewnut shell oil was also found unsatisfactory in this respect; moreover, the spary men did not like it as it has an irritant effect on the skin.

SUMMARY AND CONCLUSIONS.

A new bio-assay method for testing space sprays using conventional type of a living room has been described. Toxic properties of allethrin, synthetic pyrethrin-like compound and pyrethrum sprays incorporating n. propylisome, piperonyl butoxide and cashewnut shell oil as synergists against mosquitoes both as space and residual sprays, have been discussed. As space spray, allethrin compares favourably with the natural pyrethrins. Although claimed to be more stable than natural pyrethrins, the residual toxicity of allethrin was found to be as poor as natural pyrethrins. Both piperonyl butoxide and cashewnut shell oil were found to increase the toxicity of pyrethrum when used as a space spray, but under field conditions on mud surfaces no residual toxicity against mosquitoes was obtained.

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Proforma for testing of space sprays.

Cubic space of the testing chamber = 1500 c. ft.

[illegible]

APPENDIX II.

The test insects *Culex fatigans* Weidmann required for routine insecticidal evaluation are reared in the insectories of the Malaria Institute of India, Delhi. The insectory rooms are located in the basement where temperature ranges from 75° to 85°F and humidity from 70 per cent to 80 per cent. The colony is maintained in a cage prepared from cellotax board (2×2×2 feet). The female mosquitoes are fed on sparrows (*Passer domesticus*). The egg rafts are collected in petri dishes containing small quantities of water. The larvae are reared in large enamelled photographic trays (12×18 inches) containing hay infusion as larval food. Hay infusion is prepared by putting about 45 gm. of hay in a tray containing 3 to 4 litres of water. This is kept for 48 hours for the development of infusoria after which mosquito larvae are introduced. The pupae are placed in breeding cages (12×12×12 inches) for hatching. The adult mosquitoes are held for 48 hours and fed on glucose.

THE DISTRIBUTION OF INSECTICIDES ON SPRAYED SURFACE AND SPECIFICATIONS FOR STIRRUP PUMP SPRAYERS.*

BY

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[July 27, 1951.]

INTRODUCTION.

SPRAYING equipment must fulfil many conditions from both the theoretical and the practical point of view. The efficiency of a sprayer lies in its ability to distribute the insecticide as evenly as possible in order to kill the maximum number of insects with a minimum of material.

Spraying operations can be carried out either by interrupted or continuous action.

An interrupted spray action suggests covering a surface by first treating a portion of an area with a certain quantity of insecticide. Flow of the insecticide is then interrupted until the nozzle is re-directed to a new portion of the area, at which time insecticidal flow is allowed to continue. Coverage of the adjoining areas may slightly overlap.

As it is impracticable to direct the nozzle to the right place with every discharge, the alternative method which can be used is the continuous spray action. Here the nozzle is moved with a constant speed at a prescribed distance from and parallel to the surface. The direction of movement can be vertical or horizontal and, whichever procedure is used, does not materially affect the application.

The interrupted spray theoretically permits a more uniform coverage than the continuous spray. Even distribution of spray liquid is possible under conditions only when the pyramids of spray liquid are either triangles, rectangles, squares or regular hexagons. Obviously such nozzles cannot be constructed.

On the other hand it is apparently much easier to construct a nozzle which gives a cone-shaped pattern. Here again the circles that such a nozzle will produce with each discharge do not cover the entire surface unless they partially overlap each other.

*This document was submitted for consideration of the Expert Committee on Insecticides of the World Health Organization at its 3rd session held at Savannah, Ga., U.S.A., from July 27 to August 4, 1951. Revised specifications approved by the World Health Organization are reproduced in original. The specifications chart does not strictly conform to these specifications but has been included for ready reference.

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The smallest overlap can be obtained when the discharge is so directed that the centres of the circles form triangles with side equalling $R\sqrt{3}$ when R is the radius of the circle. Thus the overlapped zones may be covered with a double dosage.

Besides, the number of discharges which must be given, and the labour which is involved with interrupted spray action, do not suggest its practicability. Continuous spray action may, therefore, be preferred.

In both cases, more even distribution of the insecticide on the surface may be achieved by changing the form of spray. The fan—or flat-shaped spray is often considered as the answer to this problem. But it is obvious that the flat-shaped spray is obtained by altering the form of the cone-shaped spray. The distribution of insecticide on the surface could be the same as when a cone-shaped spray is used. Improvement can be obtained only when it is possible to form a uniform spray pattern on the surface that is bordered by two parallel lines. Even so it is still doubtful whether perfection of distribution can be obtained in any variation of this nozzle type.

Another reason for an uneven spray distribution is due to the change in pressure, since the difference between the maximum and minimum pressures may be considerable; a factor depending to a considerable extent upon pump manipulation. Still another deformation depends upon wear and tear of the nozzle orifice both by usage and maltreatment by the workmen.

Many other factors may be responsible for an uneven spray. For instance, the sprayman may move the nozzle with varying speed, or may change the distance between the nozzle and the surface. Considerable variation may also occur if the two adjoining strips do not overlap properly.

Summing up, it may be expected that the ideal of an even distribution is not achieved by the existing methods of application. However, better results may be achieved if the nozzle material used is such that possibility of abrasive deformation is reduced to a minimum, and the nozzle is so constructed that the spray pattern on the surface is bordered by two parallel lines as far as possible. The most important variation in all sprayings is due to the personal factor, which may be remedied by thorough training of the spraymen.

SPECIFICATIONS FOR STIRRUP PUMP TYPE SPRAYERS AS APPROVED BY THE EXPERT COMMITTEE ON INSECTICIDES, WORLD HEALTH ORGANIZATION.

There are two types of stirrup pumps—single—and double-barrelled—and the following specifications are applicable to both. Experience has shown that both units give satisfactory results. Specifications are given in general terms without exact measurements since satisfactory units of both types may have minor variations.

In general, the device shall be so designed that delivery will be as nearly uniform as possible over the entire operative cycle; the committee recommends that there shall not be more than 35 per cent variation between maximum and minimum delivery under uniform operation.

All metals, gaskets, tubing, and other parts coming into contact with the spraying liquids shall be chemically resistant to them. The weight of the sprayer with all auxiliaries, shall be not more than 3.18 kg. (7 pounds) for single barrel and 5.44 kg. (12 pounds) for double-barrelled units.

Barrel.—The barrel shall be smooth bore, with ability to withstand consistent rough-usage in the field. It shall be capable of withstanding a pressure of the order of 13.6 atmospheres (atm.) (200 pounds per square inch (psi). without distortion.

One end of the barrel shall be fitted with a heavy-duty, plunger shaft housing-guide, and the other with a combined strainer assembly and foot valve.

In a single-barrel pump, the barrel shall be not more than 56 cm. (22 inches) in length, and in a double-barrelled pump, not more than 20 cm. (8 inches). The bore shall be not less than 1.8 cm. (0.7 inch) nor more than 3.2 cm. (1.25 inch).

The pump inlet 'Crow feet' shall be sufficiently strong to withstand a crushing load of 68 kg. (150 pounds) without damage.

Plunger assembly.—The plunger assembly shall be capable of being easily removed for emergency field cleaning and repairs.

The plunger shaft shall be made highly torque-resistant (Appendix I).

The pump handle shall be securely attached to the plunger shaft in such a manner that it will not tire the operator with unnecessary strain during the downward stroke of the piston. It shall be either 'D' or 'T' type and shall have a total length of not less than 15 cm. (6 inches) available for the grip of the operator's hands. The handle may be of wood or metal.

A device shall be provided which will limit upward and downward travel of the plunger shaft within the barrel in order to avoid damage to either member (plunger shaft or barrel) at the end of its stroke. Sufficient overlap shall be provided within the barrel to maintain stability of the plunger shaft along its longitudinal axis.

The cup leather of the plunger assembly shall be of chrome leather impregnated with graphite and shall be a good fit within the barrel wall.

Valves.—Valves shall be air and liquid tight.

The travel of the liquid inlet valve shall be limited by an appropriate strap.

A suitable valve shall be fixed at the intercommunicating aperture between the pump and air chamber in the case of double-barrelled pumps.

A relief valve opening at a pressure of 3.4 atm. (50 psi.) shall be fitted either into the unit or near the attachment of the hose with the barrel.

Strainer.—A strainer shall be provided at the inlet to the pump in the barrel base. The openings in this strainer shall have no dimension greater than 90 per cent of the smallest dimension of the nozzle orifice. The total open area of the

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strainer shall be not less than 0.5 cm.² (0.08 sq. inch). The strainer shall be mounted in such a way, or shall itself have sufficient structural strength, that it will not be subject to accidental puncture. The strainer shall be easily removable and replaceable.

Delivery outlet.—The delivery-outlet hose-attachment shall be an adaptor about 2.5 cm. (1 inch) long, screwed or welded to the unit.

Hose.—The hose shall meet the World Health Organization specifications for this item (Appendix II).

It shall not be less than 1 cm. (0.38 inch) inside diameter and shall be 3 m. (10 ft.) long, unless otherwise specified by the purchaser.

Lance.—The lance shall be of rigid seamless construction and easily detachable for the purpose of cleaning.

The joint shall be leak-proof.

The lance shall be approximately 60 cm. (2 feet) long. Extension lances, if specified, shall be of similar length and construction.

Cut-off valve.—The cut-off valve shall meet the World Health Organization specifications for this item (Appendix III).

Nozzle.—The nozzle shall comply with the World Health Organization specifications for this item (Appendix IV).

Stirrup.—The stirrup comprises the barrel clamp, vertical bracket, and foot rest.

The vertical bracket shall be made of mild steel of not less than 1.3 cm. (0.5 inch) in diameter or of an alternative material of equal strength. The design shall be such that the vertical bracket can be readily clamped on the barrel so that its height is adjustable. It shall be 6.3 cm. (2.5 inches) from the pump barrel and attached at right angles to the foot rest.

The foot rest shall not be less than 12.7 cm. (5 inches) in diameter.

Plunger-shaft packing.—The plunger-shaft packing shall be chemically resistant and so held in place that leakage of the liquids shall not occur under a pressure of 4.08 atm. (60 psi.).

Liquid passages.—No liquid passage within the unit may be less than 0.25 cm.² (0.04 square inch) in cross-sectional area, exclusive of the nozzle.

PERFORMANCE OF STIRRUP PUMP TYPE SPRAYER.

Stirrup pump pressure discharge is related to length of the working stroke and to the number of strokes per minute. The length of the working stroke in a double-barrel stirrup pump is about 4.5 inches (11.43 cm.), the average pumping speed is about 27 strokes per minute. In the case of the single-barrel stirrup pump, length of the working stroke is about 12.5 inches (31.7 cm.) and the aver-

PLATE VI

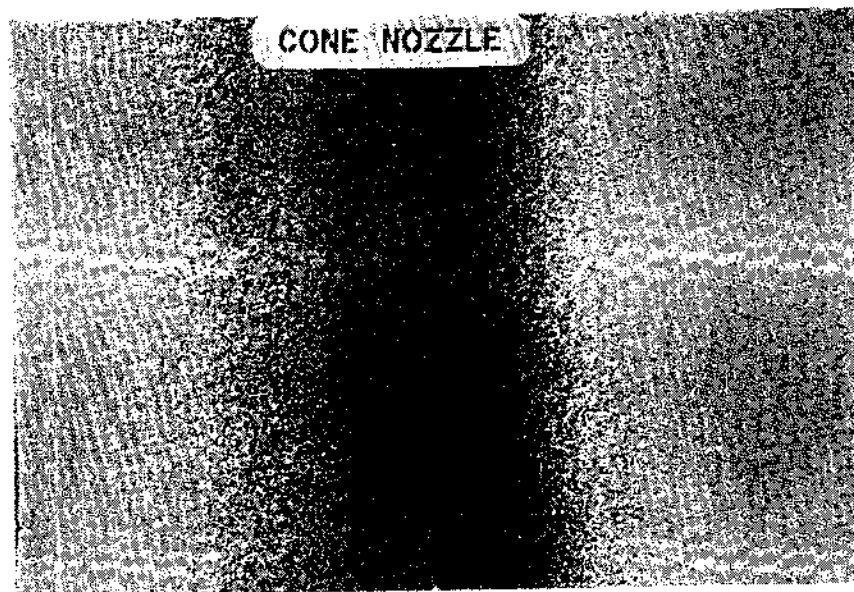


Fig. 1. - Spray pattern with cone nozzle.

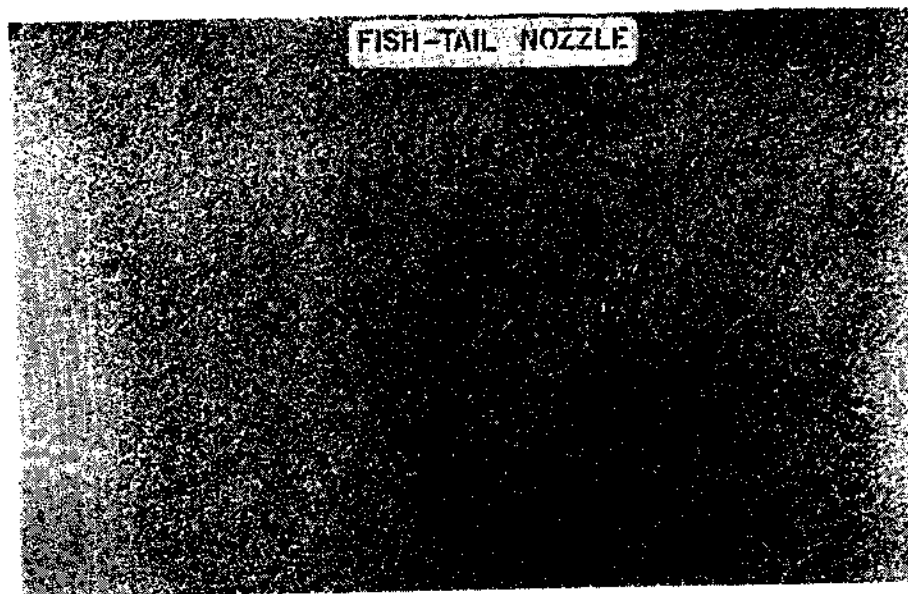


Fig. 2. - Spray pattern with fish-tail nozzle.

age pumping speed is about 15 strokes per minute, at which speed the maximum pressure developed on both cone and flat spray nozzles is in the order of 75-100 lbs.

The discharge rate of a flat nozzle with a bore of 0.039 inch and a cone-shaped nozzle of 0.046 inch bore are 713 and 675 cubic centimetres per minute respectively.

In any unit of time (say five minutes) the variation in the discharge rate from the nozzle is negligible though the variations in the discharge rate on the up and on the down stroke of the piston are considerable. This is due, not only to the variations in pump assembly characteristics but also the human factor involved. The actual output will depend on two factors—the bore of the nozzle and the pressure exerted. The smaller the bore, the greater the pressure developed with a constant pumping speed.

In the application of residual sprays, emphasis should be laid on the 'personal factor' because some of the inherent shortcomings in spraying equipment may be remedied to a certain extent by slightly overlapping each swath, thus compensating for the scattered droplets which appear at the edges of the spray columns (Plate VI, Figs. 1 and 2).

The droplet size varies considerably from 50 to 600 μ with both cone and flat type nozzles.

If the discharge rate is from 360 to 720 c.c. per minute, it is possible to cover the surface properly without undue haste. It has been observed that with identical equipment five men operating 2 stirrup pumps can spray about 56,300 sq. ft. in 2.5 hours while 8 men operating 3 stirrup pumps can spray an area of 81,189 sq. ft. in 3 hours. This indicates that a five-man spray team with two stirrup pumps is a more efficient unit.

SUMMARY.

Spraying equipment must fulfil many conditions from both the theoretical and the practical point of view. These conditions have been described and possibilities of improving upon the existing spraying equipment have been discussed. Specifications for stirrup pump sprayers as approved by the World Health Organization have been included along with a specification chart of the stirrup pump commonly used in India. The performance of the stirrup pump has also been discussed.

ACKNOWLEDGEMENTS.

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The specification chart has been prepared through the courtesy of the Central Water & Power Commission, New Delhi.

APPENDIX I.

TEST FOR STRENGTH OF STIRRUP-PUMP-TYPE SPRAYER PLUNGER-SHAFT.

The plunger shaft, stripped of all its parts, shall be placed in a horizontal position and supported across two iron or steel rods 2.5 cm. (1 inch) in diameter and 40 cm. (16 inches) apart between centres. A load of 22 kg. (48.5 pounds) shall be applied for not less than 5 minutes at a point on the upper side of the plunger shaft midway between the supports. After removal of the test load, there shall be no permanent deflection of the shaft.

APPENDIX II.

SPECIFICATION FOR CHEMICAL-RESISTANT COTTON- REIN FORCED RUBBER HOSE.

GENERAL.

Hose covered by this specification shall include sizes (inside diameter) 6 mm. to 13. mm. ($\frac{1}{4}$ to $\frac{1}{2}$ inch).

MATERIALS.

Rubber.—The tube and cover shall be made of synthetic rubber or a mixture of synthetic and natural rubber with not more than 35 per cent of the latter permissible.

Cotton.—Reinforcements shall be made of cotton duck or cotton yarn. The material shall be evenly and firmly woven and free from unsightly defects such as knots, and lumps, as well as from irregularities of twist.

Tube and cover.—The tube and cover shall be smooth, free from pitting, and uniform in thickness.

CONSTRUCTION.

Type.—The hose shall consist of a rubber tube, reinforced with cotton duck and a rubber cover. The whole shall be vulcanized together.

Reinforcement.—Plies of cotton duck shall be applied on a bias of 45° with edges lapped at least 1.3 cm. ($\frac{1}{2}$ inch) (not sewed), and shall be well frictioned on both sides with a rubber compound which shall firmly join the plies to the rubber tube and cover, and to each other.

Physical requirements.—The hose shall meet or exceed the following requirements :—

	Inside diameter of hose.	
	6 mm. ($\frac{1}{4}$ inch)*	9-13 mm. ($\frac{3}{8}$ - $\frac{1}{2}$ inch)
Tolerance in inside diameter, plus or minus	0.4 mm. (0.015 inch)	0.8 mm. (0.03 inch)
Thickness		
Tube, minimum.	1 mm. (0.04 inch)	1 mm. (0.04 inch)
Cover, minimum.	0.5 mm. (0.02 inch)	0.5 mm. (0.02 inch)
Fabric reinforcement plies, minimum ..	2	2
Tensile strength		
Initial ..	54.4 atm. (800 psi.)	54.4 atm. (800 psi.)
Decrease after ageing, per cent ..	35	35
Friction**		
Between tube and plies, minimum ..	3.6 kg. (8 pounds)	3.6 kg. (8 pounds)
Between plies, minimum ..	4.5 kg. (10 pounds)	4.5 kg. (10 pounds)
Between plies and cover ..	3.6 kg. (8 pounds)	3.6 kg. (8 pounds)
Decrease after immersion, per cent	50	50
Hydrostatic pressure test***		
Burst test before immersion, minimum	54.4 atm. (800 psi.)	54.4 atm. (800 psi.)
Burst test after immersion, minimum	20.4 atm. (300 psi.)	20.4 atm. (300 psi.)
Swelling, after immersion, maximum per cent.	30	30

*The specifications in this column will apply to all hoses up to and including 8 mm. ($\frac{1}{4}$ inch) inside diameter.

**The rate of separation shall be not greater than 2.5 cm. ($\frac{1}{4}$ inch) per minute under the specified load.

***Where the hose is purchased exclusively for use on compression hand sprayers or other sprayers with similar operating pressures, a 50 per cent reduction in hydrostatic pressure requirements is allowed.

TESTING.

The purchaser shall test an adequate number of random samples to satisfy himself that the article meets the specifications. Tests shall be carried out as indicated below:—

TESTS FOR HOSE.

1. *Tolerance, inside diameter.*—This dimension should be measured by means of a barrel-type expanding feeler-gauge.

2. *Thickness.*—This dimension shall be determined by means of a micrometer after carefully separating the cover and tube from the reinforcement by means of a sharp knife or razor.

3. *Tensile strength.*—This test may be performed by attaching one end of the sample to a solid support and hanging from the lower end weights as follows:

$$W = \left(\frac{\pi D^2}{4} - \frac{d^2}{4} \right)$$

Where:

W is the weight required in pounds
D is the outer diameter in inches
d is the inner diameter in inches
P is the tensile strength in psi.

4. *Ageing*.—The sample to be tested shall be placed in an oven in which the temperature is maintained at $78^{\circ} \pm 1^{\circ}\text{C}$ ($172.4^{\circ} \pm 1.8^{\circ}\text{F}$). The sample shall be so suspended that it will not be in contact with any metal part of the oven. The sample shall be removed and subjected to appropriate tests after 48 ± 0.25 hours.

5. *Friction*.—A cylindrical section, 2.5 cm. (1 inch) in length, shall be cut from the hose sample. This section shall be placed upon a metal rod, the diameter of which is approximately 80 per cent of the tube diameter.

A cut shall be made the full length of the cylindrical section. The depth of this cut will depend upon the test to be carried out i.e. through (1) cover, (2) first ply of reinforcement, and (3) second ply of reinforcement. The cut plies shall be stripped (peeled, pared) back a sufficient distance to permit attachment of a proper clamp. The clamp shall securely bind the separated portion of the ply throughout its full length. Weights as indicated in above shall be hung from the clamp, (Fig. 1.)

6. *Hydrostatic pressure test*.—Samples of hose not less than 50 cm. (20 inches) in length shall be coupled to a hydraulic pump. Water shall be pumped through the tube. While the tube is full of water, the open end shall be closed with a metal plug and secured with compression clamp. The full indicated burst pressure shall be applied to the sample under test in a period of not more than 5 seconds.

7. *Immersion*.—Where immersion is required, the sample shall be completely immersed in technical grade xylene. Care should be exercised to avoid formation of air pockets inside the tube and to keep the sample from contact with the surfaces of the container. The sample shall be left immersed for a period of 72 ± 0.25 hours at a temperature of 21° – 27°C (70° – 80°F). After completion of immersion, the sample shall be dried by hanging in air at $21^{\circ} \pm 6^{\circ}\text{C}$ ($70^{\circ} \pm 10^{\circ}\text{F}$) for 24 hours.

8. *Swelling*.—Swelling shall be determined by measuring the inside and outside diameters of the sample before and after immersion.

APPENDIX III.

SPECIFICATIONS FOR CUT-OFF VALVES.

GENERAL.

Several types of cut-off valves are available in the commercial market. Others are in process of development. These specifications are not intended to restrict purchase of any satisfactory type of valve but rather to set general standards.

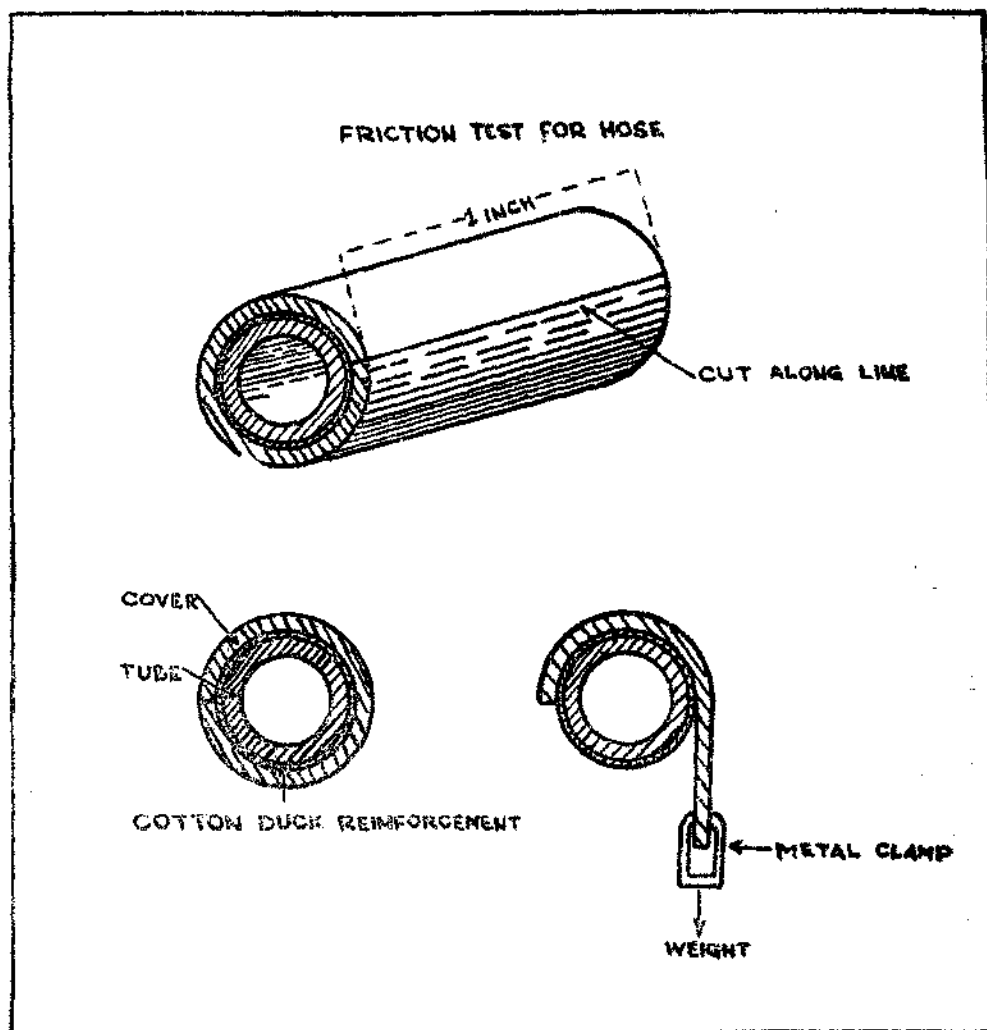


FIG. 1
Friction Test for hose.

MATERIALS.

Metal parts.—All metal parts of the valve shall be of brass or aluminum, or of stainless steel which shall contain not less than 8 per cent nickel.

Gaskets.—All gaskets and other parts not made of metal shall be chemically resistant to the action of insecticide formulations.

CONNEXIONS.

All valves shall be equipped with screw threads on the inlet and outlet ports. The outer surface of the valve adjacent to the screw threads shall have opposing flat or hexagonal faces to permit the use of a wrench on them. A bayonet type of connector may be supplied on one or both ends of the valve, but such units shall be removable and not constructed as an integral part of the valve body.

MAINTENANCE.

The valves shall be so constructed that inner and outer parts are readily accessible and replaceable without the use of special tools.

DESIGN.

Liquid passages.—There shall be no liquid passages in the valve through which the entire liquid volume must pass that have cross-sectional areas of less than 0.25 cm² (0.04 square inch).

Cut-off valve handle.—The handle shall be conveniently located on the valve to permit actuation by the hand. Pressures upon it shall be as follows:—

Lever movement.—The force required to move the handle on a lever-type valve from the closed to open positions should not require a torque of more than 200 inch-ounces (1.04 foot-pounds or 0.1438 kilogram-mètres).

Lateral movement.—Valves depending upon direct lateral movement for their actuation shall not require a force greater than 227 g. (8 ounces) if such force is designed to be exerted by one thumb or finger. Where such force may be exerted by the entire hand, the force required shall not exceed 0.9 kg. (2 pounds).

Locking device.—A device to hold the handle in the open position shall be provided. It shall be readily locked or released from the locked position by the simple movement of one finger of the operator's hand.

Shape.—The valve handle and all associated parts shall conveniently fit the operator's hand without discomfort.

Tests.—It is recommended that, if feasible, durability tests be performed as indicated below:—

Durability tests for cut-off valves:—

Cut-off valves shall be tested under simulated natural conditions of operation. The valve shall be mounted on a test rack in such a manner that the body of the valve is held in a rigid position. A test fluid consisting of a 5 per cent DDT-xylene emulsion shall be applied to the inlet of the valve under a static pressure of 3.4 atm. (50 psi). The outlet of the valve shall be carried through a spray nozzle desired to deliver 0.76 litre (0.2 US gallon) per minute under the stated pressure. The nozzle may discharge directly or through appropriate piping into a reservoir from which the emulsion is pumped back under pressure to the inlet port of the valve. Where such recycling is used, the emulsion shall be changed after each 48 hours of test. The testing apparatus shall be so arranged, by cams, solenoids, or other devices, that the valve mechanism will be actuated at a rate of not more than 15 cycles per minute. The mechanism shall open and close the valve in a period of not less than 1/5 second nor more than 1/2 second. Valves shall be deemed to have met this specification if, after

50,000 complete cycles of operation under the above conditions, there is no evidence of structural damage, deformation, or failure of the interior or exterior of the valve, and if no drip or leak through the valve or around the packing is evident during the entire test period.

The above tests shall be repeated with the input pressure to the valve at 6.8 atm. (100 psi) and the valve operated at this pressure for 500 cycles. The same criterion of acceptance shall apply to this latter test.

Technical improvements.—The following technical improvement is recommended for probable inclusion in the specification at a later date:

Drip-control valve.—A combination drip-control and cut-off valve with the cut-off situated at the nozzle instead of at the base of the lance has been developed and is commercially available. This type is considered a distinct advance in cut-off valve design, and its use is to be encouraged commensurate with further favourable usage and availability.

APPENDIX IV.

DISCHARGE EQUIPMENT.

Nozzle tip should be capable of producing a flat fan spray of uniform pattern with a spray angle between 60° and 65°. Rate of discharge should be between 0.76 litre (0.2 gallon) per minute and 1.52 litre (0.4 gallon) per minute at 2.8 atm. (40 pounds per square inch) pressure. When continuously operated over a one-minute period at pressure between 2.8 atm. (40 pounds per square inch) and 2.1 atm. (30 pounds per square inch), the nozzle should not produce dripping from the spray orifice at a rate in excess of five drops over the period of test. The nozzle must be designed with flat or hexagonal faces to facilitate removal and replacement. Nozzles should be of stainless steel or of material of equivalent hardness and resistance to corrosion.

A SURVEY OF THE ECONOMIC STATUS OF VILLAGERS
IN A MALARIOUS IRRIGATED TRACT IN MYSORE STATE,
INDIA, BEFORE AND AFTER D.D.T. RESIDUAL
INSECTICIDAL SPRAYING.*

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INTRODUCTION.

REDUCTION of malaria morbidity and mortality is the first aim of antimalaria measures. A by-product of this achievement is the mobilization of the potential of the protected population to improve its economic condition.

✓ * The work on which this paper is based was performed under the auspices and with the support of the Division of Medicine and Public Health of the Rockefeller Foundation in cooperation with the Bureau of Malariology of the Mysore State Health Department.

The authors wish to thank the Director, Malaria Institute of India, Delhi, for detailing a unit consisting of one Malaria Assistant (Mr. M. G. Narayandas) and one Insect Collector. They were responsible for furnishing pre-spraying entomological data, which constitutes part of the back-ground on which this paper is based.

The extent of such improvement would be proportionate to the severity of pre-existing malaria and the degree of effort expended by the inhabitants for self-betterment.

Objective means for assessing economic levels of rural Indian populations are not easily found. As elsewhere, the rate of illiteracy is high in the scattered villages of Mysore State. Sketchy records are kept by the head-man (*Patel* or *Shanbhogue*), who is appointed by Government for the purpose of collecting taxes and generally supervising local affairs. These records, while not of unimpeachable character, may be used as rough measures of the local economy. Personal inquiry is likewise productive of only the approximate truth, since it is met with the assumption that it will somehow result in increased taxation. Villagers, however illiterate they may be, have active minds in which shrewdness plays a major rôle.

In the present study, trained medical personnel were sent into villages in Mysore State for the purpose of eliciting data that would permit an objective, although rough, appraisal of economic conditions. It is the intention of this paper to present a series of findings concerning a group of rural Indian villages in an irrigated tract in Mysore State. In a preliminary survey it was found that the area supported hyperendemic malaria. Certain villages were treated with DDT residual spray, while for purposes of comparison other villages were left unsprayed. One year after the first application of DDT the economic survey was made. An appraisal was made of the economic status of families in both sprayed and unsprayed villages at the time of the inquiry and one year previously. The resultant data were then grouped so that the progress of the material welfare of families in treated and untreated villages could be compared.

METHODS.

The site of the work to be described is the Sreenivasapura Area, Channarayapatna Taluk, Hassan District, Mysore State, India. Small villages, each with a population of only a few hundred people, are located about a mile apart in the general neighbourhood of an irrigation canal. Most of the dwellings are of sun-dried mud walls with crude tile roofs, the poorest ones being unplastered and covered with thatch instead of tiles. In some of the villages there are well-built houses of a distinctly higher quality.

The canal, which originates in the Hemavati River, has been in operation for about 65 years. The Sreenivasapura Area is watered by a branch of the canal known as the Ramadevara North Ane Channel. Irrigation is seasonal; water is turned into the canal on the fifteenth of June each year and allowed to flow until the following fifteenth of January. The chief crops dependent on irrigation are rice and sugar cane. These are the major crops of the area; however, cocoanuts and dry crops like ragi are also cultivated.

A rapid preliminary malaria survey was conducted in August 1950 and covered 22 villages with a total population of approximately 4,614 persons. Four hundred and twenty-four children under the age of 12 were given spleen examinations. The spleen rate was 75.4 per cent; the average enlarged spleen size was 3.32 (Darling's classification).

Anopheline mosquitoes were collected at specially designated catching stations for a period of five months, September 1950 through January 1951, to appraise mosquito densities and the likely important vectors. During this period 4,384 female *Anopheles* were identified, of which 1,360 were *A. culicifacies*, 878 were *A. fluviatilis* and 2,146 were distributed among 13 other species.

The villages were arbitrarily divided into three groups; each received different dosages of DDT and was on different time schedules for respraying. Several villages were chosen at random to be omitted from DDT spraying in order to furnish comparison data. Monthly infant blood surveys, semi-annual spleen and blood surveys of older children and weekly entomological collections were made in the treated and comparison villages beginning with the first application of DDT in September 1950.

The varying dosages of DDT and their residual effectiveness are not of importance to the contents of this paper, since schedules were designed in each instance to interrupt malaria transmission. Reduction of malaria and of mosquito populations was observed in all groups of treated villages (see below, "Results").

An economic survey was conducted during September and October 1951 by representatives of the Malaria Investigation Centre of the Bureau of Malariology, Mysore State Health Department. The fact that these doctors wanted intimate information about family—and especially financial—affairs of the people was received with astonishment. It was only after several hours of conversation, argument and persuasion with the head-man of a village, accompanied by a major fraction of the population, that a few of the inhabitants began to have any confidence in the harmlessness of the doctors' intentions. But when a working relationship had been established, the persons volunteering information did so in a fairly straightforward manner. In fact, the general congregation of villagers during the questioning was sometimes helpful, as the spectators occasionally prompted the faulty or reluctant memory of a volunteer.

The selection of families for interrogation was accomplished by identical means in the sprayed and unsprayed villages. It is difficult to know whether the investigation became biased at this point, for the selection

was based principally on the willingness of a family representative to disclose his private affairs and on the opinion of the investigators as to the honesty of the representative's report. The only predetermined criterion used in the process of selecting families was that various economic levels, or income groups, must be represented. Thus, in each village families were chosen from groups having an annual income of less than Rs 500, between 500 and 1,000, between 1,000 and 1,500, and above Rs. 1500. The survey covered 29 families in the sprayed area and 31 families in the comparison area. "Families" were considered to include all the residents of a given dwelling. ✓

The distribution of families by income group for the area as a whole was 60 per cent in the lowest category, 25 per cent in the next group, 10 per cent in the third, and 5 per cent in the highest. These percentages varied somewhat from village to village, but the sprayed and unsprayed villages were roughly similar in this respect. In the selection of families for the economic survey, it happened that in the sprayed villages 15, 4, 3 and 7 families respectively fell into the foregoing categories, while in the comparison villages the corresponding distribution of families into income groups was 16, 5, 3 and 7.

Each family in both categories of villages was asked standard questions pertaining to health and economics in the year preceding DDT application and in the year immediately following application.

The information elicited by the inquiry included first of all the costs to a family arising directly from clinical malaria. Since prior to the original survey none of the cases, fatal or otherwise, had been diagnosed on a parasitological basis, there is great possibility of error in accepting statements concerning this. However, it is obvious from consideration of the malaria survey data that the villagers must have had close acquaintance with the disease and its manifestations. It was also learned that they claimed the ability to differentiate between ordinary malaria illness and sickness produced by plague, pneumonia, cholera and other enteric fevers. While no precise validity can be attached to these data, qualitatively at least they may have some significance.

Costs arising from an attack of alleged malaria were tabulated under headings of medical treatment, spiritual care and funerals. It must be understood that these peoples, when ill, seek not only to cure the body but also to appease the god or spirit who has imposed this form of punishment. The priest attempting this appeasement must be paid as well as the local medicine man. Furthermore, a funeral is extremely costly, since the bereaved family receives relatives and friends from great distances and provides them with food during the lengthy funeral service.

These expenses were sometimes left unpaid, in which case the indebtedness of the family was tabulated as an economic liability. Sometimes ✓

the family paid a debt by selling livestock or land to raise the money and this sacrifice of property was listed as an economic loss.

The number of days of productive labour lost by ill members of a family was learned insofar as possible and was assessed in terms of prevailing rates of wages for hired labour. In case of the death of a working member of a family, the lost man-days of labour during the illness and throughout the remainder of the year were estimated and similarly assessed.

Basic information concerning the annual revenue of each family had to be obtained in order to classify the family by income group. This was calculated on the basis of crop yields at prevailing rates of value. This was the most delicate question of all, since there was a natural hesitation on the part of everyone to admit the actual amount of rice or other crop produced. If the yield were said to be poor, Government would demand less of it for the rationing programme. It is to be presumed, therefore, that lingering suspicion of the questioners' motives led to considerable distortion of these figures.

It was learned during the course of the inquiry that it is an established practice in these villages to import labour from adjacent non-irrigated farming areas during those phases of rice culture when a maximum effort is required to handle the crop (planting, weeding, harvesting and threshing). Families in the sprayed and comparison villages were therefore questioned as to their past and current expenditures for imported labour, since a saving in this category could be credited to antimalaria work if decreased man-days of illness enabled a family to get along with less outside help.

It was further determined that the acreage of land under various types of cultivation might also be an indication of a family's condition with respect to available man-days of labour per year. Hence the current and past number of cultivated and fallow units of land per family was ascertained.

The costs of DDT spraying were not borne directly by the inhabitants of the treated villages. Theoretically, these might be deducted from the total of economic advantages resulting from this procedure.

RESULTS.

It may be generally stated that during the year following DDT application the incidence of malaria declined in the sprayed villages but remained essentially the same in the unsprayed areas. Spleen rates in the

sprayed villages were 34.2 per cent at the time of the economic survey, a figure less than half the original one (Table I).

TABLE I.

Spleen rates of children 2 to 12 years of age inclusive in sprayed and unsprayed villages before and one year after DDT application. About sixty per cent of the children examined were the same on both occasions.

Number of villages		Approximate numbers of children examined	Palpable spleens in pre-DDT period (percentage)	Palpable spleens one year later (percentage)	Ratio
Sprayed villages	20	425	75.4	34.2	0.454
Comparison villages	5	140	46.9	46.9	1.000

Of 121 infant blood films taken in sprayed villages at the time of the economic survey, none showed malaria parasites, while of 59 films taken in the unsprayed area three, or 5.36 per cent, were positive. This difference, while suggestive, is not statistically significant.

Entomological comparisons between sprayed and unsprayed areas are presented in Table II. The incidence of anophelines in sprayed structures was practically nil throughout the year, while normal densities continued to prevail in the unsprayed villages. *Anopheles culicifacies* and *A. fluviatilis* were taken at the latter stations in the usual proportions.

TABLE II.

Sample of results of weekly mosquito collecting in repeatedly sprayed and unsprayed structures, expressed as anophelines caught per man-hour from March to May 1951.

Week	1	2	3	4	5	6	7	8	9	10
Sprayed	0.0	0.0	0.0	0.17	0.08	0.0	0.0	0.0	0.17	0.0
Unsprayed	15.5	32.8	16.0	45.0	17.3	12.7	25.5	31.8	23.5	6.75

The villagers' reports on their clinical experience with malaria before and after DDT application will now be considered. Table III summarizes the claimed incidence of deaths from malaria during the year preceding and the year following DDT application to the sprayed and comparison areas. While there was a sharp drop in malaria deaths in both areas, it was more than twice as marked in the sprayed areas as in the unsprayed regions. The per-family incidence of deaths in treated villages was 12.4 per cent that of the pre-DDT figure but declined to only 28.7 per cent in untreated villages.

TABLE III.

Annual deaths attributed to malaria in surveyed families living in sprayed and unsprayed villages before and after DDT application.

	Total deaths		Deaths per family		
	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Ratio
Sprayed	24	3	0.828	0.103	0.124
Unsprayed	18	5	0.561	0.161	0.287

The per-family expenditures for medical treatment, spiritual care and funerals likewise decreased in both groups of villages, but in this instance a really significant difference could be observed. Costs in the sprayed villages were 14.6 per cent of the pre-DDT figure, while in comparison villages they were 71.9 per cent of the pre-DDT level (Table IV).

TABLE IV.

Annual per family expenditure (in rupees) attributed to malaria.

	Medical treatment		Spiritual care		Funerals		Total		Ratio
	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	
Sprayed	210.9	27.1	3.3	0.0	173.8	31.0	397.0	58.1	0.146
Unsprayed	143.1	120.5	38.4	26.3	68.0	24.2	250.4	180.0	0.719

Estimates of earnings lost through illnesses and deaths caused by alleged malaria infection are presented in comparative form in Table V. The decline in such losses is more or less parallel to that in illness expenditures; in the treated villages losses amounted to 10.4 per cent of the estimated pre-spraying cost, while in the untreated villages they came to 77.5 per cent of the pre-DDT expenditure.

TABLE V.

✓ *Annual per-family lost earnings (in rupees) attributed to malaria.*

	Illnesses		Deaths		Total		Ratio
	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	
Sprayed	418.6	47.4	237.4	20.7	656.0	68.1	0.104
Unsprayed	206.3	175.8	70.6	38.7	276.9	214.5	0.775

Indebtedness and sale of land and/or livestock to pay debts arising from illness or death attributed to malaria are summarized in Table VI. These liabilities decreased to 9.2 per cent of the pre-spraying total for families in sprayed villages but to only 72.4 per cent for those in unsprayed villages.

TABLE VI.

Per-family summary of values (in rupees) of land and/or livestock sold annually because of expenses attributed to malaria, or of indebtedness incurred annually to pay for such medical expenses (Table IV).

	Sale of land and/or livestock		Indebtedness incurred		Total liabilities		Ratio
	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	
Sprayed	189.3	23.1	212.1	13.8	401.4	36.9	0.092
Unsprayed	0.6	3.6	146.0	102.6	146.6	106.2	0.724

Costs of imported labour are presented in Table VII. These are derived from study of a single sprayed village. Figures refer to the village as a whole, not to the surveyed families. The fact that in some cases the imported

labourers had to be fed is not included. A further item not included in this tabulation is the fact that another village, formerly an importer of labour, exported labour after DDT application. Comparison villages reported no change with respect to importation of labour.

TABLE VII.

Annual expenditures (in rupees) in wages for imported labour needed because of shortage of local manpower in a representative sprayed village.

Planting		Weeding		Harvesting		Threshing		Total Expenditures		
Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Ratio
5343.8	1716.6	2112.3	187.5	1865.0	750.5	1500.0	0.0	10821.1	2654.6	0.245

In Table VIII, there is a statement concerning the acreage of farm-land lying fallow before and after the DDT programme. In the sprayed villages 60 per cent of the formerly fallow land had been put back into production, while only 3.3 per cent of such land had become profitable in the comparison villages.

Table VIII.

Annual per-family acreage of farmland lying fallow.

	Wet crops		Dry crops		Total		
	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Pre-DDT	Post-DDT	Ratio
Sprayed ..	0.2	0.0	1.8	0.8	2.0	0.8	0.400
Unsprayed ..	0.1	0.1	2.9	2.8	3.0	2.9	0.967

DISCUSSION.

The reasons for the decline in malaria prevalence in the unsprayed areas were not determined. There is a possibility that widespread natural decline of malaria was taking place, but the studies here reported were confined to the sprayed and comparison villages themselves. Since the comparison villages were close to the sprayed ones, the possibility that the unsprayed

area received some benefits from the nearby antimalaria measures must be considered. Any factor diminishing the transmission of malaria in a given area may reduce malaria prevalence in bordering areas. A third possibility remains: the decline of malaria in the comparison villages was a result of both of the above-mentioned forces operating simultaneously.

The decrease in malaria prevalence in the comparison villages was minor, however. Spleen rates were unchanged; parasites were detected in infant blood films; anopheline mosquitoes continued to be prevalent. Nevertheless there was a strikingly lower incidence of deaths attributed to malaria and a considerably lessened expenditure for treatment of alleged malaria illness. Improvement in economic conditions as outlined, was also appreciable, although on a much smaller scale than in the sprayed villages.

Since this uncertainty exists as to whether there was really a natural decline in malaria in the general region, one should refrain from attributing the entire improvement in the economy of the sprayed villages to the beneficent action of residually sprayed DDT. The benefits of DDT may have extended to the comparison villages, but since this is unmeasurable, it will not be considered in the evaluation which follows. Instead, only the difference between the amount of improvements in the two village groups will be discussed.

Savings in the sprayed villages during the year in which DDT was routinely applied are summarized and totalled in Table IX. The per-family total is in itself sufficient to move even the poorest families up to the second category of the income groups adopted for the purposes of this study and to advance many of the families surveyed to the top category. Even the families formerly at the top of the register, with incomes in excess of Rs. 1500, could scarcely fail to notice a great difference, saving almost Rs. 800.

TABLE IX.

Difference between annual per-family costs (in rupees) attributed directly to malaria in sprayed and unsprayed villages.

COSTS							
Item	SPRAYED FAMILY			UNSPRAYED FAMILY			DIFFERENCE BETWEEN SAVINGS
	Pre-DDT	Post-DDT	Saving	Pre-DDT	Post-DDT	Saving	
Medical care, spiritual care, funerals (Table IV)	397.0	58.1	338.9	250.4	180.0	70.4	263.5
Lost earnings (Table V)							
Illness ..	418.6	47.4	371.2	296.3	175.8	30.5	340.7
Deaths ..	237.4	20.7	216.7	70.6	38.7	31.9	184.8
Total ..	1053.0	126.2	926.8	527.3	394.5	132.8	794.0

We shall proceed here to a consideration of the per capita benefit of DDT spraying to the members of these families and to the inhabitants of the villages in general. The average family or household surveyed in these studies consisted of 8.4 individuals. Hence the per capita benefit consisted of an annual saving of Rs. 94.5.

At this point one must return to Table VII, which shows savings in wages for imported labour. These figures refer to a representative sprayed village and may be taken to apply to all sprayed villages. The population of this village was 274 persons. Since a saving of Rs. 8,166.5 was realized in the year following application of DDT, the per capita benefit on this score alone was Rs. 29.4. Thus the total per capita benefit following DDT application may be stated as Rs. 123.9. Savings resulting from the yield from formerly fallow lands will not be used to further inflate estimated savings.

Per capita costs for applying DDT to the villages are estimated at Rs. 1.7. This includes labour, materials and depreciation of equipment on an annual basis. Thus the net gain per capita, or the savings per capita, is Rs. 122.2. The return on the investment is over seventy-fold and according to the above rough estimate is actually 71.9-fold. This compares well with Viswanathan's (1951) fifty-fold results in Bombay State.

The reduction of data such as these to a per capita basis is convenient for certain purposes, but it may mask the truly astonishing magnitude of the over-all benefit. The sprayed villages, with a population of 4,614 persons avoided a financial loss of Rs. 5,63,830 in the year during which DDT was applied, after costs for the DDT programme had been paid. Even if there were an error as great as fifty per cent in the survey data and subsequent statistical analysis, a saving of over Rs. 2,50,000 is still a substantial one.

Obvious economic benefits can be rather readily appraised, as has been done. There must be less concrete advantages as well. Physical well-being, relief from chronic apprehension, better social life, increased efforts for education—all these can result from an improved economic status and, in turn, be catalysts of further economic improvement. A yearly follow-up survey in these villages may well reflect such a happy course of events.

SUMMARY AND CONCLUSIONS.

1. An economic survey was made, largely on a family basis, in a group of villages in an irrigated tract in Mysore State, India. Some of the villages had been periodically sprayed with DDT during the previous year; other villages had purposely been omitted from the spraying programme to serve as a source of comparison. A preliminary survey established the presence of malaria in the entire area in hyperendemic intensity. A resurvey one year after institution of the DDT programme indicated marked reduction of malaria in the sprayed area and persistence of malaria in the comparison area. The

purpose of the economic survey was to ascertain whether reduction of malaria endemicity in the sprayed villages was accompanied by changes in the economic situation of the protected inhabitants.

2. Families at all income levels were interrogated in sprayed and in comparison villages. They were questioned about their economic status in the year preceding the DDT era and during the first year after DDT application. Village records were also used as a source of information. In both instances the data were regarded by the investigators as general indicators of trends rather than as absolute criteria.

3. In the sprayed villages decreased expenditures for medical and spiritual care and for funerals were incurred during the year following DDT application. Fewer sick-days annually occurred. Sale of lands and livestock and other forms of indebtedness were reduced. There was a sharp drop in expenditures for wages for imported labour assisting in the cultivation and harvesting of rice. Some acreage, formerly fallow for want of local labour, was returned to cultivation.

4. During the same period there was a small improvement under the above headings in the comparison villages. The causes of this improvement were not determined.

5. The difference between economic conditions in the sprayed villages before and after the application of DDT cannot be attributed to insecticidal effort without certain reservations. The conditions under which this study was made did not permit the establishment of adequate controls. Intrinsic differences between sprayed and comparison villages could not be evaluated. Despite the lack of background and detail, however, there is repeated evidence of different trends in the two village groups indicating strong likelihood of a positive DDT effect.

6. The difference between savings in the sprayed and unsprayed villages was summarized in terms of per capita annual economic improvement of residents of sprayed villages in the year following DDT application. It was found that this amounted to slightly more than seventy times the cost of the DDT programme.

ACKNOWLEDGEMENTS.

The authors are greatly indebted to Dr. D. K. Viswanathan, Poona, and to Dr. Hugo Muench, Harvard University, for reading and criticising this paper.

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SEASONAL VARIATION IN THE BREEDING PLACES OF
ANOPHELES FLUVIATILIS JAMES IN WYNAAD
AND ITS RELATIONSHIP TO ERADICATION
OF THE SPECIES.

BY

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[September 24, 1952.]

INTRODUCTION.

Anopheles fluviatilis, a vector of considerable importance in the peninsular India, is essentially a stream breeder. Russell and Jacob (1942) while studying the habits of this species in the Nilgiris found that in the west it almost disappeared during July to September, the months of heavy rainfall, while in the east where the rainfall was more evenly distributed, it was found throughout the year.

The present report deals with observations carried out between 1938 and 1947 on the breeding habits of *A. fluviatilis* with special reference to the monsoon period, in the Wynaad, west of the Nilgiris. The object underlying is to emphasize the important rôle of breeding in stagnant water collections for the survival of the species during the adverse monsoon season.

TOPOGRAPHY AND CLIMATE.

The Wynaad is a hilly taluk of the Malabar District of the Madras State, with an average elevation of about 3,000 feet above sea level.

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Its climate is generally cool and the year may be conveniently divided into 3 seasons (Table I) based on the amount of precipitation.

TABLE I.

Meteorological indices (means) at Kalpatta, Wynaad (Stevenson's Screen), December 1944 to November 1945.

Season	Months	Mean maximum temperature ° F	Mean minimum temperature ° F	Mean relative humidity 4 p.m., per cent.	Total rainfall in inches
Cold	Dec.-Jan.	86.8	56.8	57.3	4.08
Premonsoon ..	Feb.-May.	90.0	64.7	66.0	13.61
Monsoon	Jun.-Nov.	80.2	65.4	90.3	100.86

In general, the temperature and humidity conditions are favourable for malaria transmission throughout the year. As will be seen later, the monsoon period with a total of 100.86 inches rainfall is adverse to the breeding of *A. fluviatilis* due to the streams being converted into torrents.

Since *A. fluviatilis* prefers streams as its breeding places, the water temperature of a stream was recorded throughout a year and the mean values are given in Table II.

TABLE II.

Water temperature in a stream recorded during 1945-1946.

Month	Temperature			
	Mean maximum ° F.	Mean minimum ° F.	Variation ° F.	Mean ° F.
1945				
Jun.	87.0	79.3	7.7	83.2
Jul.	83.1	79.5	3.6	81.3
Aug.	85.2	81.1	4.1	83.2
Sep.	86.6	80.4	6.1	83.5
Oct.	85.9	79.7	6.2	82.8
Nov.	82.2	74.9	7.3	78.6
Dec.	76.3	70.8	5.5	73.6
1946				
Jan.	77.2	65.2	12.0	71.2
Feb.	81.5	68.9	12.7	75.2
Mar.	88.1	72.1	16.0	80.1
Apr.	88.9	73.2	15.6	81.1
May.	90.6	74.1	16.5	82.4

The highest diurnal variation (16.5°F.) in the stream was observed in May and the lowest (3.6°F) in the monsoon month of July. The water temperature *per se* during the monsoon period did not appear unfavourable for the breeding of the species as compared with the favourable non-monsoon period.

BREEDING PLACES OF *A. FLUVIATILIS*.

During the period 1938-1947, several types of breeding places were searched and a total of 9,327 larvae of *A. fluviatilis* was collected from all of them. Their distribution in the different places is shown in Table III.

TABLE III.

Incidence of A. fluviatilis larvae in different types of breeding places (1938-1947).

Type of breeding places	Number of <i>fluviatilis</i> larvae captured.	Percentage to total collected in all breeding places.
Streams	5,248	56.3
Ricefield channels	2,090	22.4
Contour drains	956	10.3
Wells	131	1.5
Shallow earthen wells	824	8.8
Small tanks	13	0.15
Marsh	6	0.07
Ricefields	30	0.3
River bed pool	27	0.3
Seepage stagnation	2	0.02

Breeding places like streams, ricefield channels and contour drains with flowing water accounted for 88.9 per cent of the larvae while the remaining 11.1 per cent were found in stagnant water collections like wells, ricefields, marsh and pools in the river bed.

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The data were analysed (Table IV) to determine the seasonal distribution of larvæ in different types of breeding places.

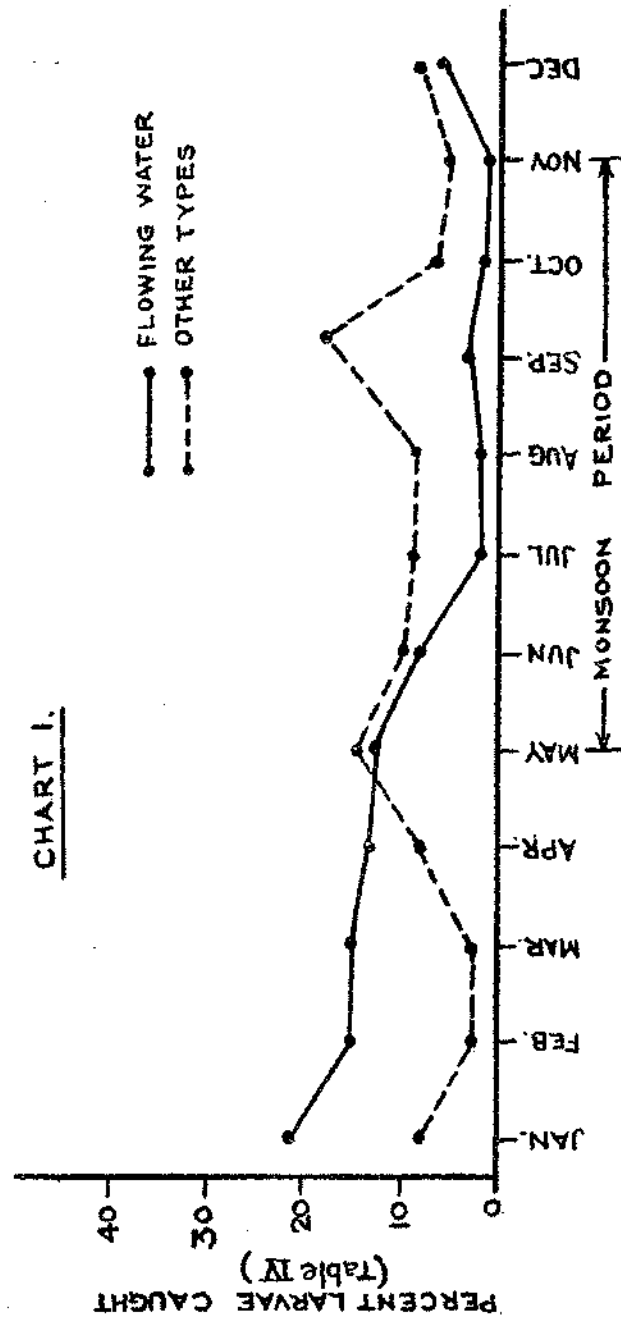
TABLE IV.
Seasonal distribution of A. fluviatilis larvæ in different breeding places.

Month	Total collected from all types of breeding places	Flowing water		Others	
		Number collected.	Percentage collected during the month to total collected during the entire period	Number collected	Percentage collected during the month to total collected during the entire period
Jan.	1,817	1,734	20.8	83	8.0
Feb.	1,292	1,257	15.2	35	3.4
Mar.	1,383	1,248	15.1	35	3.4
Apr.	1,188	1,100	13.3	88	8.5
May	1,227	1,084	13.1	143	13.4
Jun.	755	658	7.8	97	9.4
Jul.	191	99	1.2	92	8.9
Aug.	248	153	1.8	95	9.3
Sep.	424	254	3.1	170	16.5
Oct.	174	113	1.4	61	5.9
Nov.	131	83	1.0	48	4.6
Dec.	597	511	6.2	86	8.3
Total	9,327	8,294	..	1,033	..

A fall in the number of larvæ collected from all types of breeding places was noticeable with the onset of monsoon in June, this being more marked in the flowing waters. The density of breeding during the rest of the monsoon period remained comparatively higher in stagnant type of breeding places than in the flowing waters. During the post and pre-monsoon periods *A. fluviatilis* was seen breeding mainly in the flowing types of breeding places (Chart 1).

Covell and Harbhagwan (1939) found *A. fluviatilis* breeding in a shallow earth well in Wynaad, during the monsoon period. Based on this finding a thorough search was carried out in similar places during the monsoon period of 1940 and 1941 in two areas in Wynaad, namely Kalpatta and Sultan's Battery, fifteen miles apart.

During the monsoon period of 1940, 126 wells, 132 field wells and 42 small tanks in Kalpatta and 36 wells, 56 field wells and 21 small tanks in Sultan's Battery were surveyed weekly. During the following monsoon, 76 wells, 60 field wells and 21 small tanks in Kalpatta and 35 wells, 76 field wells and 11 small tanks in Sultan's Battery were searched. The results



A. fluviatilis larva catches in different breeding places.

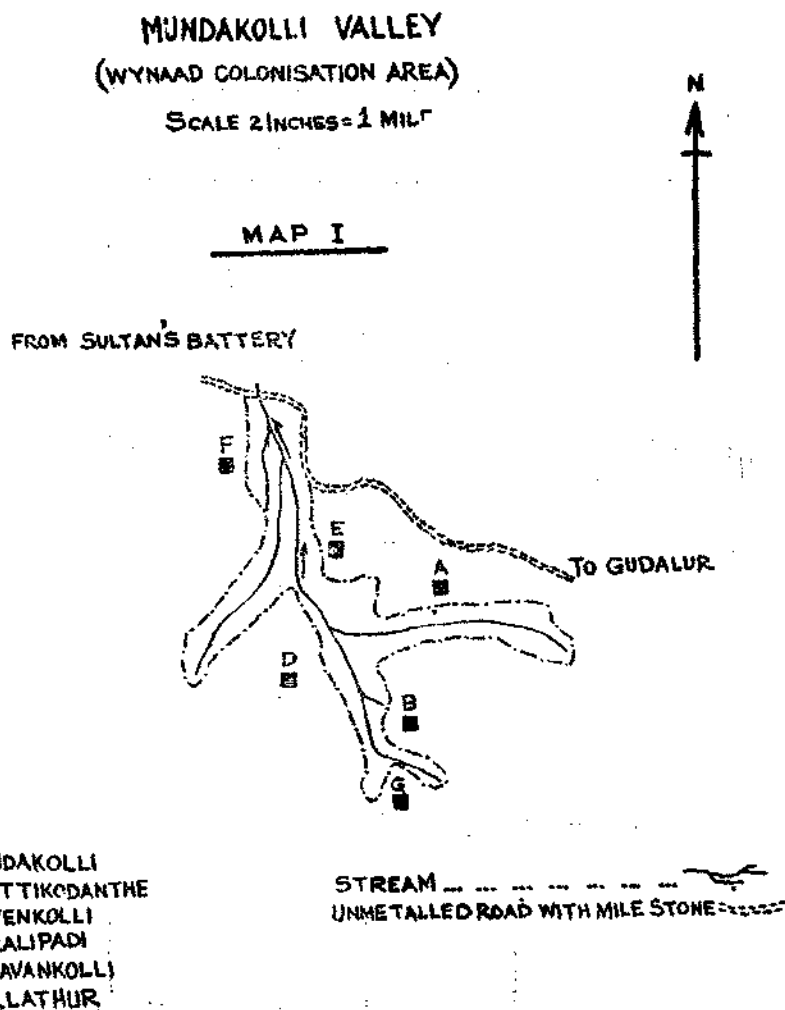
TABLE V (contd).

Month	Total number of larvae of all species collected	Deep wells				Shallow field wells				Small Tanks		
		Searches.		Number of larvae captured		Searches		Number of larvae captured.		Searches	Number of larvae captured	
		Number	Number positive for <i>fluviatilis</i>	<i>fluviatilis</i> .	other species	Number	Number positive <i>fluviatilis</i> .	Number <i>fluviatilis</i> .	other species.		<i>fluviatilis</i>	other species.
1941.	511	118	2	4	44	220	3	23	429	25	..	38
Aug.	..	..	..	..	..	..	..	..	..	..	..	..
Sep.	405	111	1	2	68	199	1	2	301	19	..	36
Total for 1941.	1,765	374	4	7	126	710	11	46	1,447	84	..	192
Total for 1940 and 1941	2,856	555	6	13	395	993	20	75	2,171	129	1	290
Total for both areas 1940 and 1941	9,189	2,401	11	21	3,520	2,541	22	83	4,784	735	1	886

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 are shown in Table V. Out of a total of 9,189 anopheline larvæ collected in the two seasons, only 105 were of *A. fluviatilis*. Comparatively more larvæ were captured in the Sultan's Battery area than in Kalpatta.

AGE DISTRIBUTION OF *A. FLUVIATILIS* ADULTS AND THEIR DENSITY DURING THE MONSOON.

In the monsoon period representing the season of minimum prevalence of *A. fluviatilis*, an attempt was made to observe any existing co-relation between larval breeding and adult densities, during this period in 1945, in a valley about six miles from Sultan's Battery on Gudalur Road called for the purpose of this paper Mundakolli valley which is the name of one of the six small hamlets situated on the slopes of the valley (Map 1.)



Ten anopheline species (*jeyporiensis*, *subpictus*, *vagus*, *aconitus*, *barbirostris*, *hyrcanus*, *jamesi*, *majidi*, *tessellatus*, and *fluviatilis*) were captured from human dwellings and cattlesheds in the valley from May to October. *A. fluviatilis* was found only in human dwellings along with a few *A. jeyporiensis*, *subpictus* and *vagus*. The others were collected only from the cattlesheds. The figures regarding *A. fluviatilis* are given in Table VI.

TABLE VI.

Collection of adult *Anopheles* from human dwellings in Mundakolli area.

Month	Number of searches	Number of <i>Anopheles</i> captured		Percentage of <i>fluviatilis</i> to total
		Total	<i>fluviatilis</i>	
May. ..	9	79	77	97.5
Jun. ..	25	196	193	98.5
Jul. ..	31	166	162	97.6
Aug. ..	27	89	86	96.6
Sep. ..	23	62	62	100.0
Oct. ..	3	7	7	100.0
	118	599	587	97.9

During the same period larval catches were made from breeding places in the valley and the results are given in Table VII.

TABLE VII.

Monthly abstract of collections of larvæ of *A. fluviatilis* in Mundakolli Valley.

Month	Streams and Channels.			Ricefields, marsh and wells.		
	Searches		Average number of larvæ per positive search	Searches		Average number of larvæ per positive search
	Total	Positive		Total	Positive	
May ..	5	5	24	..	..	..
Jun. ..	30	12	39	..	..	..
Jul. ..	54	2	3	62	Nil	Nil
Aug. ..	52	1	2	60	1	1.0
Sep. ..	57	1	2	75	Nil	Nil
Oct. ..	16	1	2	17	Nil	Nil

It is seen from the above that after June there was an abrupt fall in the prevalence of *A. fluviatilis* larvæ in the valley. A similar decline in the prevalence of adults was seen only from August (Table VI).

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In order to determine the age composition of adult *A. fluviatilis* in the valley during the monsoon, 611 specimens were dissected from June to October and the results are furnished in Table VIII.

TABLE VIII.
Abdominal condition of A. fluviatilis captured in the Mundakolli Valley, June to October 1945.

Month	Number with abdominal condition in stage.				Percentage of IV stage to total
	I	II	III	IV	
Jun. ..	4	65	45	19	14.3
Jul. ..	10	138	71	37	14.4
Aug. ..	6	87	28	6	4.7
Sep. ..	1	75	11	1	1.1
Oct. ..	Nil	7	Nil	Nil	Nil

NOTE : I. No blood-No mature ovaries.
II. Full fresh blood.
III. Ovaries partially developed.
IV. Ovaries fully mature.

A decrease in the number of *A. fluviatilis* with partially and fully mature ovaries was noticeable from August to October, with the progress of the monsoon. It would appear that adult mortality was high during adverse period.

DISCUSSION.

Viswanathan (1950) reviewing the available evidence, has forcefully indicated that *A. fluviatilis* species may comprise more than one biological race with different vectorial capacities and host predilection in different parts of India. The study under report deals with *A. fluviatilis* of the foot-hill region, where it is a vector of hyperendemic malaria.

Although the water temperature in streams was not unfavourable during the monsoon, the species suffered adversely during the heavy rains as clearly indicated by an abrupt fall in numbers of larvæ and adults caught during this period.

A. fluviatilis in the Wynaad not being able to breed in its favourite breeding places during the monsoon, tides over the adverse period by breeding in water collections not resorted to normally. Such breeding is at a low level as shown by the small numbers of larvæ captured in wells, field wells

and tanks during the monsoon. It was further seen that larval prevalence in these breeding places was not consistent at every survey. Ramachandra Rao (1945) while definitely incriminating ricefields, as important sources of the species in Bombay State, mentioned that in several papers published on the habits of the species in the Western Ghats extending from North Kanara to Travancore, the status of ricefields as breeding places has been considered, but the findings have not been in agreement. The same author also observed a difference in the degree of prevalence of this species in ricefields between two seasons viz. August 1942 to December 1943 and January to December 1944. The conflicting findings were perhaps due to the fact that *A. fluviatilis* as found in the present observations was tiding over conditions extremely adverse to it.

Breeding places of *A. fluviatilis* during the monsoon are never permanent and not sources of continuous output of the species. Nevertheless such breeding places play an important rôle for the survival of the species during the period adverse both to its larvæ and adults by maintaining themselves wherever possible.

Conditions are generally favourable for the longevity of the imago during the monsoon period. Nevertheless the observations recorded in the Mundakolli Valley show that the number of mosquitoes gravid and semi gravid become less and less during the months of August and September. The Mundakolli Valley being a spot very favourable for a *A. fluviatilis* it was feasible to observe this but in other areas although identical conditions may prevail, *A. fluviatilis* occurrence is generally so low during the monsoon period that it becomes imperceptible. The fact that adults of this species were captured in the area during the monsoon period and occasional breeding places were also observed to exist during the same period, explains to some extent the rôle of such breeding places in the life history of the species.

Vedamanikkam (1949) recorded a marked decrease in the prevalence of *A. fluviatilis* larvæ following two successive years of indoor D.D.T. residual spray in the Wynaad colonisation area. This was observed in places where although ideal conditions for breeding of this species existed in many areas, larvæ were not found in them and it was specially conspicuous in areas known to yield many in the years prior to D.D.T. residual spray. It may be safely said that in Wynaad the highly anthropophilic *A. fluviatilis* breeding close to human dwellings, does not spend more than three or four days without resting sufficiently long to take the lethal dose of D.D.T. inside human dwellings, although one cannot assert that *A. fluviatilis* of this locality does not rest outside human dwellings at all. Hence the low incidence of the species observed in Wynaad after two years of D.D.T. indoor residual spray.

Muirhead-Thomson (1947) indicated that the dramatic fall in the house catch following treatment with D.D.T. as indoor residual spray cannot be

accepted as sufficient proof of mosquito reduction but when, as has been found in Wynaad, a conspicuous reduction in the prevalence of larvæ almost to nil collection together with a dramatic fall in adult captures, it may be safely assumed that the species is fast becoming extinct in the area.

The monsoon period represents the weakest link in the life chain of *A. fluviatilis*. The species is able to thrive by breeding in places other than streams during the monsoon period and is able to increase its numbers with the return of the favourable season. *A. fluviatilis*, with its highly specialised breeding places, has to take recourse to temporary and chance breeding places for the perpetuation of the species during the adverse period, but with D.D.T. it has not been known to develop so far any such device or method to survive its toxic effect so that the species seem to die out and chances for the survival of the individual that might continue the species during the next favourable season, are little. The results of observation on the effect of D.D.T. indoor residual spray on this species has been convincing enough to show that this species is highly susceptible to its toxicity and so perhaps capable of being eradicated by D.D.T. residual spray.

Hence it is suggested that before any resistance to this insecticide is developed, this carrier of hyperendemic malaria in the Western Ghats of India be fought with all the strength at our disposal in the entire area, where it holds its sway.

SUMMARY.

1. The breeding habits of *A. fluviatilis* with special reference to the monsoon period are described.
2. The results of study into the habits of *A. fluviatilis*, both adult and larvæ, in a valley in the Wynaad Colonisation area, are given.
3. The rôle of chance breeding places during the monsoon period is discussed and a possibility of the eradication of the species in the Western Ghats of India is suggested.

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4

MALARIA CONTROL MEASURES IN THE TERAI AREA
UNDER THE TERAI COLONIZATION SCHEME,
KICHHA, DISTRICT NAINITAL: 1949 TO 1951.

SECOND REPORT.

BY

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(October 9, 1952.)

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- II. METEOROLOGY.
- III. POPULATION.
- IV. ORGANISATION.
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VIII. DISCUSSION AND CONCLUSION.

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X. ACKNOWLEDGMENT.

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INTRODUCTION.

The problems of malaria control, organisation and the initiation of the control scheme in the Terai area under the Terai Colonisation Scheme, District Nainital, was described in 1950 by the senior author (Srivastava, 1950). The present report deals with the progress of the scheme during the three years, 1949-51.

I. TOPOGRAPHY AND HYDROGRAPHICAL FEATURES.

The topographical features have greatly changed since 1948. A vast area of jungle, 16,370 acres in extent, has been cleared by the Central Tractor Organization and has been made available for large-scale cultivation of food and other crops. The total area under cultivation has been increased from 9,703 acres in 1947 to 26,073 acres in 1951.

The sub-soil water level which was 3 to 3½ feet below the surface in 1947 fell much below that level in 1951. This fall was chiefly due to scarcity of rains during the latter period and possibly also due to improved drainage of the area.

II. METEOROLOGY.

The climate of the Terai differs from that of the adjoining plains country in great variations between the day and night. The maximum temperature of 105° to 106°F. is reached in the months of May and June, while the minimum temperature of about 43°F. is recorded in December. The relative humidity ranges from 42 to 88 per cent and is the lowest in April and May and highest in August and September. Average monthly records for the meteorological data for 1949, 1950 and 1951 are shown in Table I

III. POPULATION.

The population of the Terai is on the increase due to a considerable number of immigrants settling in new villages. This has been largely due to the success of malaria control measures, and since the previous report, twenty new villages have sprung up with a population of 4,000 settlers, most of whom are displaced people from Pakistan or ex-service men. In addition, three colonies of permanent modern buildings have been constructed to accommodate Government officials.

IV. ORGANIZATION.

Antimalaria staff.—A junior clerk, a stores clerk and four more insect collectors were added in 1949-50 to the strength of the staff existing in 1947-48. The present staff position is as follows:—

Name of post.	Number
Antimalaria officer	1
Malaria supervisors	5
Accounts clerk	1
Junior clerk	1
Stores clerk	1
Senior malaria field workers	5
Malaria field workers	25
Mistri	1
Laboratory assistants	2
Insect collectors	6
Motor driver	1
Motor cleaner	1
Peon	1
Orderlies	2
Sweepers.	2

TABLE I.
Annual record of meteorological data for the year 1949-1951.

MONTHS	1949					1950					1951				
	Maximum temperature, °F.	Minimum temperature, °F.	Relative humidity per cent	Total rainfall in inches.	Maximum temperature, °F.	Minimum temperature, °F.	Relative humidity per cent	Total rainfall in inches.	Maximum temperature, °F.	Minimum temperature, °F.	Relative humidity per cent	Maximum temperature, °F.	Minimum temperature, °F.	Relative humidity per cent	Total rainfall in inches.
Jan.	78.3	48.3	70.6	Nil.	76.77	47.67	76.0	1.10	67.00	44.10	81.5	67.00	44.10	81.5	0.04
Feb.	78.2	51.2	66.4	2.13	76.62	45.11	63.3	0.50	77.90	48.25	71.28	77.90	48.25	71.28	0.45
Mar.	88.1	60.8	60.5	0.93	87.0	55.8	61.2	1.83	85.16	58.55	58.14	85.16	58.55	58.14	1.23
Apr.	99.5	70.2	50.01	0.5	92.2	62.9	42.2	Nil.	91.12	66.65	50.7	91.12	66.65	50.7	0.4
May.	100.5	78.4	57.3	2.5	105.5	75.0	43.0	0.40	100.20	72.36	53.96	100.20	72.36	53.96	0.8
Jun.	105.6	81.07	68.0	1.89	100.5	77.07	69.2	5.12	100.60	77.70	63.78	100.60	77.70	63.78	0.02
Jul.	93.66	78.73	80.7	10.75	91.4	77.2	58.9	21.51	93.30	78.80	80.0	93.30	78.80	80.0	2.75
Aug.	90.44	78.18	85.88	17.38	85.2	77.2	87.8	17.35	91.03	71.14	85.38	91.03	71.14	85.38	17.08
Sep.	92.0	75.93	81.71	12.97	85.6	77.4	82.2	3.40	89.70	75.40	82.26	89.70	75.40	82.26	6.74
Oct.	92.83	68.74	74.41	0.46	82.8	69.2	70.18	Nil.	91.70	67.20	76.9	91.70	67.20	76.9	Nil.
Nov.	83.2	53.8	84.38	Nil.	77.3	59.3	70.6	Nil.	83.40	55.73	73.9	83.40	55.73	73.9	0.45
Dec.	78.77	44.18	69.95	Nil.	75.2	43.3	68.2	Nil.	75.10	46.00	79.5	75.10	46.00	79.5	Nil.
				49.51				51.21							29.94

The antimalaria officer also controls the working of the mobile dispensaries in his area and has the following staff :—

Medical officers	2
Compounders	3
Dressers	3
Driver	1
Cleaner	1

The function of the mobile dispensaries is to render medical aid for common ailments including malaria treatment to the villagers at their own homes and to remove emergent cases requiring hospitalization to fixed dispensaries at Kichha and Rudrapur.

For purposes of malaria control work, the entire area was divided into three sub-areas or sectors with approximately equal number of villages in each. Each sector was placed under one malaria supervisor who was supplied with the necessary number of field workers and requisite quantities of insecticide and spraying equipment. He was responsible for entire spraying operations and maintaining records of work within his sector.

V. TECHNIQUE.

A. INDOOR RESIDUAL SPRAY.

D.D.T. water suspension prepared with gum acacia and gelatine in accordance with the formula recommended by the Malaria Institute of India was used in 1949 in the strength of 2.5 per cent giving a dose of approximately 50 mg. per sq. ft. as in 1948. In 1950, this was replaced by D.D.T. emulsion, also prepared in accordance with the formula recommended by the Malaria Institute of India using aromex and soft soap, and used in the same strength as water suspension. In 1951, besides using emulsion, Geigy's 50 per cent wettable powder in 2.5 per cent water suspension was also used in a few places.

It was noticed that with emulsion there was greater absorption of the fluid in porous mud walls but blockage in the pumps was nil. With locally made water suspension from technical D.D.T. or from Geigy's 50 per cent wettable powder, absorption into the walls was comparatively little but blockage in the pumps was of frequent occurrence and consequently increased the number of idle hours. Besides, the use of emulsion was observed to have some repellent effect on mosquitoes for two to three days after spraying operations but this was not so with suspension. The residual effect was observed to be comparatively less with emulsion than with suspension. Emulsion was observed to cause smarting of eyes and nostrils of the field

workers and had a rapid corrosive action on rubber hoses and washers of spray pumps.

Different types of sprayers were tried but double and single barrelled double action stirrup pumps gave satisfactory and efficient service. The nozzle used in the spray pump was of an aperture of $1/24$ to $1/16$ inch.

In the year 1949, the frequency of indoor residual spraying was spaced at intervals of six weeks in all villages, staff quarters, hutments and other structures except in temporary camps and thatched gang-huts which were sprayed at three weekly intervals due to the prevalent practice of frequent replacement of thatch. Spraying operations were discontinued in November and December.

In 1950, the number of applications was reduced to five, namely two in spring and three in autumn at six weekly intervals. In 1951, four rounds of spray were found sufficient. The first was carried out in February-March, second in July-August, third in September-October and fourth in November-December. Dosage employed at each spraying was approximately 50 mg. D.D.T. per sq. ft. as before.

B. PALUDRINE PROPHYLAXIS.

In 1949 and 1950, great difficulty was experienced in proper and timely distribution of paludrine for prophylactic purposes amongst the local inhabitants of the operational villages (Table II). In a majority of cases, on the days fixed for administration of prophylaxis, people were either absent from their villages or were reluctant to take the course. It was never possible to administer the treatment to more than 50 per cent of the population in any case. Women folk in particular were the greatest defaulters; purdah prevalent amongst them was the chief handicap.

In 1951, paludrine prophylaxis was generally discontinued. It was carried out from July to November, only in certain Government and public institutions like the police, schools and State farms. The total number of paludrine hydrochloride tablets (0.3 gm. each) distributed was 20,544. The number of malaria cases receiving curative treatment as a routine in 1951 was negligible.

VI. EPIDEMIOLOGICAL INDICES.

(i) SPLEEN EXAMINATION IN CHILDREN.

Malarionetric survey were carried out twice a year between May-June and November-January in 66 villages. Occasionally a few villages were missed. Marked progressive reduction in spleen rate among children was

TABLE II.
Distribution of paludrine hydrochloride.

Months.	Total number of persons to whom paludrine was given			Total number of tablets given		
	1949	1950	1951	1949	1950	1951
Jan.	18,967	..	Paludrine distribution discontinued, do	56,954	..	Paludrine distribution discontinued.
Feb.	16,875	..	do	48,778	..	do
Mar.	21,402	27,026	do	62,744	76,557	do
Apr.	27,682	..	do	83,031	..	do
May	29,101	..	do	83,143	..	do
Jun.	27,241	..	do	77,101	..	do
Jul.	33,020	49,653	do	90,393	1,29,901	do
Aug.	41,673	45,862	do	1,01,508	1,26,505	do
Sep.	37,514	35,773	do	99,769	96,556	do
Oct.	17,131	26,240	do	47,535	71,391	do
Nov.	..	10,011	do	..	27,337	do
Dec.	..	..	do	..	..	do
Total	2,71,296	1,94,565		7,31,156	5,28,297	

observed. The average figures during each year for the villages are shown in Table III.

TABLE III.

Child spleen rates.

Period	May to June			November to January		
Year	Number of villages	Number examined	Spleen rate, per cent	Number of villages	Number examined	Spleen rate, per cent
1949	32	465	57.4	41	995	39.1
1950	33	941	19.7	33	913	9.9
1951	48	998	8.4	51	1005	10.5

The average enlarged spleen was determined by Christophers method in all the years regularly in 37 villages in 1949, 34 villages in 1950 and 44 villages in 1951. The 1951 figures showed an increase over those of the previous years in eight villages due to imported cases, and in three the figures were static during the 3 years. In the remaining villages, the average enlarged spleen had decreased.

(II) PARASITE RATE IN CHILDREN.

The parasite rates in children during the six surveys are shown in Table IV. The cause for the increase during 1951 was due to the new arrivals from highly endemic areas of the West Punjab who were found to be harbouring previously acquired infections.

TABLE IV.

Child parasite rate.

Period	May to June				November to January			
Year	Number of villages.	Number examined.	Number positive	Parasite rate, per cent	Number villages.	Number examined	Number positive	Parasite rate, per cent.
1949	33	382	5	1.3	41	1042	12	1.1
1950	33	984	10	1	34	912	14	1.5
1951	49	901	18	2	52	991	61	6.1

(III) INFANT PARASITE RATE.

That fresh transmission was mostly eliminated in the area during the three years was clear from the results of the examination of blood smears from 3,814 infants during 1951. Fortyseven of the smears were positive to malaria parasites and the species prevalence was found to be 56.25 per cent *P. falciparum* and 43.75 per cent *P. vivax*. The details are summarised in Table V.

TABLE V.
Infant parasite rate in 1951.

Month.	Number examined.	Number positive.	Infant parasite rate, per cent.
Jan.	252	2	0.79
Feb.	266	2	0.75
Mar.	215	2	0.93
Apr.	227	4	1.76
May	211	1	0.47
Jun.	225	2	0.88
Jul.	220	2	0.91
Aug.	227	5	2.2
Sep.	260	11	4.23
Oct.	304	5	1.64
Nov.	315	7	2.33
Dec.	292	4	1.37

(IV) SPECIES PREVALENCE OF PARASITES.

Plasmodium vivax was found to be the commonest infection up to the year 1950 but it subsequently went down and was superseded by *P. falciparum* in 1951. The proportion of various types of parasitic infections found during the three years were as follows:—

Percentage species prevalence.

Infection	1949	1950	1951
<i>P. falciparum</i>	33.3	38.7	70.9
<i>P. vivax</i>	50.0	55.7	28.5
<i>P. malariae</i>	16.7	4.2	0.6

Malaria morbidity statistics were collected from the Civil Dispensary at Kichha and the Colonisation Mobile and Static Dispensaries at Simla Pistaur and Rudrapur. Diagnosis of malaria was generally based after positive findings of malaria on blood examination, except for Simla Pistaur Dispensary from where blood films were not regularly received. Kichha and Rudrapur dispensaries regularly supplied blood films of clinically diagnosed malaria cases for confirmation only in 1951. Although generally there had been fall in malaria attendance no definite inference can be derived from the dispensary figures shown in Tables VI and VII.

TABLE VI.

*Percentage of malaria cases to total cases who attended
Kichha Dispensary, Naini Tal, during 1949-51.*

Months	Percentage of malaria cases to total cases			
	Average of 5 years 1944-48	1949	1950	1951
Jan.	26.8	11.9	0.5	1.6
Feb.	22.6	8.1	0.5	1.1
Mar.	20.2	8.8	0.2	0.9
Apr.	21.9	12.0	0.1	0.2
May	23.5	9.9	0.1	0.4
Jun.	21.0	6.1	0.0	0.5
Jul.	22.3	5.5	0.1	0.8
Aug.	32.2	8.8	0.4	0.4
Sep.	40.6	12.5	1.0	9.1
Oct.	59.0	15.3	4.7	11.1
Nov.	47.8	9.8	2.0	11.8
Dec.	31.1	8.8	1.4	5.8

TABLE VII.

*Percentage of malaria cases to total cases attending the
dispensaries at Simla Pistaur and Rudrapur in 1951.*

Months	Percentage of malaria cases to total cases	
	Mobile dispensary Simla Pistaur	Rudrapur Hospital
Jan.	8	4
Feb.	6	4
Mar.	5	3
Apr.	5	4
May	4	4
Jun.	7	8
Jul.	4	7
Aug.	10	8
Sep.	10	6
Oct.	14	5
Nov.	13	5
Dec.	7	5

 (V) ENTOMOLOGICAL DATA.

Regular collections of mosquitoes were undertaken from a number of unsprayed cattlesheds and human dwellings within the sprayed villages earmarked as catching stations and also from similar stations situated in villages outside the sprayed areas (otherwise known as 'check' villages) for comparison. Altogether fifteen species* of adult anophelines were identified in the area during the period under report.

After a study of the collected entomological data it has been surmised that, *A. minimus* is neither the chief nor the sole vector of Terai and in fact at any time cannot have possibly taken a very active rôle in the transmission of malaria for the following reasons :—

1. Larvæ are very scarce.
2. Adult density and seasonal prevalence were found to be insignificant.
3. The little rise in density that was observed never coincided with any instance of high transmission.
4. A few specimens, which were available for dissection, did not show infection either in glands or guts.

(VI) SPOROZOITE RATE.

It has been gathered from previous records (Clyde, 1931 ; Das, 1930) that *A. minimus* was incriminated as vector in Bazpur area. On the contrary, during the months of April to September in 1949 four specimens of *A. fluviatilis* out of twenty one specimens dissected showed sporozoites in their salivary glands and also in 1951 one out of nineteen of this species dissected between July and September showed sporozoites in the glands. Moreover, in May 1949, an outbreak of B.T. malaria occurred in a group of unprotected labourers and on investigation *A. fluviatilis* was detected with sporozoites in gland in the locality. So it is not illogical to conclude that *A. fluviatilis* is a confirmed vector in the Terai tract of Naini Tal District. The details of dissection are shown in Table VIII. 

**A. culicifacies*, *A. fluviatilis*, *A. minimus*, *A. aconitus*, *A. vavuna*, *A. subpictus*, *A. vagus*, *A. annularis*, *A. pallidus*, *A. gigas*, *A. splendidus*, *A. barbirostris*, *A. hyrcanus*, *A. stephensi* and *A. maculatus*.

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TABLE VIII.✓
Result of dissection.

Year	Species	Number dissected.	Number showing salivary gland infection.	Sporozoite rate per cent.	
1949	<i>A. culicifacies</i> ..	156	0	0	*Two were found in the quarter July-September and the other two in October to December.
	<i>A. fluviatilis</i> ..	36	4*	11.1	
	<i>A. minimus</i> , ..	1	0	0	
1950	<i>A. fluviatilis</i> ..	76	0	0	
	<i>A. culicifacies</i>	104	0	0	
	<i>A. minimus</i> ..	1	0	0	
1953	<i>A. culicifacies</i> ..	81	0	0	*Found in the quarter July to September.
	<i>A. fluviatilis</i> ..	61	1*	1.6	
	<i>A. minimus</i> . ..	<u>99</u>			

A. culicifacies bred lustily in autumn months but has not presented any infective specimens to date. Even so, in analogy of the fact that, since this species is the chief established vector in the plains of U. P., and since the Terai tract of Naini Tal District is flush with the northern boundary of the Bareilly District, which categorically belongs to U. P. plains, it is reasonable to consider *A. culicifacies* as a probable vector. Further, *A. fluviatilis* is comparatively more prevalent in spring than autumn and *A. culicifacies* breeds more profusely in autumn months than any other time. The malaria morbidity curve shows two peaks, one in spring and one in autumn, the latter being higher. In order to explain this dual rise in the curve, the probable supplementary rôle of *A. culicifacies* in autumn can not be ignored.

(VII) COST OF CONTROL.

The cost of D.D.T. indoor residual spray and paludrine prophylactic treatment during the period under review is summarised in Table IX. All the items incidental to the spraying operations are included.

TABLE IX.

Cost of Control.

Year.	Number of sprayings in 10 months.	Total cost of spraying operations, Rupees.	Per capita cost per year of spraying operations, Rupees.	Cost per capita per spray, Annas.	Total cost of prophylactic paludrine per year, Rupees.	Per capita per year cost of paludrine distribution, Rupees.	Number of weekly treatments administered.
1949	7	41,304.71	4.00	9.00	19,930.19	1.94	52
1950	5	21,833.16	1.46	0.48	11,513.7	0.73	21
1951	4	16,113.56	1.07	0.44	0	0	0

VIII. DISCUSSION AND CONCLUSION.

As described already, there have been changes in the topography and hydrographical features in the Terai during the period under review. The destruction of the jungle and extension of agriculture have changed the environment.

The antimalaria organisation has undergone minor changes as a result of experience as well as due to the extension of the scheme. The mobile dispensaries have increased the scope for finding cases of malaria in addition to increase in the facilities for modern treatment of diseases, which were deplorably lacking in the rural areas.

The colonisation area has lately presented certain epidemiological problems affecting the malaria control operations. The change over in population, arrival of new communities from endemic and non-endemic areas and in many instances the extremely temporary nature of their habitations have all prompted constant vigilance of the malaria organisation. Although it has not yet been assessed as to how much these factors have been responsible for the malaria transmission in spite of 4 years of malaria control operations, there is no doubt about their contribution towards the presence of fresh malaria and efforts are being made to tighten up the measures.

The infant parasite rates, and *P. falciparum* being the most prevalent species, speak for themselves in reference to the present status of malaria

transmission/in the area. As against the above, the dispensary figures at Kichha, Simla Pistaur and Rudrapur indicate that there has been a reduction in the malaria cases. The increase in the spleen rates in certain villages requires investigation. At the same time, how much of this is due to immigrant population in these villages has to be assessed.

Looking at the results generally, there is no doubt that there has been an overall amelioration in the malaria morbidity. The number of small industries like rice mills under construction and the reactivation of sugar mills which had remained defunct for want of labour in the past, are all signs that the malaria hazard is not what it used to be in the past.

The incrimination of *A. fluviatilis* as a vector, is a new finding and not in accordance with the results of investigations in the past. *A. minimus* which was believed to be the vector in the area has almost disappeared and it is to be doubted whether the species was ever present in adequate numbers and played a vectoral rôle

IX. SUMMARY.

Four years of malaria control in the colonisation area of the Terai show a general decline of malaria incidence in the area. Transmission of the disease has not been totally eliminated and the probable causes for it are discussed.

There is evidence of improvement in the economic status of the people as judged from industrial and agricultural developement in the area.

Expenditure shown in the report included capital and recurring items, incidental to actual operation and not during the non-operation periods. The per capita cost of D.D.T. spraying per year has reduced from Rs. 4/- in 1949 to Rs. 1.46 in 1951. The use of paludrine suppressive treatment was discontinued in 1951.

X. ACKNOWLEDGMENT.

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D.D.T. AS A MOSQUITO LARVICIDE AND ITS APPLICATION IN HIGH SPREADING OILS ON LARGE EXPANSES OF WATER SHEETS.

BY

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[October 10, 1952.]

INTRODUCTION.

D.D.T. as a larvicide and adulticide was introduced during World War II. As an adulticide, it appeared from the results obtained that it was an instant and startling success.

During the war, expense was no object, and whole islands were sprayed from the air. The results obtained in many cases thoroughly justified the means. However aerial spraying of D.D.T. solutions as a larvicide did not prove to be effective under all circumstances. Tyssul Jones (1949) assessing the effectiveness of aerial sprayed solutions of D.D.T. in Burma has shown that, where vegetation was present, it had a marked filtering effect on the spray, so that, although the control in open water was good, in those areas where there was a vegetative cover the percentage kill was much less marked.

Reid (1948) has quoted "Parts of Kuala Lumpur were sprayed from the air with 5 per cent D.D.T. in kerosine at the end of September 1945 and it was found that while the mosquito larval breeding places in flat open country were reduced by over 90 per cent, the much more dangerous breeding places in ravines were reduced by only 45 per cent."

Many papers have appeared to show the effectiveness of the ground application of D.D.T. solutions and these papers indicate that this effectiveness is obtained with very minute quantities of the toxic substance.

Ribbands (1945) who carried out his experiments in water contained in rectangular tanks, 4 x 3 feet in area, showed that D.D.T. solutions at 1 pint to the acre gave a 100 per cent kill of both anopheline and culicine larvæ in 24 hours. Even at a dosage of $\frac{1}{2}$ pint to the acre a 90 per cent

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mortality against anopheline larvæ was obtained but the effect on culicine larvæ at this rate of application was much less marked.

Puri and Pal (1947) working in Delhi, Northern India, found that D.D.T. in oil solutions is very effective in doses of 4 to 6 c.c. per 10 square yards (approximately 3 to 5 pints to the acre).

Puri and Krishnaswami (1947) found that on still water 0.6 c.c. of a 5 per cent solution of D.D.T. per 10 square yards (approximately $\frac{1}{2}$ pint to the acre) was 100 per cent effective against anopheline larvæ.

D.D.T. IN HIGH SPREADING OILS AS A LARVICIDE.

Reid (1948) says "the economy of the oil remains at the present time the principal advantage of D.D.T. for ordinary work. Instead of using 25 gallons to the acre..... a gallon or less will suffice, and the cost of D.D.T. and oil is likely to be one-tenth of that of the large quantity of ordinary oil."

Some of the earlier work and certainly later work showed that, whereas one or two ounces of D.D.T. per acre of treated water surface would give control of mosquito breeding, there were certain disadvantages of using these very small quantities of the chemical.

The first of these disadvantages was the less marked effect on culicine larvæ as compared to the effect on anopheline larvæ.

The second disadvantage was that whereas these smaller dosages of D.D.T. per acre of water surface would give a 100 per cent control of anopheline larvæ after 24 hours, breeding recommenced almost immediately and many 4th stage larvæ were present in the treated area 6 days after the application of D.D.T.

The recommended dosage is now 4 ounces of D.D.T. per acre. Reid (1950) stated in his summary that "A dose of 4.3 ounces of D.D.T. per acre applied as a 5 per cent solution in malariol at 0.5 c.c. per square yard ($= \frac{1}{2}$ gallon per acre) was selected for further trial in a large pond, and gave good results. Control, 24 hours after treatment, averaged 99 per cent in three trials and the number of large larvæ (4th stage) present six days after treatment was only one-third of the number before treatment began. Some of these large larvæ were kept, but none pupated the following day."

A 5 per cent solution of D.D.T. in an oil and applied at the rate of $\frac{1}{2}$ gallon per acre is equivalent to 4 ounces of D.D.T. per acre of treated water surface. The difficulty revolves around the application of this small quantity of oil. The knapsack sprayer which was designed for the application of oil at the rate of 18 to 20 gallons per acre cannot economically be used to apply an oil at the rate of $\frac{1}{2}$ gallon per acre. Various other methods have been suggested such as pouring the oil on to the water surface at discreet distances, or the use of slit pumps. These, however, are impracticable.

The breeding places of mosquito larvæ are of two main types:—

- (a) Large expanses of water such as lakes, ponds, rivers and streams.
- (b) Small discontinuous water surfaces such as puddles, water in hoof prints, seepages, etc.

In this paper an applicator for applying D.D.T. in high spreading oils to large expanses of water has been described. Work is in progress with the application of D.D.T. in high spreading oils to discontinuous water surfaces.

Most of the work carried out hitherto has been designed to show the advantages and efficiency of the use of D.D.T. in oil for the control of mosquito breeding, in addition to determining the minimum dosage of D.D.T. to assure adequate control. Reid in Malaya has done a considerable amount of valuable work in this direction. He has carried out field experiments with D.D.T. in oil as an anopheline larvicide on still and flowing water. However, in order to determine the correct dosages and to show the advantages of D.D.T. in oil over ordinary oiling, he chose ditches and ponds the whole of the water surface in which was covered with oil. In other words these were confined spaces, although of considerable extent, which ensured that all the water surface was covered with an oil film provided that there was sufficient oil applied. In his paper on the application of D.D.T. in oil to still water (Reid, 1950) he states "The larvicide was applied by pouring on out of a measuring cylinder at one point in the middle of each ditch, or at two points equidistant from either end of the ditch and from each other. The oil spread of its own accord, penetrating amongst the water hyacinth and usually reached the ends of the ditches within about 15 minutes."

The emphasis has been on the dosage and not the means of applying that dosage. The present author, accepting the efficiency of a 5 per cent solution of D.D.T. in a high spreading oil in controlling mosquito breeding, has been concerned in designing a suitable piece of equipment for applying such a solution at the rate of $\frac{1}{2}$ gallon per acre.

Mosquito breeding in large ponds, rivers, etc., is usually confined to the vegetative margins which vary considerably in extent. It was soon apparent that pouring on large quantities of the high spreading oil at long distances was inapplicable in many cases. For example if the oil was applied in this way to ponds with a narrow vegetative border it would start spreading out in all directions. However, on reaching the open water the speed of spread was very rapid with the result that there was insufficient lateral spread through the vegetation to give a complete film between the points of application. It was therefore essential that small quantities of oil be applied at shorter distances. On the other hand it was necessary that the quantity of oil to be applied at each point be kept within practical limits.

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✓ 1 c.c. of a high spreading oil will cover at least 2 square yards of water surface if allowed to spread unimpeded. Preliminary work indicated that 5 c.c. of oil applied at 8 to 10 paces intervals would give a complete cover water surface in vegetative margins of ponds up to at least 10 yards in width.

A large number of experiments were thus carried out on ponds having vegetative borders of different habits of growth and width in order to determine that this rate of application was practical. For this work the apparatus consisted of tube to hold the necessary 5 c.c. of oil. This was attached to one end of a long stick which could be pushed into the vegetation so that the oil could be poured directly on the water surface thus avoiding any waste of oil. The following experiments are described from many carried out.

TRIAL AREAS.

Three ponds differing in the nature of their vegetative cover were taken, using the method of application mentioned above. The method of determining the larval population before and after oiling is common to all; hence it will be described now in order to avoid repetition.

METHOD OF DETERMINING LARVAL POPULATION.

The trial areas were divided into 10 yard lengths, each marked with a bamboo pole, the areas between the poles representing a test point. At each test point 6 dips before and numerous dips after oiling were taken using an ordinary soup ladle. In all cases the searcher, in addition to collecting at the sides of the ponds, waded out as far as possible to determine the effectiveness of the oil film at the outer edge of the vegetative cover. There are disadvantages attached to the use of a dipper for the estimation of larval population. Dipping would be useless where the larvæ are of a "shy" nature. However the ease with which the larvæ were collected before oiling, and their absence or rarity after oiling, in spite of numerous dips, gave a good indication of the efficiency of the oil film.

FIRST POND.

It was selected because there was a border of very thick grass, 18 to 24 inches in height and averaging 4 to 5 yards wide, running more or less parallel to the side of the pond from which it was separated by an open channel of water approximately 2 feet wide.

Larval population.—There was an occasional larva found in the open channel and the number found in the water covered with grass border was by no means large. ✓/

The number of larvæ collected in the 10 test points over a distance of 100 yards is given in Table I. Most of the larvæ were 3rd and 4th stage.

TABLE I.

Larval population at each test point per 6 dips before, and numerous dips 24 hours after, the application of 5 per cent D.D.T. in a high spreading oil.

Test point	Before oiling		24 hours after oiling	
	Anophelines	Culicines	Anophelines	Culicines
1	6	4	0	0
2	7	8	0	0
3	5	3	0	0
4	10	4	0	0
5	3	7	0	0
6	10	8	0	0
7	6	4	0	0
8	8	7	0	0
9	9	8	0	0
10	8	4	0	0
Total	72	57	0	0

It will be seen from this table that 24 hours after oiling no larvæ were found.

SECOND POND.

This pond had a totally different type of vegetation. It was about 330 yards in circumference and one side 100 yards long was taken for test. This side had a border of mixed vegetation extending for 6 to 10 yards into the pond. In some parts the vegetation consisted of water hyacinth plants growing so closely together that the water surface could not be seen. In other parts there were grasses and reeds which again were so dense in growth that the water surface was invisible.

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Larval population.—The number of larvæ collected at 10 test points over a distance of 100 yards is given in Table II.

Most of the larvæ were 3rd and 4th stage.

TABLE II.

Larval population at each test point per 6 dips before, and numerous dips 24 hours after, the application of 5 per cent D.D.T. in a high spreading oil.

Test point	Before oiling		24 hours after oiling	
	Anophelines	Culicines	Anophelines	Culicines
1	3	4	0	0
2	3	0	0	0
3	3	1	0	0
4	4	0	0	0
5	7	0	0	0
6	3	0	0	0
7	5	1	0	0
8	4	0	0	0
9	6	2	0	0
10	7	3	0	0
Total	45	11	0	0

24 hours after the application of the oil no larvæ were found.

THIRD POND.

This trial illustrates the limitations of a high spreading oil containing D.D.T. for the control of mosquito larvæ and applied even at shorter distances.

Provided the vegetation rises vertically through the surface of the water, it will not impede the spreading of an oil. Mechanical barriers, lying horizontally on the surface of the water, either in the form of grass stems or other objects, will however prevent the spread of an oil film.

This pond had a circumference of about 1,100 yards. A length of 260 yards of one side was selected for test. The bank along this length differed markedly in its topography. For the first 100 yards it was about

1 to 2 feet in height but for the remaining distance the height varied from 12 to 25 feet. The whole bank was densely covered with a lallang type grass, the underground stems of which penetrated through the face of the bank and passed out on to the water surface for about 4 to 5 yards. These stems formed an irregular network partly on and partly below the surface of the water.

Larval population.—The number of larvæ collected in the 26 test points is given in Table III. The anopheline larvæ were actually counted, but the culicine larvæ were so numerous that the collector did not record the actual number. The figures given are a very conservative average.

TABLE III.

Larval population at each test point per 6 dips before, and 24 hours after, the application of 5 per cent D.D.T. in a high spreading oil.

Test point	Before oiling		24 hours after oiling	
	Anophelines	Culicines	Anopelines	Culicines
1	0	12	1	0
2	3	12	2	3
3	0	12	0	4
4	10	12	2	0
5	5	12	0	10
6	4	12	0	6
7	3	12	1	4
8	4	12	1	5
9	8	12	0	0
10	4	12	0	0
11	6	12	0	0
12	5	12	0	1
13	7	12	0	0
14	6	12	1	1
15	7	12	2	0
16	4	12	1	4
17	6	12	2	3
18	10	12	0	0
19	10	12	0	0

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TABLE III (Conold.)

Test point	Before oiling		24 hours after oiling	
	Anophelines	Culicines	Anophelines	Culicines
20	6	12	2	3
21	5	12	1	2
22	4	12	2	3
23	3	12	1	2
24	7	12	0	0
25	5	12	0	0
26	6	12	0	0
Total	153	312	10	51

The presence of the mechanical barriers prevented the oil from forming a complete film; a number of large uncovered areas remained. The results in the above table reflect the discontinuous nature of the oil film since all the living larvæ were taken from the areas uncovered with oil.

In order to demonstrate that the incomplete control was due to the incomplete film, and not to other factors, another area of the same pond was cleared of the grass stems for a distance of 50 yards. The oil was applied in the same way and the results before and 24 hours after application, are given in Table IV.

TABLE IV.

Larval population in the area cleared of grass stems, per 6 dips before, and numerous dips 24 hours after, the application of D.D.T. in a high spreading oil.

Test point	Before oiling		24 hours after oiling	
	Anophelines	Culicines	Anophelines	Culicines
1	21	2	0	0
2	26	2	0	0
3	25	4	0	0
4	17	7	0	0
Total	89	15	0	0

APPLICATOR FOR HIGH SPREADING OILS CONTAINING
D.D.T. FOR USE ON LARGE EXPANSES OF WATER.

The application of a high spreading oil containing 5 per cent D.D.T. at small doses and at short distances gave the necessary good results except in that area of water surface which had mechanical barriers in the form of grass stems lying on the water surface. The *rate* of application proved efficient but the *method* was from the practical point of view most unsatisfactory. An applicator, cheap, efficient and easy to handle was a necessary requirement.

This applicator had to fulfil two important functions :—

- (1) It should deliver approximately 5 c.c. of the oil solution.
- (2) It should be of such a construction that it could deliver this quantity of the solution direct to the water surface so that waste on heavily vegetated water surfaces could be avoided.

The following piece of equipment was therefore designed.

The applicator is connected to a tank on the operator's back by means of oil resisting tubing (Fig.1). The applicator is so designed that it sucks in oil on one stroke of the piston and forces oil out on the other stroke.



Fig. 1. Applicator gun connected to tank on operator's back by means of rubber tube.

It consists of a cylinder containing a double acting piston and at one end of the cylinder there are situated two non-return valves (Fig. 2 and 3). On the forward stroke of the piston, valve A opens allowing the oil to pass through to the outlet pipe and valve B closes preventing the egress of oil back into the tank. On the reverse action of the piston, valve A closes and valve B opens allowing ingress of oil from the tank, but preventing the liquid from passing back from the outlet pipe.

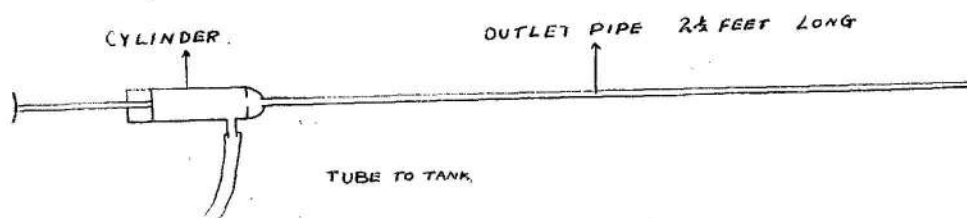


Fig. 2. Complete applicator.

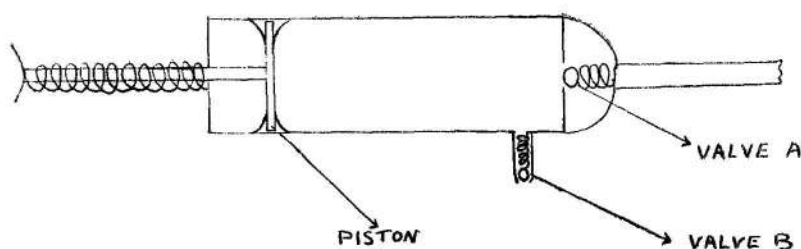


Fig. 3. System of non-return valves.

The rod operating the piston has round it a strong spring, thus ensuring the return of the piston from each forward stroke (Fig. 3).

The capacity of the cylinder and the stroke of the piston is such that for every complete stroke of the piston approximately 5 c.c. of oil are ejected.

The outlet pipe is 2.5 feet long thus enabling the oiler, from a standing position, to push the pipe through any thick vegetation so that the oil can be applied direct to the water surface (Fig. 4).



Fig. 4. Advantage of the applicator gun when applying high spreading oils to water surfaces covered with vegetation.

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The oil is ejected in the form of a jet and not a spray and there is sufficient force to project it about 20 to 30 feet, should this be necessary.

TWO FURTHER TRIALS USING THE APPLICATOR.

Only two of many are described.

As far as the oiling labourers were concerned this was a completely different way of applying oils for the control of mosquito larvæ, but it was surprising how quickly they realised what was required of them.

One of these trials was carried out on a pond and the other on a roadside ditch.

TRIAL ON POND

This pond was several acres in extent and at one end had a very good vegetative cover. The length of one side, used for the trial was 100 yards with the vegetation, mostly grasses, extending for about 10 yards into the pond.

Larval population.—The number of larvæ before and 24 hours after oiling is given in Table V. The larvæ were mostly 3rd and 4th stages.

TABLE V.

Larval population per 6 dips before, and numerous dips 24 hours after, the application of 5 per cent D.D.T. in a high spreading oil.

Test point	Before oiling		24 hours after oiling	
	Anophelines	Culicines	Anophelines	Culicines
1	18	79	0	0
2	27	33	0	0
3	59	13	0	0
4	18	2	0	0
5	14	3	0	1
6	17	3	0	0
7	22	5	0	0
8	26	0	0	0
9	12	7	0	0
10	21	74	1	0
Total	234	225	1	1

✓ One squirt of oil at 10 pace intervals was applied. 24 hours after the application of D.D.T. in high spreading oil, there was only 1 anopheline and 1 culicine larva present in the treated area.

TRIAL ON ROAD SIDE DITCH.

Culicine breeding was very intense. Each dip yielded about 12-15 larvæ. The results of 6-dips at each of 7 points are given in Table VI.

TABLE VI.

Larval population per 6 dips before, and numerous dips 24 hours after, the application of 5 per cent D.D.T. in a high spreading oil.

Test point	Before oiling		24 hours after oiling	
	Anophelines	Culicines	Anophelines	Culicines
1	2	95	0	3
2	1	84	0	0
3	2	87	0	0
4	1	86	0	2
5	1	74	0	0
6	3	98	0	0
7	2	101	0	1
Total	12	625	0	6

✓ One squirt of oil at 10 pace intervals was applied. It will be seen from the results in Table VI that the percentage kill after 24 hours was of a very high order.

DISCUSSION.

Reid (1948) and Tyssul Jones (1950) have drawn attention to the application of straight oils to large sheets of water. On large ponds, rivers etc., straight oils are applied at the recommended dosage only to the sides where mosquito larvæ are normally found. However, the oil will naturally spread out over the general surface with the result that, after a comparatively short period of time, the film remaining at the sides may be too thin to ensure effective control.

D.D.T. in high spreading oils does not suffer this disadvantage since the oil merely serves as a vehicle for the toxic substance, D.D.T., and films of monomolecular thickness containing this substance are still lethal to mosquito larvæ.

Apart from the economics of the problem, D.D.T. in high spreading oils is more efficient than straight oils used for the control of mosquito breeding. The difficulty has been to find the necessary means of applying the small quantities of oil ($\frac{1}{2}$ gallon per acre) required.

The applicator described above goes a long way in meeting this difficulty, designed, as it is, for ease of handling and provided with a long outlet pipe which can be pushed into the vegetation thus avoiding waste of oil on the foliage, as would certainly occur with any form of spray.

The use of the applicator is confined to large expanses of water such as lakes, ponds, rivers, streams, etc. The limitation in the application of high spreading oils containing D.D.T. at short intervals of distance is confined to those water surfaces which have mechanical barriers lying on them. Trials in which the overall kill of mosquito larvæ has been reduced in such water surfaces have been described. In addition to grass stems, sticks, etc., thick scum would also greatly impede the spread of an oil applied in this manner. However such impediments are found only on a very small percentage of water surfaces which have to be controlled.

SUMMARY.

(1) Some practical field trials have been described demonstrating the efficacy of small doses of D.D.T. in high spreading oils when applied in the correct manner.

(2) An applicator designed to produce the small quantity of the oil containing 5 per cent D.D.T. (at $\frac{1}{2}$ gallon per acre) has been described.

(3) The limitation of applying small quantities of oil at intervals of distance has been referred to.

(4) The advantages of D.D.T. in high spreading oils over the straight oils has been briefly discussed.

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FREE AMINO ACID PATTERN OF BLOOD OF NORMAL AND MALARIAL CHICK INFECTED WITH *P. GALLINACEUM*.

BY

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DISORDERS of amino acid and protein metabolism have been studied in a number of diseases by the application of chromatographic methods (Biochemical Society Symposia No. 3, 1950). Preliminary investigations on the other forms of liver diseases by Young and Homberger (1947), on progressive muscular atrophy by Ames and Risley (1947) and on hepato-leuticular degeneration by Uzman and Brown (1948) have also been reported. Of special interest was the almost constant finding of Dent (1947) about the substantial rises in the blood levels of methionine and cystine in some forms of liver diseases.

In the course of systematic studies on the derangement of the nitrogen metabolism of chick during malarial infections, the authors were interested in investigating the changes in blood in both normal and pathological conditions. Since the malarial parasite is a fast developing intracellular organism and as it needs protein for its development, a study on the free amino acids was undertaken with a view to throw light on the changes occurring during the rapid synthesis of the parasite protein.

Circular chromatographic technique as described by Giri and Rao (1952) has been used throughout and the method adopted for the extraction of the free amino acids was essentially the same as described by Giri *et al.* (1952).

METHODS AND MATERIALS.

Two months old chicken of white Leghorn or Rhode Island red breed were used in this investigation. They were kept in separate cages and were fed with the usual diet twice a day. Blood samples for the experiment were taken by heart puncture using potassium oxalate as anticoagulant. Throughout the experiment, blood was taken only in the mornings before giving any

(411)

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diet. After taking blood from the normal birds, for detecting free amino acids before infection, the same were infected intramuscularly into the pectoral muscle with *P. gallinaceum*, a strain maintained in the laboratory. Usually on the eighth or tenth day the birds reached the acute stage of infection and showed a very high degree of parasitæmia. Blood from such birds was taken and free amino acids were identified by circular paper chromatographic technique.

EXTRACTION OF FREE AMINO ACIDS.

(a) *Whole blood*.—Whole blood taken by intra cardiac puncture was mixed with potassium oxalate used as an anticoagulant. For each c.c. of blood taken, three c.c. of 95 per cent distilled alcohol were added and after thorough shaking, it was centrifuged. The supernatant was then shaken with three volumes of chloroform with subsequent centrifugation and the aqueous top layer was removed for chromatographic analysis.

(b) *Plasma*.—The same procedure was adopted as for whole blood.

(c) *Erythrocytes*.—Whole blood was centrifuged at high speed for 30 minutes and the plasma removed completely. Distilled water was added to the erythrocytes left in the tube to make up to the original volume of blood taken and this was ground well in an agate mortar and pestle with chemically purified sand. To this, alcohol in the proportion of 1 : 3 was added and after grinding the cells well the whole was centrifuged in a centrifuge tube. The alcohol layer was then transferred to a centrifuge tube and treated with chloroform as above for obtaining the aqueous extract.

The above process was repeated for normal and parasitised blood, and each of the final aqueous extracts containing free amino acids were diluted to the same volume.

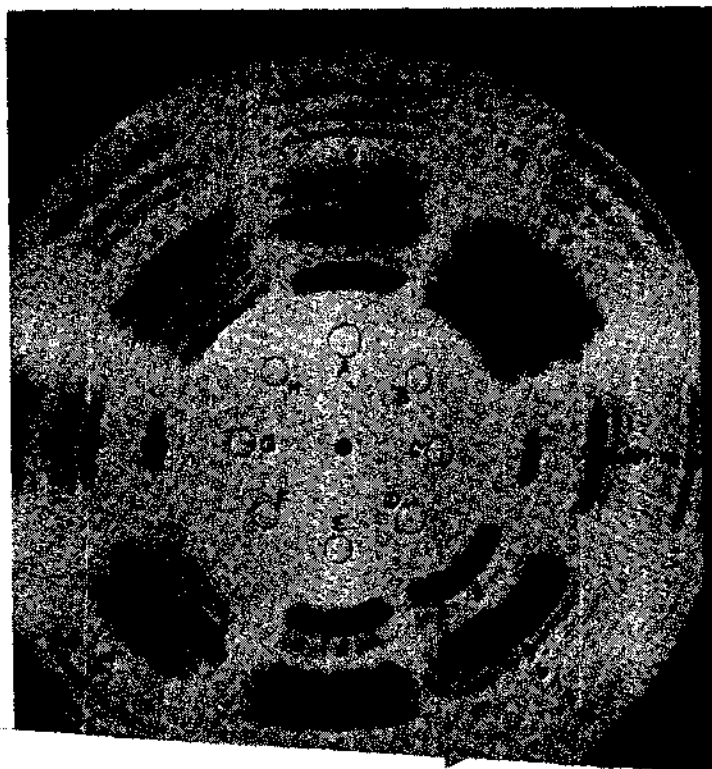
The amount of the final aqueous extract used for running the chromatograms was the same (80 μ l.) in all cases. The chromatograms were run as described by Giri and Rao (*loc. cit.*) using whatmann No. 1 filter papers.

The chromatograms were developed using n-butanol-glacial acetic acid-water mixture (40 : 10 : 50) as the developing solvent. The chromatogram after drying in air was sprayed with ninhydrin in acetone (0.1 gm. in 100 c.c. of 95 per cent distilled acetone) and dried at 60°C. for 15 to 20 minutes. Coloured bands in the form of concentric arcs developed on the paper indicating the presence of the amino acids and they were identified by running known amino acids side by side and confirmed by carrying out specific tests for some amino acids.

One of the typical chromatograms showing the amino acid patterns of the whole blood, plasma and erythrocytes of normal and infected chicken with *P. gallinaceum*, is shown in Fig. 1.

IDENTIFICATION OF BANDS.

Most of the bands separated were easily identified by running known amino acids side by side and by specific tests for each band. Arginine and methionine were identified by the technique of cutting sections and brushing the paper with specific reagents, as described by Giri *et al.* (1952). The presence of arginine (band. 2), histidine (between band. 1 and 2), glutamine (diffused band. 3), glutamic acid threonine (band. 4), alanine (band. 5), tyrosine (band. 6), valine-methionine (band. 7), phenylalanine (band. 8) and leucine-isoleucine (band. 9), can be seen in Fig. 1. The presence of glutamine was indicated by its disappearance on acid hydrolysis with the simultaneous increase in the intensity of glutamic acid band. Band 1 could not be identified with certainty. This band is very prominent in whole blood and erythrocytes, and is destroyed on acid hydrolysis. Further investigation is in progress on the identification of this band.



One of the significant observations made in the course of this investigation is the large increase in the concentration of glutamic acid in whole blood and erythrocytes in infected condition. Quantitative determination of the intensity of the glutamic acid bands by the method described by Giri *et al.* (1952 a) showed that the glutamic acid concentrations in infected erythrocytes is approximately five times the concentration present in normal.

Since malarial parasite rapidly increases in size and number in the blood of the host, they must synthesize large amounts of protein. Since they are intracellular organisms they may make use of the free amino acids present in the cell for the protein synthesis. Transaminase which has been found to be present in blood may play a rôle in this process and during this rapid protein synthesis, glutamic acid is formed in excess on account of its important rôle in the transaminase reactions. Further investigation on the reactions involved in the formation of glutamic acid in high concentrations in parasitised blood is in progress.

SUMMARY.

1. Changes in the free amino acids present both in normal and parasitised whole blood, plasma and erythrocytes, have been studied by circular paper chromatographic technique.
2. Glutamic acid was found to increase nearly five times in the infected conditions uniformly in whole blood, and cells.
3. The presence of increased amounts of free glutamic acid in parasitised conditions may probably be due to the transaminase reactions in the rapid process of protein synthesis in the parasitised erythrocytes.

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THE DISTRIBUTION OF HUMAN PLASMODIA IN INDIA.

BY

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Two decades have passed since Knowles, Senior White and Das Gupta (1930) published their studies on the parasitology of malaria in which they discussed by species the world distribution of human plasmodia. Since then, in India a tremendous malaria consciousness has developed and as a result of the large scale control activities undertaken, a change in the distribution of the species might well be expected. Additional factors influencing the distribution were possibly the World War II and mass migration of people consequent to partition of the country.

Knowles *et al.* (*loc. cit.*) remarked "The outside world may believe that, with the exception of a few distinguished and well-known workers, India is doing little or nothing to study and cope with her vast problem of malaria, but workers in this country know better. The truth is that there is an immense amount of malarial investigation being carried out all over India by trained and reliable workers but the information so laboriously collected does not get into the journals ; what little of it is published is published locally, chiefly in the form of Government reports, difficult to come by and only available to local workers ; the main bulk of this information, however, is not even published ; it exists in manuscript form in the offices of the different Directors of Public Health." The state of affairs described in the above quotation, has not changed much through the two decades.

It is now estimated that at present about twenty five million people in India are receiving protection from malaria and it is anticipated that a total of seventy five million will be protected during the current year under the National Malaria Control Plan. Provision has also been made for giving cover to a total of one hundred and twenty five million people by March 1955.

In view of the impending developments it was considered desirable to collate available data and record the present distribution of the species of human plasmodia in the country so that it may be possible to compare any subsequent changes that may occur.

For the present compilation, a questionnaire with a proforma was sent to different malaria organisations in the country, and where available, full use of published data has been made. The data thus collected have been analysed and presented in Table I and Appendix I.

TABLE I.
Percentage species prevalence in the States of India.

State	<i>P. vivax</i>		<i>P. falciparum</i>		<i>P. malariae</i>	
	1930	1950	1930	1950	1930	1950
Andamans ..	52	81	43	19	5	0
Assam ..	36.3	32	53.9	59	9.9	9
Bengal ..	*41.4	43	46.3*	54	12.3*	3
Bihar ..	††49.4	56	49.4††	43	1.2††	1
Bombay ..	†55.4	41	39.7†	61	5.9†	8
Coorg ..	28	80	48	20	24	0
Delhi ..	54.4	60	44.4	40	1.2	0
Hyderabad ..	35.6	55	54.5	33	9.7	12
Kashmir and Jammu ..	..	96	..	0	..	4
Junagadh ..	..	90	..	10	..	0
Kutch ..	..	68	..	31	..	1
Madhya Bharat ..	..	35	..	63	..	2
Madhya Pradesh ..	59	65	30.2	31	11.8	4
Madras ..	57.8	46	35.2	43	7	11
Mysore ..	69.4	34	28.6	57	2	9
Nepal ..	..	62	..	38	..	0
Orissa ..	††49.4	43	49.4††	53	1.2††	4
Punjab ..	58.4	61	40.6	38	1	1
Rajasthan ..	..	65	..	30	..	5
Rajkot ..	..	8	..	92	..	0
Saurashtra ..	..	66	..	34	..	0
Travancore ..	..	..	..	..	..	..
Cochin ..	..	17	..	26	..	57
Uttar Pradesh ..	..	39	..	60	..	1
Vindya Pradesh ..	70	54	26.1	38	3.9	8
India (General)	47.1	54.0	45.9	40.1	6.9	5.9

†These figures are average of totals for Sind, Bombay and Goa.

††Figures are for Bihar and Orissa combined.

*Figures are combined for East and West Bengal.

Presentation of the data on a map is similar to that of Knowles *et al.* (*loc. cit.*) but the map now prepared (Map 1.) shows on comparison with that of 1930 (Map 2), a considerable amount of additional information available at present.

Diagnosis of the species of malaria parasites has been made by various workers and their methods of staining and standards of identification differ. The degree of accuracy in the identification cannot, however, be correctly assessed. Mixed infections have been taken into consideration only where the species identification was made.

A summary of the available data for each State is presented below.

ANDAMANS.

The parasite rate for the area is 17.4 per cent., *P. vivax* and *P. falciparum* accounting for 81 and 19 per cent respectively. *P. malariae* seems to be totally absent at the present time.

ASSAM.

The total parasite rate for the State is 31 per cent. Of this, 32, 59 and 9 per cent respectively are the proportions in which *P. vivax*, *P. falciparum* and *P. malariae* are distributed. It is clear that *P. falciparum* continues to predominate in Assam as concluded by Knowles *et al.* (*loc. cit.*) The two exceptions appear to be the Khasi and Jaintia Hills and Sibsagar, where *P. vivax* predominates. In Sibsagar *P. vivax* has increased to 56 per cent from 38.1, while *P. falciparum* has decreased to 36 from 40.9 per cent. *P. malariae* is 9 per cent of the total while it used to be 9.9 per cent.

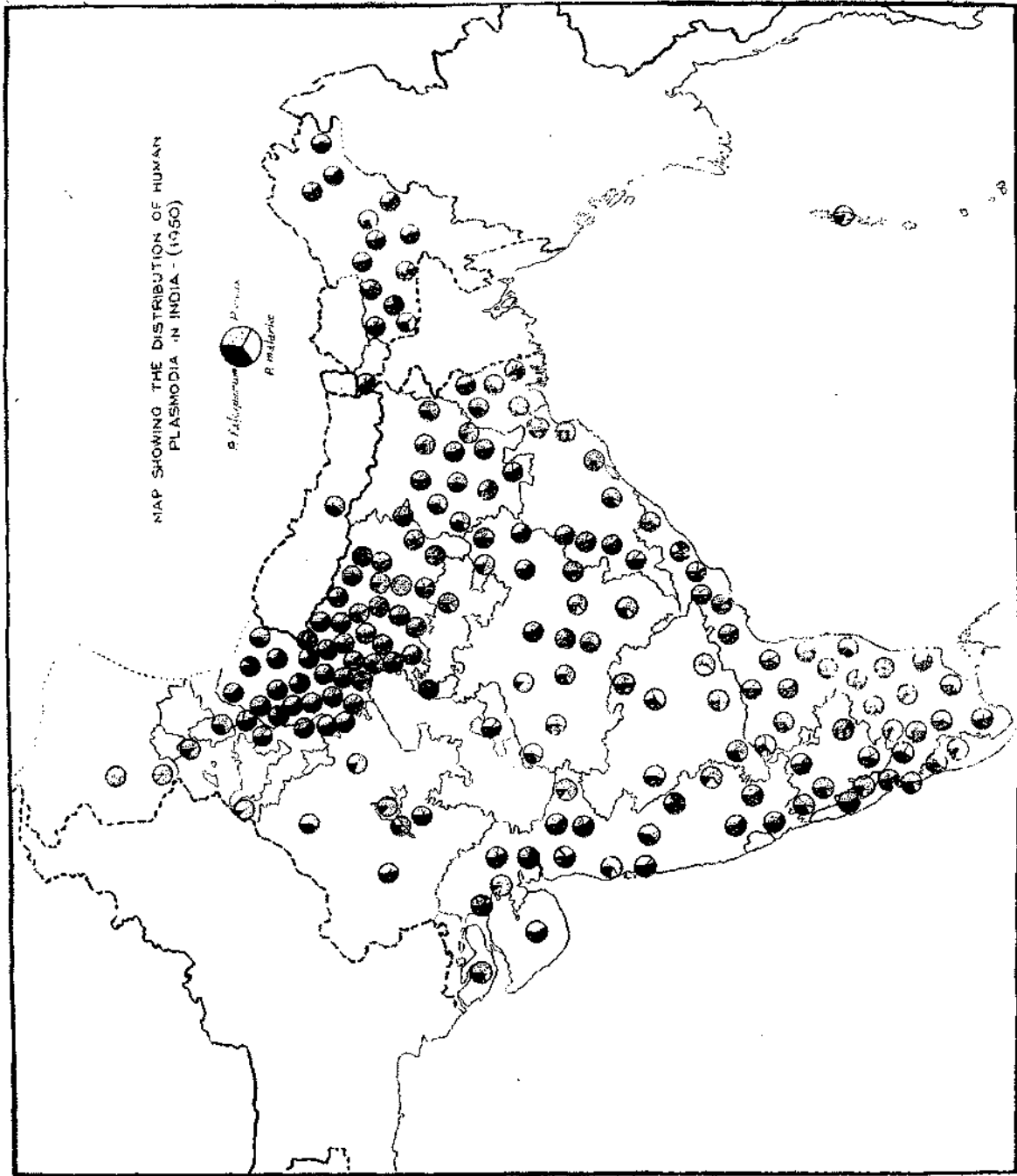
BENGAL.

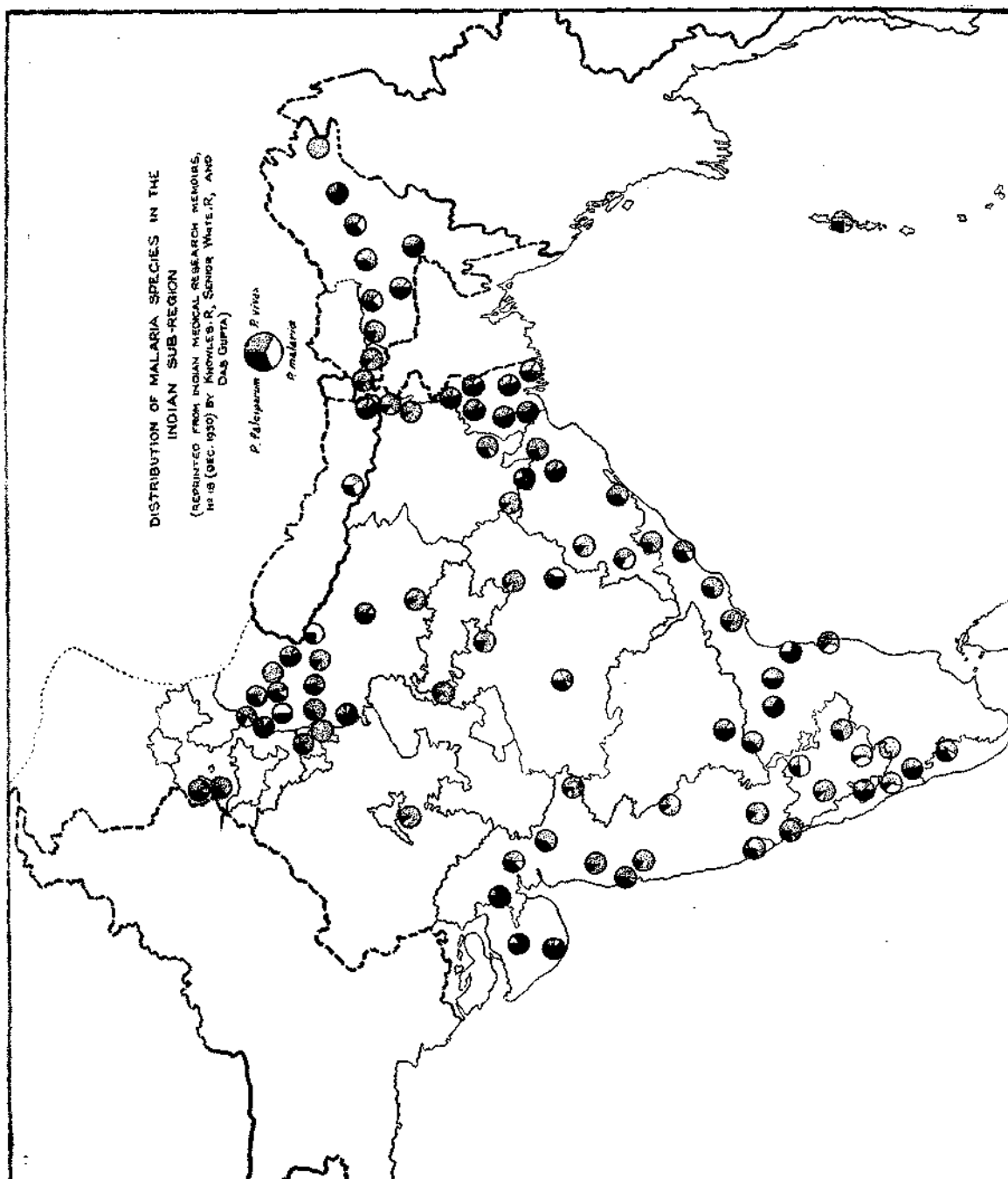
The data from Bengal in the present compilation is meagre and covers the areas designated West Bengal by Knowles *et al.* (*loc. cit.*). The parasite rate for the State is 30.7 per cent and the percentage prevalence of the three species are 43, 54 and 3 respectively for *P. vivax*, *P. falciparum* and *P. malariae*. *P. falciparum* continues to predominate in the State with three exceptions in Burdwan, Hooghly and Midnapur. The prevalence of *P. malariae* is only 3 per cent compared to 12.3 in 1930 and has been recorded only in the 24-Parganas and Darjeeling in the present series.

BIHAR.

P. vivax continues to be the predominating parasite as before. Ranchi, the hill station of the State, showed little malaria in the past and the parasite

Map 1





prevalent was almost exclusively *P. vivax*. Hazaribagh, Monghyr and Singhbhum show a higher prevalence of *P. falciparum*, but it must be remembered that these are industrial areas and labour is imported from outside the State. Singhbhum, however, showed predominance of *P. falciparum* according to the previous report also. The parasite rate for the State is 31.2 per cent, the species prevalence being 56, 43 and 1 per cent respectively for *P. vivax*, *P. falciparum* and *P. malariae*.

BOMBAY.

With the advent of D.D.T., great inroads have been made to prevent malaria transmission and the antimalaria operations are rapidly assuming a State-wide basis. The figures for purposes of this compilation are from the areas where no control measures have been carried out in the State.

The parasite rate for the State is 13.5 per cent as compared to 45.6 in 1930 and the species prevalence is 41, 51 and 8 per cent respectively for *P. vivax*, *P. falciparum* and *P. malariae*.

P. falciparum predominates in Khandesh West, Nasik, Kolaba, Sholapur, Belgaum, Dharwar, North Kanara, Kaira, Panch Mahals and Broach, while in Khandesh East, Thana, Bijapur, Poona and Ahmedabad, *P. vivax* predominates. The highest prevalence of *P. malariae* is in Dangs, Khandesh East, Thana and Bijapur.

Knowles *et al.* (*loc. cit.*) reported a predominance of *P. vivax* in the State, while at present *P. falciparum* is predominating, and *P. malariae* is as high as 30 per cent in some areas. *P. malariae*, however, seems to be a parasite of low prevalence (8 per cent) in the whole State. In some of the unsprayed villages in North Kanara, the parasitological picture has changed and *P. falciparum* predominates now, while in 1915 as quoted by Knowles *et al.* (*loc. cit.*) this species played a minor rôle and *P. vivax* formed as high as 75 per cent of all parasites.

HYDERABAD (DECCAN).

Knowles *et al.* (*loc. cit.*) concluded that *P. falciparum* was the predominating species in this State and also indicated that the data were unreliable. The present compilation indicates that *P. vivax* is the predominating species. As high a rate as 50 per cent has been recorded for *P. malariae* in Nizamabad, and is the only instance of this species predominating over the other two. The parasite rate for the entire State is 11.9 per cent, the species prevalence being 55, 33 and 12 per cent for *P. vivax*, *P. falciparum* and *P. malariae* respectively.

MADRAS.

The parasite rate for this State is 10.5 per cent as against the figure 36.1 of Knowles *et al.* (*loc. cit.*). The distribution of *P. vivax* and *P. falciparum* would appear to be about equal. The highest figures in the country for *P. malariae* are in this State, viz. 75 per cent in Nilgiris East and above 20 per cent in Bellary, Kurnool, North Arcot, Trichinopoly and Nilgiris West. It is from this last district that an infection of *P. ovale* was recorded by Raman (1940).

MADHYA BHARAT.

The only figures available for this area are from the Hamidia Hospital in Bhopal from 1945 to 1949. The predominating species is *P. falciparum* (63 per cent). These figures are likely to be representative for the rest of the State.

MADHYA PRADESH.

The antimalaria organisation and its operations in this State are comparatively recent. More data, however, are available regarding the parasitology of malaria than that gathered by Knowles *et al.* (*loc. cit.*). The parasite rate for the State is 21.3 per cent as against 45 of the previous authors. *P. vivax* continues to predominate as before; *P. malariae* is present in most districts with the exception of Bilaspur, Chanda and Chindwara, and as high a rate as 18 per cent of this species is recorded in Drug.

MYSORE.

A considerable amount of control operations have been carried out in this State. The parasite rates for each district are low, the rate for the whole State being 5.3 per cent as compared to 48.6 per cent recorded by the previous workers. Thirty-two per cent of infections in Nagenahalli was *P. malariae* in 1928 and this species prevailed to the extent of 43 per cent in 1933, almost occupying an equal status with *P. vivax*.

ORISSA.

Knowles *et al.* (*loc. cit.*) referred to the area as malarious. The available data for the State indicate a predominance of *P. falciparum*. *P. malariae* is recorded as 17 per cent in Ganjam. The parasite rate for the State is 18.4 per cent, the species prevalence being 43, 53 and 4 per cent respectively for *P. vivax*, *P. falciparum* and *P. malariae*.

PUNJAB (INDIA).

The State in India where most epidemiological investigations have been carried out is the Punjab. As mentioned in the report by Knowles *et*

al. (loc. cit.) *P. vivax* still remains the predominating species in normal years, while *P. falciparum* takes the lead during epidemic years. Their statement that "Quartan malaria is almost (but not quite) absent from the Panjab" remains true as shown by an isolated record of 4 per cent for this species in Ferozepur. The parasite rate for the State is 16.4 per cent as against the previous figure of 85.3.

RAJASTHAN.

The control organisation for this State has been set up recently and little has been carried out by way of antimalaria operations. The area is known to be liable to fulminant epidemics. Figures available for 1949 to 1950 indicate a high prevalence of *P. vivax*. The parasite rate for the State is 12.5 per cent. There are no previous figures available for comparison. *P. malariae* is recorded to be 11 and 12 per cent in Jodhpur and Udaipur respectively.

UTTAR PRADESH.

The average parasite rate for the State as a whole is 24.7 per cent, the prevalence of *P. vivax*, *P. falciparum* and *P. malariae* being in the proportion of 39, 60 and one per cent respectively. *P. malariae* is mainly in the central and southern parts and it is present only in 14 out of the 46 districts in the State. The distribution of *P. vivax* and *P. falciparum* varies from district to district. The latter species is predominating in most of the districts, with the exception of Azamgarh, Benares, Etawah, Lucknow, Nainital, Pilibhit, Pratapgarh and Sultanpur.

OTHER STATES.

The data available from Coorg, Delhi, Junagadh, Kashmir and Jammu, Kutch, Nepal, Saurashtra, Travancore-Cochin and Vindhya Pradesh have also been presented in Appendix I.

DISCUSSION.

Knowles *et al. (loc. cit.)* based on the reproductive powers of the three species arrived at a formula for their normal prevalence in any area in the absence of any disturbing factors. Such a normal prevalence was estimated by them to be.

<i>P. falciparum</i>	..	..	42.7	per	cent.
<i>P. vivax</i>	..	..	39.3	per	cent.
<i>P. malariae</i>	..	..	18.0	per	cent.

Variations from the normal for each State at the present time could be seen in Table I where the State-wide average figures for the prevalence of each species are depicted.

With regard to *P. malariae*, Knowles *et al.* (*loc. cit.*) considered 18 per cent to indicate normal prevalence. Districts in the different States where the prevalence of this species is 18 per cent or more, are listed in Table II. The most marked changes in the species prevalence are seen with regard to *P. malariae*. Normal and higher prevalence are confined to a single focus in Assam, the rest being situated in peninsular India. It is difficult to explain this unless it be that localised intensive control measures in the last 5 years have brought this most chronic of all infections in the forefront, the other two being relatively under control.

TABLE II.
Prevalence of P. malariae in India.

Serial Number.	Place	State	Percentage prevalence of <i>P. malariae</i> (1950)
1	Mikir	Assam	27
2	Drug	Madhya Pradesh	18
3	North Arcot	Madras	28
4	Bellary	"	27
5	Kurnool	"	22
6	Nilgiris West	"	26
7	Nilgiris East	"	75
8	Trichinopoly	"	40
9	Dangs	Bombay	30
10	Bijapur	"	20
11	Kandesh East	"	23
12	Thana	"	20
13	Nizamabad	Hyderabad	50
14	Nagonahalli	Mysore	43
15	Cochin	Travancore-Cochin	44
16	Travancore	"	70

It is well known that in the same area all conditions being identical, the predominant species of parasite will vary from season to season, namely, in the spring and the autumn. In the present compilation this point of difference has not always been clearly defined, but since the data in general cover a period of five years, it could be taken as fairly representative of the existing conditions. It should be interesting to follow up changes in the pattern of distribution of the species in India.

ACKNOWLEDGEMENT.

It is obvious that this compilation has been possible only due to the ready collaboration of the Officers-in-Charge of different organisations in the country. Their names are given in Appendix I. The authors are grateful to them.

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APPENDIX I.

Species prevalence in the districts of the different States in India.

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Andamans & Nicobar (Data supplied by Dr. P. R. Sondhi, S. M. O., Port Blair)						
	1949-50	6654	1151	81	19	..
Assam (Data supplied by Dr. S. C. Dutt, D.P.H., (Assam.)						
Cachar ..	1932	187	79	33	64	3
Darrang ..	1933-34, 1947-48	5901	1468	31	57	12
Garo Hills ..	1933	131	92	9	86	5
Goalpara ..	1947-48	683	351	36	55	9
Kamrup ..	1946-48	3200	1519	21	75	4
Khasi & Jaintia Hills ..	1940, 1947-48	1916	602	61	33	6
Lakhimpur ..	1932-47	15565	6528	24	71	5
Mikir ..	1949	110	38	32	41	27
Naga Hills ..	1946	105	68	25	67	8
Newgong ..	1934, 1947-48	2202	700	35	60	5
Sadiya Frontier ..	1940-47	1574	657	22	69	9
Sibsagar ..	1944, 1947-49	10469	1210	56	36	8
		42043	13312	32	59	9
Bengal (Data supplied by Dr. S. S. Kent, C.M.O., E. I. Ry., Dr. A. K. Adhikari, Malari- ologist, B. N. Ely., and Dr. P. C. Sen, Malaria Officer, West Bengal).						
Burdwan ..	1949	492	170	54	46	0
Darjeeling ..	Gilroy (1939)	529	273	17	75	8
Hoogly ..	1949	633	179	70	30	0
Midnapur ..	1945-50	2	2	50	50	0
Nadia ..	1949	607	164	34	66	0
24-Parganas ..	1945-49	6754	1986	34	59	7
		9017	2774	43	54	3

APPENDIX I.—(contd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Bihar	(Data supplied by Lt.-Col. A. N. Duggal, D.P.H., Bihar, and Dr. S. S. Kent, C. M. O., E. I. Rly.)					
Dhanka ..	1949	54	30	77	16	7
Gaya ..	1949	291	112	54	46	..
Monghyr ..	1949	931	288	25	75	..
Muzaffarpur ..	1949-50	..	..	95	5	..
Palamau ..	1949	356	92	60	32	2
Patna ..	1949	54	11	64	36	..
Purnea ..	1949-50	..	..	55	45	..
Ranchi ..	..	..	..	25	75	..
Santhal Parganas	1949	159	54	69	31	..
Singbhum ..	..	..	..	34	66	..
		1845	587	56	43	1
Bombay	(Data supplied by Dr. D. K. Viswanathan, A.D.P.H., Malaria, Bombay.)					
Ahmedabad ..	1947-48	1500	168	92	2	6
Belgaum ..	1948-49	118	22	32	68	..
Bijapur ..	1947-48	2860	371	70	10	20
Breach ..	1949-50	5447	531	28	72	Less than 1
Dangs ..	1949-50	902	219	8	62	30
Dharwar ..	1948-49	508	16	44	56	..
Kandesh West ..	1948-49	1569	430	34	66	..
Kandesh East ..	1948-49	6327	971	67	11	22
Kaira ..	1947-48	5122	435	32	65	3
Kolaba ..	1949-50	1945	292	29	65	6
Nasik ..	1947-49	7860	1301	14	74	12
North Kanara ..	1949-50	151	11	36	64	..

APPENDIX I.—(contd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Panch Mahala ..	1949-50	6120	540	31	67	2
Poona ..	1948-49	593	72	62	38	..
Sholapur ..	1947-50	444	162	15	85	..
Thana ..	1947-48	828	68	56	24	20
		42294	5708	41	51	8
Coorg	(Data Supplied by Dr. C. B. Kariapa, Malaria Officer, Coorg.)					
	1948	573	42	80	20	..
Delhi	(Data supplied by the Officer-in-Charge, A.M.O., Delhi State).					
Rural ..	1947-50	1180	184	60	40	..
Hyderabad	(Data supplied by Dr. A. Abraham, Chief Malaria Officer, Hyderabad)					
Adilabad ..	1948-49	1188	427	45	50	5
Hyderabad ..	1948-49	207	24	75	25	..
Nalgonda ..	1948-49	312	46	67	33	..
Nizamabad ..	1948-49	830	168	21	29	50
Rajchur ..	1948-49	4181	136	69	25	6
		6718	801	55	33	12
Kashmir & Jammu	Jacob (1950)	159	23	96	0	4
Junagadh	Sinton (1931)	56	14	90	10	0
Kutch	M.I.I. Delhi 1950	359	94	68	31	1
Madhya Bharat Bhopal	1945-49	4589	502	35	63	2
Madhya Pradesh	(Data supplied by Dr. R. Subramaniam, Deputy Director of Health Services, M.P., Dr. A. K. Adhikari, Malariaologist, B. N. Rly., Dr. S. Roy Chowdhury, Asstt Director, Coalfield Malaria Organization).					
Amravati ..	1948	776	277	59	39	2
Bastar ..	1949-50	1258	166	73	23	4
Bilaspur ..	1945-50	358	84	41	59	..
Chanda ..	1948-49	973	65	73	27	..

APPENDIX I.—(contd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Chindwara ..	1949-50	127	10	90	10	..
Drug	1950	384	123	68	14	18
Nagpur	1947-50	1452	254	61	33	6
Nimar	1947-50	969	283	64	35	1
Raigarh	1949-50	1277	169	79	20	1
Raipur	1946-50	1093	177	77	20	3
Surguja	1949-50	545	32	36	50	14
Khairagarh ..	1946-50	1804	795	23	71	1
Korea	1946-50	396	93	84	15	1
		12670	2684	65	31	1
Madras ..	(Data supplied by Dr. K. S. Viswanathan, A.D.P.H. Malaria, Madras, and the C.M.O., M. & S. M. Rly.)					
Anantapur ..	Not available	330	36	66	34	..
North Arcot ..	"	4990	146	25	47	28
South Arcot ..	"	285	25	52	48	..
Bellary	"	988	122	35	38	27
Chingleput ..	"	103	6	66	34	..
Chittur	Subramaniam & Chetty (1949)	..	..	55	33	12
Coimbatore ..	Not available	2376	131	50	50	..
Cudappah	"	104	14	43	50	7
East Godavari ..	"	344	85	34	64	2
West Godavari ..	"	280	12	50	50	..
Guntur	"	2173	207	25	70	5
S. Kanara ..	Ramakrishnan <i>et. al.</i> 1948)	179	36	42	56	2
Kistna	Not available	438	75	45	55	..
Kurnool	"	885	177	47	31	22
Malabar	"	16035	1271	59	28	13

APPENDIX I.—(contd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Madras (Contd.)						
Mathurai	Not available	623	87	42	45	13
Madras	1945-49	4519	647	84	16	..
Nellore	Not available	2541	472	45	47	8
Nilgiris West** ..	Russell and Jacob, (1942)	273	91	32	42	26
Nilgiris East	„ (1942)	371	169	10	15	75
Ramnad	Not available	554	29	57	43	..
Salem	„	1252	183	46	51	3
Tanjore	Russell, <i>et al.</i> (1938)	..	..	72	26	2
Tinnevely	Not available	1154	99	38	57	5
Trichinopoly	„	112	23	51	9	40
N. Vizagapatam	„	1187	245	48	37	15
S. Vizagapatam	„	614	163	18	82	..
		42719	4551	46	43	11
Mysore (Data supplied by Dr. B. A. Rao, Supdt., Bureau of Malariaology; and Dr. Paul Bierstein, W.H.O. Team Leader, Mysore)						
Bangalore	1948-50	174	14	28	72	..
Chitaldrug	„	2285	129	43	54	3
Hassan	1945, 1949-50	270	44	62	35	3
Mandya	1949	62	1	..	100	..
Nagenahalli	Sweet (1933)	..	..	46	11	43
Narsipur	„ (1937)	1221	..	31	58	11
Shimoga.	1948	2478	148	59	28	13
Tumkur	1949	25	9	..	100	..
		6515	345	34	57	9
Nepal*	1949	373	78	62	38	0

*Unpublished reports. Malaria Institute of India.

**One case of *P. ovale* recorded (Raman, 1940).

APPENDIX I.—(contd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P.</i> <i>vivax</i>	<i>P.</i> <i>falsi- parum</i>	<i>P.</i> <i>malariae</i>
Orissa	(Data supplied by Dr. A. K. Adhikari (1946 to 1950); Dr. R. G. Panigrahi, Provincial Malaria Officer, Orissa.)					
Balangirapatna	1946-50	206	93	39	60	1
Balasore	1940-42	140	11	55	45	..
Cuttack	1942 and 1947	597	115	65	27	8
Ganjam	1944	73	42	33	50	17
Kalahandi	1946-50	15	3	33	67	..
Puri	1941-42	1960	213	41	55	4
Sambalpur	1946-50	768	226	34	65	1
		3819	703	43	53	4
Punjab (I)	(Data supplied by Dr. D. R. Mehta, Malariologist, Punjab (I); and C.M.O., E. P. Rly.)					
Ambala	1948	482	11	46	54	..
Ferozepur	1948-49	606	81	93	7	..
Gurgaon*	1949-50	972	335	40	60	..
Kangra.	1949	48	4	75	25	..
Karnal	MacDonald and Majid (1931)	2149	269	47	49	4
Simla		82	9	67	33	..
		4239	709	61	38	1
Rajasthan	(Data supplied by D. M. & H.S., Rajasthan; and Dr. D. P. Kapur, Malaria Officer, Ajmer.)					
Ajmer	1949-50	7509	733	92	7	1
Bikaner	1947-50	11366	1555	66	34	..
Jaipur	1949-50	783	147	85	15	..
Jodhpur	1945-46	2653	393	34	55	11
Merwara	1949	510	37	81	16	3
Udaipur	1949	1179	155	33	55	12
		24000	3020	65	30	6

* Unpublished reports, Malaria Institute of India.

APPENDIX I.—(contd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Rajkot	Sinton (1931)	53	18	8	92	0
Saurashtra	(Data supplied by Dr. Chatterji, Special Malaria Officer) 1949	225	57	66	34	0
Travancore-Cochin	(Data supplied by Dr. N. K. Thampi, D.P.H.)					
Cochin	1949	491	91	14	42	44
Travancore	(Data supplied by Dr. P. Kumaran Nair, Malaria Officer) 1946-47	267	19	20	10	70
		758	110	17	26	57
Uttar Pradesh ..	(Data supplied by Dr. R. S. Srivastava, A. D. (Malariaology,) U.P.)					
Agra	1942-49	220	87	45	54	1
Almora	1945-47	52	21	52	48	..
Allahabad ..	1948-49	74	7	14	72	14
Aligarh	1943, 45, 49	107	33	39	58	3
Azamgarh ..	1945-46	102	41	75	25	..
Bareilly	1942-46	740	32	19	81	..
Bijnor	1942, 43, 45, 48	168	37	27	70	3
Baraich	1942, 43, 45	48	12	42	58	..
Basti	1947	37	16	..	100	..
Banda	1942-46	196	51	37	63	..
Banaras	1943-46	101	34	65	35	..
Ballia	1945-47	28	16	31	62	7
Bulandshahr ..	1942-46	301	26	31	69	..
Budaun	1943-45	15	3	33	67	..
Dehra Dun ..	1942, 45, 46, 48	110	16	37	63	..
Etawah	1943-48	134	27	63	37	..
Etah	1943-45	89	22	36	64	..
Fyzabad	1944, 45, 47	124	58	40	60	..

APPENDIX I.—(contd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. mal- ariae</i>
Uttar Pradesh (contd.)						
Fatehpur	1942-49	342	143	33	65	2
Farrukhabad	1943-46	248	80	40	60	..
Ghazipur	1946-47	87	57	56	42	2
Gonda	1945,47	57	20	50	50	..
Garhwal	1945-46	28	10	10	90	..
Hamirpur	1942-48	251	74	34	66	..
Hardoi	1943,45,46,	138	50	44	54	2
Jhansi	1942,1946-47	278	99	6	94	..
Jalaun	1943-47	479	226	27	73	..
Jaunpur	1943-48	418	149	24	75	1
Kheri	1943-45	18	3	33	67	..
Kanpur	1943	17	9	33	67	..
Lucknow	1942-49	1359	130	65	32	3
Mathura	1942-48	448	145	35	64	1
Mainpuri	1942-46	147	9	11	89	..
Meerut	1942-49	366	76	37	62	1
Moradabad	1942-48	159	16	13	87	..
Muzaffarnagar	1942-46	389	137	20	80	..
Mirzapur	1943-46	43	6	16	84	..
Nainital	1945-50	7653	384	73	21	6
Pilibhit, ...	1942-46	94	16	75	25	..
Pratapgarh	1945	10	2	100	..	..
Rai Bareilly	1943	3	3	33	67	..
Shahjahanpur	1942-46	331	62	32	68	..
Unao	1943-47	131	19	47	53	..
Saharanpur	1942,43,45	146	82	32	68	..

APPENDIX I.—(concl'd.)

Place	Period of survey	Number exa- mined	Number posi- tive	Percentage prevalence		
				<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Uttar Pradesh (cont'd.)						
Sitapur ...	1942,47,48	46	16	31	56	13
Sultanpur ..	1945	41	17	82	19	..
		16366	4955	39	60	1
Vindhya Pradesh	(Data supplied by Dr. A. K. Adhikari, Malariologist, B. N. Rly.)					
Rewa	1946-1950	168	35	54	38	8

EFFECT OF PYRIMETHAMINE IN HUMAN MALARIA.

PART I.

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EFFICACY of pyrimethamine against simian, rodent and avian malaria has been amply demonstrated by Falco *et al.* (1951), Jaswant Singh *et al.* (1951); Jaswant Singh, Basu and Ray (1952) and Jaswant Singh *et al.* (1952). McGregor and Smith (1952) report that, when administered in a single dose of 0.25 to 0.5 mg./kg dosage, it possesses powerful schizonticidal action against *P. falciparum* and *P. malariae*.

The present paper records observations on the effect of pyrimethamine in the treatment of 60 cases of *P. vivax*.

Most of the patients were from the Delhi Police Force and treated in the Police Hospital, and the rest were treated at the local Jail Hospital. Thirty patients gave history of previous attacks of malaria while the other 30 were considered as fresh cases. In view of the differences in reactions observed in the two regimes of treatment, the patients have been grouped separately.

In over 70 per cent of all cases, a definite history of recent movement could be traced and in all probability infection was contracted outside the protected areas of Delhi where malaria control measures have been in progress since 1936.

All cases were diagnosed microscopically. Thick and thin blood smears were stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944).

While thirty cases were treated with a single dose of 25 mg. (base) (Regime A), the other thirty received the same dose on two consecutive days (total 50 mg. base) (Regime B), and effects of the drug in relieving clinical symptoms and on the rate of clearance of asexual parasites have been shown separately in Tables I and II.

TABLE I.

Effects of a single dose of 25 mg. pyrimethamine in the clearance of clinical symptoms and asexual parasites.

Group	Clearance of	Within hours						Total
		24	48	72	96	120	144 or over	
I*	(a) Clinical symptoms ..	9 (58.8)	3 (75.8)	5 (100)	..	..	..	17
	(b) Asexual parasites ..	6 (35.3)	8 (82.3)	3 (100)	..	..	..	17
II**	(a) Clinical symptoms ..	5 (38.4)	2 (53.8)	6 (100)	..	..	..	13
	(b) Asexual parasites ..	5 (38.4)	5 (76.9)	2 (92.3)	1 (100)	..	..	13

Figures in bracket represent percentage.

*Cases with previous history of malaria

**Cases without such history.

In both groups, relief of clinical symptoms was attained in all cases within 72 hours with as small a dose as 25 mg. But there appears to be a significant difference in the clearance rates in the two groups during the first 24 and 48 hours. Under Group I, relief of clinical symptoms was attained

in 58.8 per cent of the cases within 24 hours as against only 38.4 per cent under Group II. Further, while 75.8 per cent of cases under Group I were free from fever within 48 hours, the rate under Group II was 53.8 per cent. However, the rate of parasite clearance within 24 and 48 hours under both groups appears to be more or less similar. Clearance within 72 hours occurred in 100 per cent of cases under Group I as against 92.3 per cent under Group II.

TABLE II.

Effects of a dose of 25 mg. on two consecutive days in the clearance of clinical symptoms and asexual parasites.

Group	Clearance of	Within hours						Total
		24	48	72	96	120	144 or over	
I*	(a) Clinical symptoms	7 (53.8)	5 (92.3)	1 (100)	..	..	...	13
	(b) Asexual parasites	6 (46.1)	6 (92.3)	1 (100)	..	..	..	13
II**	(a) Clinical symptoms	4 (23.5)	5 (58.8)	2 (64.7)	4 (88.2)	1 (94.1)	1†	17
	(b) Asexual parasites	4 (23.5)	7 (64.7)	4 (88.2)	..	1 (94.1)	1†	17

Figures within brackets represent percentage.

*Cases with previous history of malaria.

**Cases with no such history.

†Pyrimethamine failed to be effective.

From Table II the rates of clearance of clinical symptoms and asexual parasites under the two groups appear to be significantly different. Whereas only 64.7 per cent of cases under Group II were free from fever within 72 hours, all cases under Group I were afebrile within that period. In the former group, one patient continued to have fever and asexual parasites in peripheral circulation beyond 144 hours, and recourse to other antimalarials had to be taken. The rate of clearance of clinical symptoms within 24 and 48 hours appeared to be about twice as fast under Group I as against Group II.

Similarly parasite clearance under Group I was much quicker than under II at all stages, 24, 48 or 72 hours. In 11.8 per cent of the cases under the latter group, parasites were detectable beyond 72 hours.

Effects of pyrimethamine on the gametocytes of *P. vivax* have been shown in Table III.

TABLE III.

Gametocyte clearance in the two groups of cases under the two regimes A and B.

Regime	Group	Clearance within hours						Total
		24	48	72	96	120	144 or over	
A	I	1	1	4 (42.8)	5	2	1	14
	II	..	..	3 (33.3)	2	1	3	9
B	I	1	3	2 (54.5)	4	1	..	11
	II	..	5	3 (53.3)	5	..	2	15

Figures in brackets show the percentage.

As compared to the rate of clearance of the asexual forms, action of the drug on the gametocyte was found to be slow. This is clearly evident under the single dosage regime series in which gametocytes were detectable in nearly 60 per cent of the cases beyond 72 hours as against in 46.2 per cent of cases under regime B. In some cases under both the regimes, gametocytes persisted well beyond 144 hours. Comparative study of the individual groups shows slight difference in the rate of clearance under regime A, which is not apparent during the first 72 hours under regime B, where the subsequent figures show at least in 2 cases under Group II persistence of gametocytes beyond 120 hours. ✓

All the sixty cases were followed up for a period varying from 5 to 7 months after treatment. Only 3 out of 60 cases relapsed, two under regime A and one under regime B, the period of latency varying between 5 and 6 weeks. //

DISCUSSION.

Except in one case, relief of clinical symptoms was observed in all the 59 patients, and 90 per cent of them were afebrile within 72 hours. Considering the small dose required for effecting a clinical cure in such a large percentage of cases, pyrimethamine seems to have a high antimalarial potentiality. But the rates of clearance of symptoms and parasites under the two regimes were not the same in Groups I and II. Irrespective of the regime, a greater percentage of cases became afebrile within 24 or 48 hours under Group I, than those under Group II during the same period. This disparity in the rate of clearance is further demonstrated under regime B in which all cases in Group I became afebrile within 72 hours as against 64.7 per cent under Group II. In two of the cases under the latter group, fever persisted beyond 96 hours and at least in one case the drug proved a failure. This difference in reaction may be due to the development of some degree of immunity in persons who had previous attacks of malaria and as such they responded better than the non-immune cases.

In *P. falciparum* and *P. malarie* infections, such difference in reaction in non-immune and partially immune population was not encountered by McGregor and Smith (*loc. cit.*).

A relapse rate of 5 per cent during an observation period of 5 to 7 months should be considered as extremely low.

The slow action of the drug on the gametocytes is apparent, but those treated with two doses of the drug disappeared somewhat earlier than those which received only one dose. This is particularly noticeable in Group I under regime B in which gametocytes had disappeared in all cases by 120 hours.

Although there were two cases of relapse under the single dose schedule as compared to only one in the other, statistically these figures are far too low to enable one to analyse the merit of one regime over the other on this aspect. As regards its effect on asexual parasites and clinical symptoms the two-dosage regime does not appear to hold any material advantage over the single dosage series.

SUMMARY.

1. Sixty cases of *P. vivax* were treated with pyrimethamine, 30 with a single dose of 25 mg., while the other 30 received one such dose on two consecutive days. //

2. Clinical cure was attained in 59 cases and only one proved refractory even with two doses. In 90 per cent of the cases, relief of clinical symptoms was attained within 72 hours. As a rule the partially immune cases were afebrile quicker than non-immune patients. ✓

3. Clearance of asexual parasites within 72 hours was observed in all partially immune cases, as against 88 to 92 per cent of non-immune cases irrespective of the regime of treatment.

4. Effect of the drug was observed to be slower against the gametocytes but under the two-dosage regime results were somewhat better.

5. Relapse rate was observed to be extremely low.

ACKNOWLEDGEMENT.

The authors wish to thank Drs. K. C. Chopra and Lajpat Rai of Police Hospital, and Dr. Bachan Singh of the local Jail Hospital for extending facilities for the conduct of these investigations. They also wish to thank Brig. J. S. K. Boyd of the Wellcome Laboratories, London, for the supply of pyrimethamine.

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 (1952) ✓

EFFECT OF PYRIMETHAMINE IN HUMAN MALARIA
(*P. FALCIPARUM*).

PART II.

BY

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IN an earlier paper, Jaswant Singh, Ray, Basu and Misra (1952) reported their observations on the effects of pyrimethamine in *P. vivax* infection. Some cases were treated with a single dose of 25 mg. while others received one such dose on two consecutive days. With a single dose treatment regime of 25 to 50 mg./kg. of pyrimethamine, McGregor and Smith (1952) had observed powerful schizonticidal action against *P. falciparum* and *P. malariae*. In 50 or 100 mg. dosage, the drug was found to be very effective by Schneider, *et al.* (1952) in *P. falciparum* infection.

The present report records observations on the plasmodicidal effect of pyrimethamine in 84 cases of *P. falciparum* infection. Out of these, 22 were treated in the Police Hospital at Delhi, while the other 62 cases were treated in two dispensaries in U.P., Terai, *viz.* Maswasi in Rampur State and Gadarpur in Nainital District.

During the years 1949 to early 1952, all malaria cases reporting to the former dispensary were treated with proguanil while in the latter, preparations of cinchona alkaloids were prescribed. Likewise synthetic antimalarial

drugs were not in use at the Delhi Police Hospital. None of the treated cases had ever taken proguanil before.

Thick and thin blood smears were stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944) and all cases were diagnosed microscopically.

The distribution of cases and dosage regime are shown in Table I.

TABLE I.
Distribution of cases.

Location	Number of cases	Dose of pyrimethamine
Police Hospital, Delhi	22	25 mg. on two consecutive days.
Maswasi Dispensary, Rampur State, U.P.	31	do.
Gadarpur Dispensary, Nainital District, U.P.	31	do.
Total	84	

The criteria of activity were determined by the relief of clinical symptoms and clearance of asexual parasites in cases treated in the hospital. As record of temperature was not possible in all cases attending the dispensaries from villages spread over wide areas, clearance of asexual parasites was taken as a guide in such cases.

The rate of clearance of symptoms and parasitæmia in the 22 hospital cases are shown in Table II.

TABLE II.
Effect of pyrimethamine on clinical symptoms and asexual parasites.

		Clearance in hours.				
		24	48	72	96	
Clinical symptoms	..	38.4	81.2	100	..	per cent
Asexual parasites	..	85.5	100.0	..	..	per cent

All cases were afebrile within 72 hours though majority of cases had relief of clinical symptoms within 48 hours. Comparatively clearance of asexual parasites occurred much earlier.

Clearance of asexual parasites in the dispensary cases are shown in Table III.

TABLE III.
Effect of pyrimethamine on asexual parasites.

Dispensary cases.	Number of cases.	CLEARANCE WITHIN HOURS				Remarks.
		24	48	72	96 or over.	
Maswasi	31	12.9	74.2	100	... per cent	
Gadarpur	31@	72.7	93.6	93.6	93.6 per cent	*2 cases proved refractory.

*Clinical symptom and parasitaemia persisted in 2 cases beyond 96 hours and other antimalarials had to be administered.

@One case had mixed infection of *P. vivax* and *P. falciparum* both of which disappeared within 48 hours.

Asexual parasite clearance occurred in 93 to 100 per cent cases within 72 hours. Within 48 hours, it was 74.2, 93.6 and 100 per cent in cases from Maswasi and Gadarpur dispensaries and the Police Hospital, respectively. Further, taking the rate of clearance within the first 24 hours, 72 to 85.5 per cent of the hospital and Gadarpur Dispensary cases became free from parasites as against 12.9 per cent amongst the Maswasi Dispensary patients.

Crescents appeared to be the least affected and action of pyrimethamine is considered to be extremely slow, and in 9 out of 17 cases gametocytes persisted beyond 120 hours. In most of them there was progressive rise in the crescent count. In 11 out of 67 cases (16.4 per cent) in whom no gametocytes were detected at the initial examination, prompt clearance of rings and appearance of immature and mature crescents were dominant features.

Table IV shows the effect of the drug on the crescents.

TABLE IV.
Effect of pyrimethamine against crescents.

Location	Number of cases.	Crescents absent initially.	Appearance within hours.					Number of cases with crescents.	Clearance within —hours.				
			24	48	72	96	Total		24	48	72	96	120 or over
Delhi Police Hospital	22	16	..	1	2	..	3	6	1	..	1	..	4
Maswasi Dispensary	31	26	1	..	..	3	4	5	..	1	..	1	3
Gadarpur Dispensary	31	25	4	..	..	..	4	6	..	1	2	1	2
Total	..	67	5	1	2	3	11	17	1	2	3	2	9

Relapse rate.—The hospital cases were followed up for a period of six months during which period two out of 22 cases had early recrudescence, one after a week, the other after 3 weeks.

The dispensaries cases were followed for a period varying from 3 to 5 months. Early recrudescence in one case from Maswasi was observed after 15 days, while in the case of mixed infection at Gadarpur area only *P. vivax* was detectable on the 16th day after completion of the course of treatment.

DISCUSSION.

From the available literature it is observed that there seems to exist a remarkable diversity of opinion on the efficacy of pyrimethamine in the treatment of human malaria. Field and his colleagues (Goodwin, 1952) reported that in their series of 80 cases of *P. falciparum*, 13 patients (16.2 per cent) proved refractory to treatment with pyrimethamine. In some of the others, severe symptoms persisted for 3 days. Chaudhuri (1952) in making similar observations reported that the drug failed to effect clinical cure in some of his *falciparum* cases. On the other extreme, Schneider *et al.* (*loc. cit.*) reported on a series of 132 cases of *P. falciparum* and *P. vivax* treated with a total dose of 50 to 100 mg. that "Daraprim was at least as rapid in its action as chloroquine". In their series of 29 cases of *P. falciparum* treated with 25 to 50 mg. of pyrimethamine McGregor and Smith (*loc. cit.*) observed that only one case failed to respond but in the others relief of clinical symptoms was attained in less than 3 days. They believed that the speed of action

in controlling fever and parasitæmia was intermediate between that of the rapidly-acting chloroquine and the more slowly-acting proguanil.

In the present series it may be observed that only 2 cases failed to respond to the treatment. Fever was controlled in the 22 hospital cases within a period of 72 hours similar to that reported by McGregor and Smith (*loc. cit.*). The rate of parasite clearance in these and in the Gadarpur Dispensary series was found to be fairly rapid during the first 48 hours. These observations appear to be similar to those observed in a series of 97 *falciparum* cases treated by Jaswant Singh, Ray and Misra (in press) with a single dose of 600 mg. of amodiaquin or chloroquine. During their earlier studies on the relative merits of chloroquine diphosphate (produced by Messrs. Winthrop & Co., U.S.A.), and resoquin (Bayer Laboratories, Germany), Jaswant Singh *et al.* (1951) reported that in 69 cases with *P. falciparum* infection treated with a total dose of 1.5 gm. base (1,500 mg.) during a period of 3 days, clearance of asexual parasites within 48 and 72 hours was attained in 92 to 96 and 96.3 to 100 per cent of cases, respectively. Thus it would appear that the speed of action of pyrimethamine in the 51 out of 53 *falciparum* cases treated either at the Delhi Police Hospital or at Gadarpur Dispensary is similar to chloroquine, as was also observed by Schneider *et al.* (*loc. cit.*).

But the 31 cases treated at Maswasi Dispensary had not responded in a manner similar to the other 53 cases. Although complete clearance of asexual parasites was attained in all cases within a period of 72 hours, the rate of 12.9 per cent during the first 24 hours is much lower when compared to 72 to 85 per cent in the other cases. Though parasitæmia was ultimately suppressed, the Maswasi Dispensary cases reacted indifferently to pyrimethamine on the first day. This tardy reaction during the first 24 hours cannot be accounted for unless it is presumed that the strain of *P. falciparum* at Maswasi area is different from that at Gadarpur and Delhi. The distance between Maswasi and Gadarpur is only about 15 miles and the malarigenic factors are almost the same.

On the other hand it is known that proguanil was extensively used in the past at Maswasi area and therefore it may be argued that the strain of *P. falciparum* which was frequently exposed to proguanil was not sensitive to pyrimethamine to the same degree as in the two other areas (Gadarpur and the Delhi Police Hospital) where previously all cases were treated with cinchona febrifuge or quinine. Evidence of high degree of cross resistance between proguanil and pyrimethamine has been demonstrated recently by Jaswant Singh, Ray, Basu and Nair (1952) and Schmidt (1952) and the initial refractoriness of the plasmodium at Maswasi area may be argued on similar lines. From Malaya, Field and Edeson (1949) and Edeson and Field (1950) reported the appearance of a strain of *P. falciparum* which was refractory to proguanil. Subsequently, Robertson *et al.* (1952) reported that the proguanil resistant strain of *P. falciparum* in Tampin area was also resistant to daraprim,

Previously this strain had been exposed to repeated doses of proguanil. The two failures in the Gadarpur Dispensary series during the present investigation cannot be explained.

Similar failures with pyrimethamine in a few cases of *P. falciparum* infection have also been reported from Assam and West Bengal by Gilroy, Hay Arthur, Norman (Goodwin, 1952) and Chaudhuri (1952). It may here be recalled that some of these observers had communicated earlier similar refractoriness with proguanil in cases previously exposed to the repeated doses of the same drug for suppressive treatment.

Pyrimethamine seems to exert little effect on the crescents. Moreover, it was evident that in 11 out of 67 cases in whom no gametocytes were detected initially, there was a progressive rise in their number. In some cases, they appeared within 24 hours and in others within 48 to 96 hours. The day to day rapid clearance of rings and appearance of large number of immature crescents lead the authors to believe that at least in some cases pyrimethamine stimulated increased gametocyte production.

Apart from the 4 cases (5 per cent) of early recrudescence there had been no relapse and this compares to the observations of the authors in *P. vivax* infection (Jaswant Singh, Ray, Basu and Misra, *loc. cit.*)

Considering the small dose required in relieving clinical symptoms, the rapid clearance of the asexual forms from peripheral circulation, comparable to drug of the 4-aminoquinoline series and the low relapse rate, the drug should be considered to have a valuable place in chemotherapy of malaria.

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STUDIES ON EXPERIMENTAL MIXED INFECTIONS IN SIMIAN MALARIA.

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JASWANT SINGH *et al.* (1951) while dealing with a mixed infection of *P. cynomolgi* and *P. inui* in *rhesus* monkeys reported that, when the animals were inoculated with samples of infected blood of *M. irus* monkey obtained from Malaya, the infection in the recipient at the initial stage was of very low grade and only one species of parasite was detectable. Subsequent passages did not materially improve the situation, but after removal of spleens from some of these monkeys, there appeared a progressive rise in infection, and the presence of a second species could be easily recognised.

Earlier, Sinton and Mulligan (1933 : 1933a) demonstrated that in mixed infections in *rhesus* monkeys, *P. cynomolgi* predominated over *P. inui* so much so that they had observed complete absence of *P. inui* during certain stages of mixed infection.

During the present studies, the authors observed the course of parasitemia in experimentally produced mixed infection of *P. cynomolgi* and *P. inui* before and after splenectomy in *rhesus* monkeys. Further, they had endeavoured to study simultaneously the periodicities of these plasmodia without isolating them.

MATERIALS AND METHODS.

Ten normal healthy *rhesus* monkeys (average weight 7 lbs. each) were utilised for these studies. Two were inoculated with 2.5×10^6 /kg. trophozoites of *P. cynomolgi* and two others received double the dose. Another lot of 4 were inoculated with *P. inui*, 2 with a dose 2.5×10^6 /kg. and 2 with 5×10^6 /kg. erythrocytic parasites.

Each of the remaining two monkeys was inoculated with a mixture of blood containing 2.5×10^6 /kg. of *P. cynomolgi* and the same number of *P. inui*. In all cases, injections were given intravenously. As a routine, Ehrlich's eyepiece adjusted to count 100 erythrocytes per field was used for enumeration of parasites. One hundred such fields were examined for determination of the number of parasitized cells per 10,000 R.B.C. Thick and thin smears were stained with the modified technique of J.S.B. (Jaswant Singh and Bhattacharji, 1944; Jaswant Singh, 1950).

For removal of the spleen, a paramedian incision was preferred after the animal was anaesthetized with kemitnal (Jaswant Singh *et al.*, 1952).

OBSERVATIONS.

P. cynomolgi.—The courses of unmixed infections due to *P. cynomolgi* and *P. inui* were studied separately. Chart 1 shows the prepatent period and the course of parasitaemia during acute infection of *P. cynomolgi* in the groups of monkeys inoculated with 2.5×10^6 and 5×10^6 parasitized cells. In both cases patent parasitaemia was evident by the following day. The prepatent period and the peak period appeared to be similar though the intensity of infection during the peak was greater in the group which received the higher dose of inoculum.

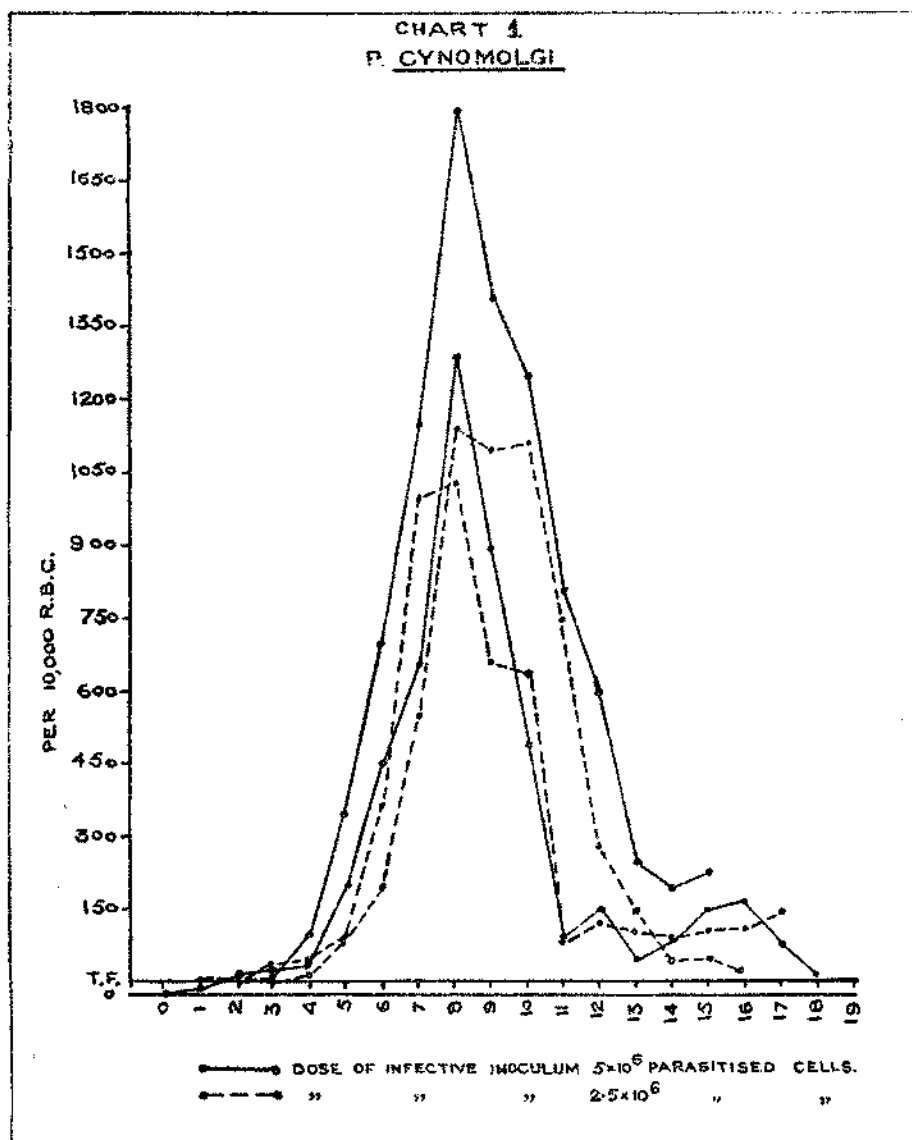
P. inui.—The course of infection in respect of *P. inui* is shown in Chart 2. In both groups patency was established by the following day and the peak was attained on the 11th day after inoculation. The intensity of infection with the lower dosage was significantly milder than the group receiving 5×10^6 /kg. parasitized cells.

Mixed infection.—The course of mixed infection in each of the monkeys was almost identical and Chart 3 shows the course of parasitaemia in one of them.

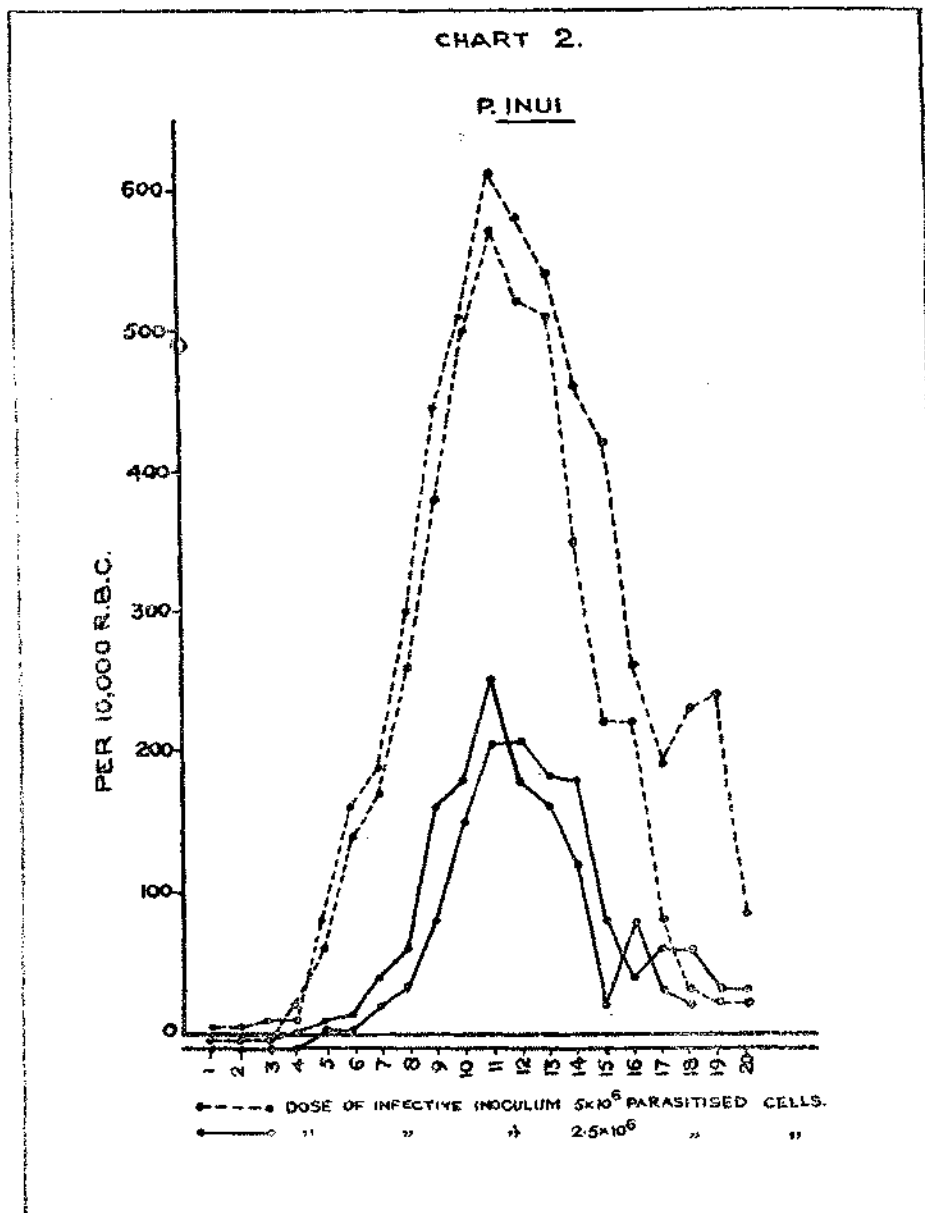
Primary infection.—The prepatent period in *P. cynomolgi* in the mixed infection was found to be the same as in unmixed infection with a dose of 2.5×10^6 parasites (Chart 1). The peak was also noted to be on the 8th day and parasite count at the peak period was not significantly different from that observed in unmixed infection.

But in case of *P. inui* the prepatent period was delayed by 5 days. Though the peak was attained at about the same period as in unmixed infection, the intensity, as determined by parasite count appeared to be significantly low.

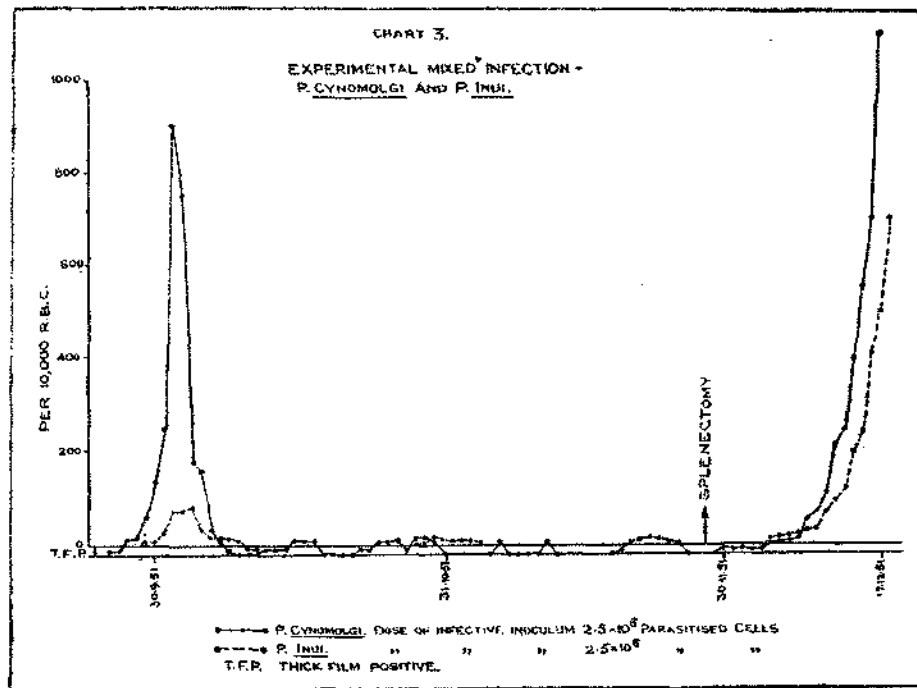
Subsequent attacks.—Shortly after the crisis both species disappeared completely from the peripheral circulation. This clearance was of a transient nature in respect of *P. cynomolgi*. Subsequently there had been several mild recrudescences at interval of 3 to 5 days till the removal of the spleen.



Prepatent period and the course of parasitemia of blood induced *P. cynomolgi* infection.



Prepatent period and the course of parasitaemia of blood-induced *P. inui* infection.



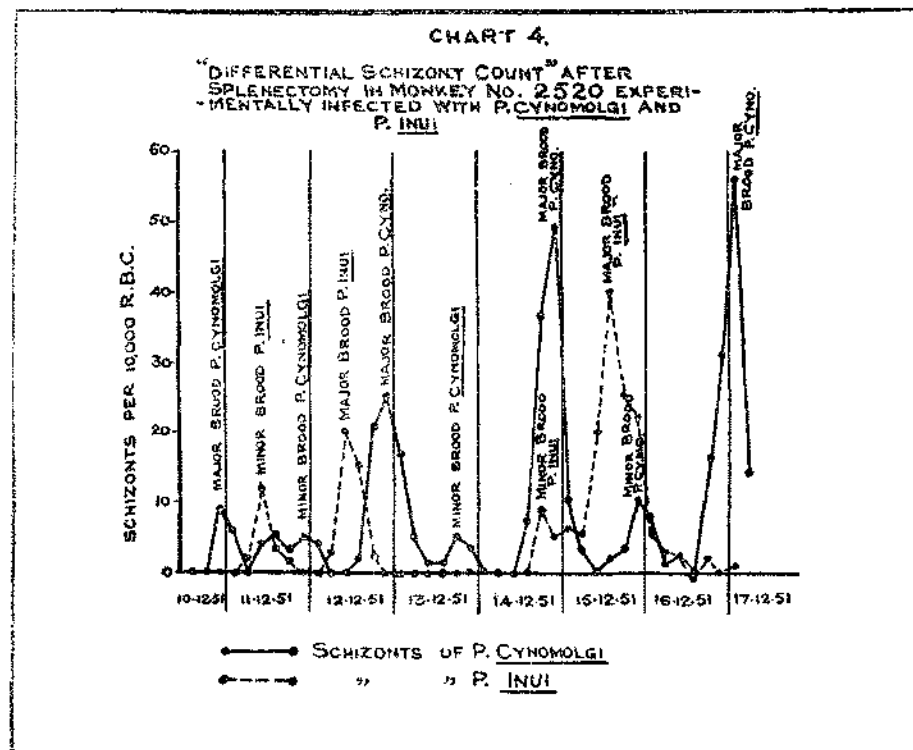
Course of parasitaemia of blood-induced mixed infection of *P. cynomolgi* and *P. inui*

Eighteen days after the initial clearance, *P. inui* was detectable again and the mild recrudescence lasted for 8 days. This species was never seen again until after splenectomy.

Parasitaemia after splenectomy.—Subsequent to the removal of the spleen, 10 weeks after the infective inoculation, both species were again detectable — *P. cynomolgi* soon after and *P. inui* about a week later. Later there was very rapid increase in parasitaemia and the intensity of infection ran almost parallel in both species.

Periodicity.—When both plasmodia were detectable simultaneously in large numbers after splenectomy, blood smears from the monkeys showing mixed infection were taken at interval of 4 hours for 8 days. The smears were then studied for determination of periodicities simultaneously in both species. Instead of counting all stages of the parasites, only the mature schizonts were considered. Differentiation of the schizonts of the two species were primarily based on the number of merozoites and the character of the pigment granules.

From Chart 4 it would be evident that each plasmodial infection had one major and one minor brood. In case of *P. cynomolgi*, the major brood seemed to precede the peak of the minor by 16 to 24 hours. In case of *P. inui*, the minor brood preceded the major by almost 24 hours.



"Differential schizont count" after splenectomy in Monkey No. 2520 experimentally infected with *P. cynomolgi* and *P. inui*.

The peaks of major or minor broods appeared at intervals of 48 hours in *P. cynomolgi* and 72 hours in respect of *P. inui*.

DISCUSSION.

Sinton and Mulligan (1933: 1933a) encountered *P. inui* in some of their *rhesus* monkeys which had previously shown only *P. cynomolgi*.

This was observed when the predominant species *P. cynomolgi* attained a low grade chronicity. Subsequently these workers demonstrated clearly the inherent character of the various plasmodia of monkeys.

During their previous studies, Jaswant Singh *et al.* (1951) did not readily encounter *P. inui* when blood from *M. irus* (with mixed infection of *P. cynomolgi* and *P. inui*) was inoculated to *rhesus* monkeys. Only after splenectomy, both species became apparent and parasitaemia increased consid-

erably. During the present studies, the authors first observed the course of unmixed infection due to *P. cynomolgi* and *P. inui* in two different doses and later studied the same for experimental mixed infection after introduction of equal doses of inoculum. Although *P. inui* was seen simultaneously with *P. cynomolgi* twice during the course of infection, the former was predominated by the latter after the first recrudescence of *P. inui*. The appearance of *P. inui* during these two occasions may be explained as due to the heavy dose of inoculum injected into the animals but even then the intensity of infection during the peak period was much milder as compared to the course of parasitaemia in unmixed infection with $2.5 \times 10^6/\text{kg.}$ dose of *P. inui* (Chart 2). Again during the first recrudescence, the peak was attained only after the peak of *P. cynomolgi* had subsided. In other words, *P. inui* became detectable when the intensity of infection due to *P. cynomolgi* was on the wane. These more or less confirm the earlier observations about the inherent predominant character of *P. cynomolgi* over *P. inui*.

The idea of counting only the mature schizonts for determination of periodicity in splenectomized monkeys is obvious. In anaemic condition and after removal of the spleen, there is considerable anisocytosis and the authors had observed that as some of the host cells seem to be enlarged even in the earliest stages of trophozoites of *P. inui*, at this stage it is not uncommon to confuse one from the other (Sinton 1934; Jaswant Singh *et al.*, 1952). In view of this, while day to day parasite count could not be continued beyond ten days after splenectomy, schizont count of 4 hourly slides did not present much difficulty.

The periodic cycle of both species was easily demonstrable in the 4 hourly slides. Were the smears taken once every 24 hours, say between 20.00 to 24.00 hours, an erroneous impression of 24 hour periodicity, might easily have been formed. Similarly the peaks of minor and major broods of *P. inui* at 12.00 hours on two consecutive days (2nd and 3rd day., Chart 4) would lead one to believe a 24 hour periodicity for *P. inui*. Similar warning was given by Mulligan (1935).

From these observations the authors consider that, in a mixed infection when one species predominates over the other and thereby makes identification difficult, firstly on account of the paucity of the species predominated by others and secondly long period of observation being necessary, removal of the spleen would make matters easier as parasites of both species show up in significant numbers. Further, for determination of the group character of the plasmodia, it would be necessary to determine the periodic cycle of the parasite, and thus the 'differential schizont count' as explained earlier, will be highly advantageous eliminating the necessity of isolation of the strain or alternatively waiting for weeks or months till the predominant species passes on to the latent phase to allow the other species to appear in suitable numbers.

SUMMARY.

1. Experimental mixed infection of *P. cynomolgi* and *P. inui* was produced in two *rhesus* monkeys, each receiving equal doses of 2.5×10^5 /kg. parasitized cells from donors.

2. The course and intensity of infection were compared with unmixed infection of these two species in doses of 2.5×10^5 /kg. and 5×10^6 /kg. parasitized cells.

3. Initially both parasites were detectable simultaneously though much less in respect of *P. inui* as compared to unmixed infection. Subsequently *P. cynomolgi* seemed to predominate over *P. inui* which completely disappeared after the first recrudescence.

4. Simultaneous increase in parasitaemia was observed after removal of spleens. Comparatively the intensity of infection was severe and anisocytosis was progressively apparent.

5. While at this stage, earlier forms were not easily distinguishable, mature schizonts could be easily identified mainly from the number of merozoites and pigments.

6. Advantage was taken of this differentiating character and schizont count was carried out at interval of 4 hours. The study of schizonts of different species at the same time has been called 'Differential schizont count'.

7. Periodicity of *P. cynomolgi* was observed to be of 48 hours while of 72 hours in respect of *P. inui*. Both were found to have a minor brood as well.

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DRUG RESISTANCE OF PRE-ERYTHROCYTIC FORMS OF
PLASMODIUM GALLINACEUM BRUMPT, 1935.

BY

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ACQUIRED resistance of *Plasmodia* to drugs has been shown to be of a stable nature. Bishop and McConnachie (1950) showed in the case of a proguanil-resistant strain of *P. gallinaceum* that the resistance so acquired remained unaffected for atleast a period of one year during which it had been maintained by serial blood passages in untreated chicks. Similarly Jaswant Singh, Ray, Basu and Nair (1952) found that a very high degree of proguanil resistance acquired by *P. knowlesi* in *rhesus* monkeys remained unaltered for a period of 8 months, during which the strain was blood passaged in untreated monkeys. Other studies by Bishop and Birkett (1947), Williamson *et al.* (1947), Hawking and Perry (1948), and Rollo *et al.* (1948) have shown that no loss in the resistance to the drug occurred when resistant strains were transmitted through the mosquitoes.

Wilson *et al.* (1952) drew attention to the question whether pre-erythrocytic stages of plasmodia also became resistant *pari passu* with the erythrocytic forms. This paper presents experimental data to prove that a strain of *P. gallinaceum* which had acquired resistance to daraprim* in its blood phase showed evidence of resistance during its pre-erythrocytic development also.

MATERIAL AND METHODS.

Vertebrate host.—Fifty-two healthy adult white leghorn and Rhode island red fowls were obtained in batches from the local Government Poultry Farm. Their weights ranged from 3 to 6 lbs.

Parasites.—The strain of *P. gallinaceum* maintained at this Institute was used in these tests. Ten and five million parasitized erythrocytes per kg. body weight of fowls were inoculated by the intravenous route, for strain maintenance and for the studies on drug resistance respectively (Table I).

The development of daraprim-resistance.—The technique adopted for developing resistance to daraprim in *P. gallinaceum* was similar to that adopted by Fulton and Yorke (1941) with plasmoquine and by Jaswant Singh, Ray, Basu and Nair (*loc. cit.*) with proguanil in simian malaria, and Bishop and McConnachie (1950a) with sulphadiazine in *P. gallinaceum*. The erythrocytic forms of the parent strain of *P. gallinaceum* were passaged serially through adult fowls to which daraprim was administered in gradually increasing doses. The details are depicted in Chart 1.

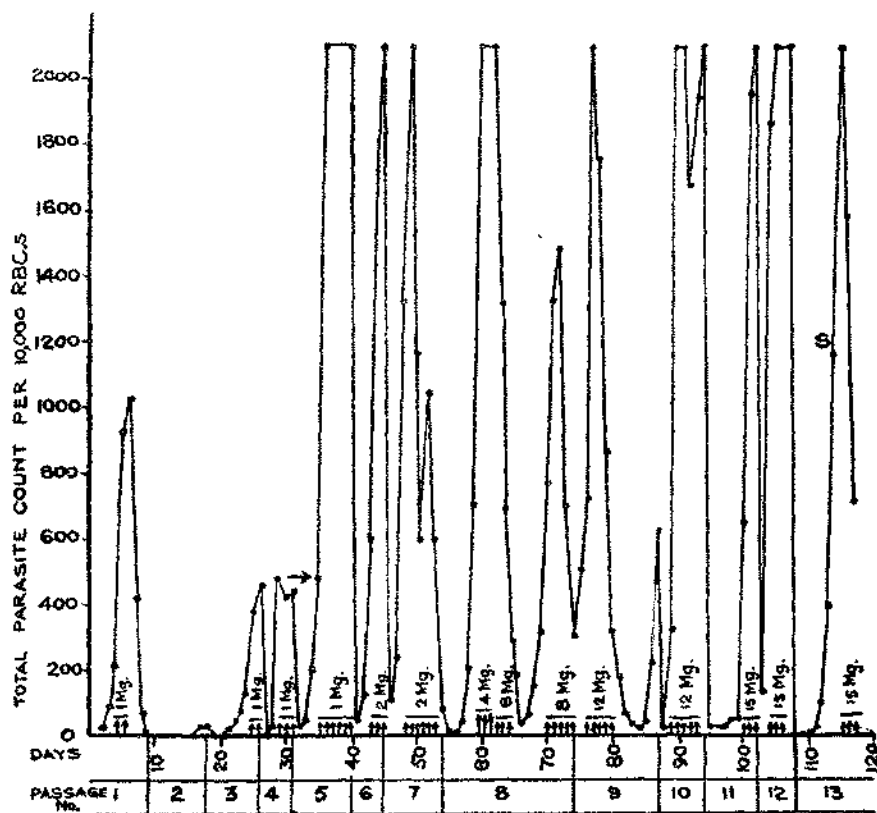
Thin blood smears stained by the Jaswant Singh and Bhattacharji (1944) technique were examined daily from the day following inoculations. Parasitised cells were counted in thin smears with an Ehrlich's eyepiece adjusted to cover a field of 200 erythrocytes. Fifty such fields were counted in each case. Hundred fields of the thin smear were examined before declaring a slide negative.

Administration of the drug.—Watery suspensions containing known quantities of daraprim were administered orally by means of a pippette to the fowls. Concentration of the drug in the suspension was so adjusted that the quantity to be given to a fowl never exceeded 5 c.c. and that it was swallowed without any difficulty.

Mosquito infections.—Two days old *Aedes aegypti* mosquitoes reared under standard conditions in the colony maintained at the Institute were used for the transmission experiments. Two fowls showing gametocytes were immobilised by being wrapped in a wire netting ($\frac{1}{2}$ inch mesh) and left inside a large breeding cage containing mosquitoes which had been starved overnight. Fully fed females were then transferred to Barraud's cages and stored in the insectary. Glucose solution soaked in cotton was placed on the cages. The temperature of the insectarium was maintained between 74 and 84°F., while relative humidity ranged from 55 to 78 per cent.

* Obtained through the courtesy of Messrs. Burroughs Wellcome & Co.

CHART 1.
DEVELOPMENT OF DARAPRIM RESISTANCE IN *P. gallinaceum*



Sample dissections were carried out from the fifth day onwards. The infected glands were crushed and drawn into a tuberculin syringe containing 0.25 c.c. of sterile normal saline and injected intravenously into fowls.

RESULTS.

COMPARISON OF THE PARENT STRAIN AND THE DRUG-RESISTANT STRAIN

(a) *Erythrocytic phase*.—The parent and resistant strains were inoculated into two batches of three fowls each. Daraprim was administered to the fowls on three consecutive days commencing from the day on which the infection was found in two per cent or more of the erythrocytes. The batch infected with the parent strain received 1 mg./kg. per day for 3 days which dose temporarily cleared the peripheral blood of parasites in the preliminary trials with that strain. The batch with the resistant strain received 15 mg./kg. per day for three days. The course of infection was as recorded in Table I.

In all the three fowls infected with the parent strain, the administration of 1 mg. of the drug per kg. per day for 3 days resulted in a temporary clearance of parasites from the peripheral blood. On the other hand, the birds infected with the resistant strain which were given fifteen times the above dose failed to show any reduction in parasitaemia; in fact, the parasites continued to multiply apparently unhampered by the drug, and two of the birds died due to heavy infection on the 11th day.

(b) *Pre-erythrocytic phase*.—Two mosquito-equivalents of sporozoites of the resistant strain were inoculated intravenously into each of the three fowls. Daraprim was administered to all the fowls at 0.3 mg./kg. twice daily for 4 days, the first dose given immediately following the sporozoite inoculation. The course of infection was as depicted in Table II. Daraprim in the dose administered failed to act as a prophylactic for the resistant strain.

DISCUSSION.

Daraprim administered at 0.3 mg./kg. twice daily for four days has been reported by Jaswant Singh, Ray, Basu and Nair (*loc. cit.*) to act as an efficient prophylactic against *P. gallinaceum*. Identical treatment failed to act as a prophylactic in the present trials with the daraprim-resistant strain. All the fowls in this group (Table II) showed overt parasitaemia on the seventh day after inoculation and the erythrocytic forms were found to exhibit the same degree of resistance as the strain on which the mosquitoes were fed. This was in accordance with the findings reported by Bishop and Birkett (1948) who actually carried out five consecutive insect passages without any loss of acquired resistance.

One of the hypotheses advanced to explain the mechanism of drug resistance is the selective inbreeding of resistant individual parasites present

TABLE I.
Effect of darsaprim on the standard and resistant strains of P. gallinaceum

Fowl number	Parasite strain	Dose of infection per Kg. (million)	Total parasite count per 10,000 R. B. Cs. on days following infection.													Remarks.
			3	4	5	6	7	8	9	10	11	12	13	15	16	
E 375	P	5	0	7	82	140	250*	112*	66*	8	1	0	0	0	0	Positive after 7 days
E 277	P	"	0	2	18	68	134	675*	765*	30*	2	0	0	0	0	Positive after 6 days
E 280	P	"	3	20	156	824*	634*	34*	20	10	0	0	0	0	0	Positive after 4 days
E 370	R	"	0	2	3	3	12	262**	480**	1364**	760	284	140	22		
E 71	R	"	0	0	8	15	140	520**	1000**	1830**	D					
E 373	R	"	8	44	160	640**	> 2000**	1830**	> 2000	> 2000	> 2000	D				

* = Darsaprim 1 mg./kg.

R = Resistant strain of *P. gallinaceum*.P = Parent strain of *P. gallinaceum*.

** = Darsaprim @ 15 mg./kg.

D = Died

TABLE II.
Observations on the prophylactic effect of daraprim.

Fowl number	Strain sporo- zoites	Prophy- lactic dose of daraprim	Parasite count/10,000 R.B.Cs. on days following infection.									
			6	7	8	9	10	11	12	13	14	15
E 381	R	@ 0.3 mg.	0	Pos.	52	288**	520**	472	D			
E 382	R	0.3 mg.	0	Pos.	142	336**	468**	280**	188	102	12	20
E 384	R	0.3 mg.	0	Pos.	38	460**	720**	388**	272	62	68	22
455	P†	0.3 mg.	0	0	0	0	0	0	0	0	0	0
456	P†	0.3 mg.	0	0	0	0	0	0	0	0	0	0

R = Resistant strain P = Parent strain. @ per kg. b.d. for 4 days. Pos. = less than 10 parasite. D = Died
 ** = Daraprim at 15mg./kilo. † = Remained negative for 40 days. Then they were superinoculated with 30,000,000
 parasitised cells, both became positive on 5th day. (Data incorporated from Jaswant Singh, Ray, Basu and Nair, 1952).

in a normal population (Cooper *et al.*, 1950). Gametocytes of such naturally resistant survivors being picked up by the vector even in the face of mass suppression can give rise to a serious problem.

Vincke (1952) reported encouraging results of weekly suppressive treatment with daraprim among a population of 209 persons in a rural area of Belgian Congo. While it was possible to ensure strict supervision as well as consumption of the proper drug regime in a small population, it would be difficult to do so in a larger community, and the chances of the parasite becoming drug-fast from inadequate suppression would be possible. The studies reported here showed that the prophylactic doses which effectively prevented the invasion of blood by parasites of the parent strain proved inadequate to confer similar protection against its drug-resistant variety.

Haphazard and inadequate suppression with antimalarials can give rise to acquired resistance to the particular drugs. Edeson and Field (1950) and Wilson *et al.* (*loc. cit.*) have reported evidence of such an occurrence in Malaya in respect of *P. falciparum* and *P. vivax* respectively with reference to proguanil. A similar resistance to proguanil acquired by *P. falciparum* in Assam was observed by Gilroy (1952).

Another matter of concern to the malariologist with reference to drug-resistance is that when parasites acquire resistance to one drug, there is a concurrent resistance to others chemically related to it. Such an occurrence has been demonstrated by Jaswant Singh, Ray, Basu and Nair (*loc. cit.*) in a proguanil resistant strain of *P. knowlesi* which concurrently showed refractoriness to daraprim. If the pre-erythrocytic parasites also show cross resistance to more than one drug, as is possible, the chemoprophylaxis of malaria can present more difficulties than at present.

SUMMARY.

Experimental proof is recorded to indicate that resistance to daraprim developed by the erythrocytic phase of *P. gallinaceum* can persist in its pre-erythrocytic phase also. The practical implications of the finding are discussed.

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A NOTE ON THE EFFECT OF PROGUANIL ON THE SPORO-
GONY CYCLE OF *P. GALLINACEUM* BRUMPT, 1935.

BY

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THE effect of paludrine on the gametocytes of simian and human malaria parasites and their subsequent development in mosquitoes has been reported by different workers. Jaswant Singh *et al.* (1951) reported that no infection of gut or gland developed in specimens of *A. annularis* fed daily for 5 days following administration of paludrine to *rhesus* monkey infected with *P. knowlesi*.

Mackerras and Ercole (1947) and Shute and Maryon (1948) found that vectors failed to develop the infection when fed on human beings with *P. falciparum* gametocytes within 8 days following a single dose of 300 mg. of proguanil. Working with two strains of *P. falciparum* (*Panamanian Pf. 6* and *South Carolina Pf. 2*), Ramakrishnan *et al.* (1952) observed that there was no infection of salivary glands among mosquitoes fed daily on gametocyte carriers treated with a single dose of 300 mg. paludrine, within

3 days of drug administration. They found that the maximum period for which conditions remained unfavourable for the development of gametocytes in mosquitoes was six days following administration of paludrine.

The present report records the effect of a single dose of 5 mg. per kg. (comparable to the human adult dose of 300 mg.) of proguanil on the development of the sexual phase of *P. gallinaceum* in *Aedes aegypti*.

MATERIAL AND METHODS.

Vertebrate hosts.— Twelve white leg horn fowls obtained from the Government Poultry Farm, Delhi, weighing between two and three lbs. each were used in the studies. Ten died in the course of investigations and data collected from the two survivors are presented here.

The parasite.— A strain of *P. gallinaceum* maintained at this Institute was used to infect the fowls. The dose of infection was always 30 million parasitised erythrocytes per kg. given intravenously. The blood smears were stained by the J.S.B. technique (Jaswant Singh and Bhattacharji, 1944) for examination.

The insect host.— Specimens of *Aedes aegypti* reared under standard conditions in the Institute were utilized. Two day old females, which had been starved overnight, were allowed to feed on the infected fowls immobilised for the purpose by being wrapped up in a broad mesh wire netting and kept inside a large cage containing the mosquitoes. Thirty mosquitoes were fed at one time, and maintained on soaked raisins in Barraud cages inside the insectarium at a temperature of 81-83°F and 75-80 per cent humidity.

Mosquitoes were first fed on two days (referred to as -2 and -1 days) prior to drug administration. Three batches of 20 mosquitoes each, were fed at 4-hourly intervals following drug on the zero day and later, batches of 30 mosquitoes each were fed daily up to the 12th day.

Sample dissections were carried out on mosquitoes from each batch from the 11th day following the infective feed to detect infections of the gut and gland. Where gland infections were encountered, two mosquito equivalents of sporozoites were inoculated into clean fowls to ensure their infectivity.

RESULTS.

Details of dissections of *Aedes aegypti* fed on the two fowls are recorded in Table I.

TABLE I.
Results of dissections of Aedes aegypti fed on the fowls.

Time of feeding	Fowl I.			Fowl II.		
	Number dissected	Number showing oöcysts	Number showing sporozoites	Number dissected	Number showing oöcysts	Number showing sporozoites.
—2 days	25	4	16	15	1	2
—1 days	20	3	2	18	2	4
plus 4 hours	9	1	1	15	0	0
„ 8 „	4	0	0	14	3	2
„ 12 „	7	0	0	15	2	2
1 day	11	3	3	10	0	0
2 „	15	1	2	8	0	0
3 „	18	3	2	12	0	0
4 „	15	0	0	15	0	0
5 „	15	0	0	6	0	0
6 „	9	0	0	15	0	0
7 „	8	1	1	15	0	0
8 „	15	0	0	15	1	0
9 „	15	0	0	12	1	2
10 „	20	0	0	9	2	1
11 „	11	0	0	17	1	1
12 „	18	0	0	15	1	1

Mosquitoes fed on both the fowls before the drug administration showed oöcysts and sporozoites. In the case of one fowl, two of the 9 mosquitoes dissected from the plus 4-hour batch showed gut and gland infection, while in the other, among 9 of the 29 mosquitoes dissected, similar infections were found only from the plus 8 and plus 12-hour batches.

In fowl I, though the plus 8 and plus 12-hour feeds failed to develop in the mosquitoes, gut and gland infections were detected among all the batches fed on three days following therapy. Among mosquitoes fed subsequently (4th to 12th day) positive dissections were recorded only from the 7th day batch. Those fed on fowl II became positive from the 8th day onward. Sporozoites from mosquitoes infected from fowls I and II were found to be infective on inoculation.

DISCUSSION.

Earlier work referred to above has proved that proguanil affects the gametocytes of both simian and human plasmodia in such a way as to inhibit their further development in the insect vector for varying periods commencing within 24 hours of drug administration. Jaswant Singh *et al.* (*loc. cit.*) observed such an effect developing in 8 hours but not within 4 hours following therapy.

In the present trials with *P. gallinaceum* the 'safe period' following a dose of paludrine, equivalent to the human dose, differed in the two fowls, though treated in an identical way. The gametocytes of fowl I continued to be infective to mosquitoes for a period of 3 days following drug administration, and later were non-infective from the 4th day, and regained infectivity from the 7th day onwards. In the second fowl, however, the blood was infective up to 12 hours following drug, but mosquitoes fed thereafter up to 7 days did not develop either oöcysts or sporozoites. These observations seemed to indicate that the absorption and metabolism of the drug varied to a marked degree in the two fowls. The belated effect observed in fowl I, was probably due to the delayed or slow rate of absorption of the drug in that bird. Carrington *et al.* (1951) showed that paludrine acted by forming metabolites in the vertebrate host. It would appear that the metabolism of the drug did not go on at the same rate in both the birds. This individual variation in the absorption and metabolism of paludrine requires further study.

SUMMARY.

Observations on the infectivity of *P. gallinaceum* in fowls treated with a single dose of proguanil are reported.

Aedes aegypti fed during 3 days prior to and 3 days following drug administration developed gland and gut infection. Those fed from 4th to 7th days failed to show either oöcysts or sporozoites, while from the 8th day onwards the gametocytes completed their development in mosquitoes.

Marked variation was observed in the behaviour of gametocytes in two fowls in their post-therapy infectivity to *Aedes aegypti*.

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NATURAL INFECTION OF *P. RELICTUM* IN WEAVER BIRD,
THE BAYA (*PLOCEUS PHILIPINUS*).

BY

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P. relictum Grassi and Feletti, 1891, is frequently found in sparrows in nature and has for some time been used at the Malaria Institute of India for preparing teaching materials. The birds are obtained from the local market and examined as a routine for plasmodial infections.

During the year 1952, 24 pigeons (*Columbia livia Gmelin*), 220 house sparrows (*Passer domesticus*), 23 red munias (*Amandava amandava*) and 14 weaver birds (*Ploceus philipinus*) were examined. The results are presented in this paper.

None of the pigeons and red munias harboured any natural plasmodial infection. Fifteen per cent of the sparrows and fourteen per cent of the weaver birds showed *P. relictum* infection in peripheral blood. While the weaver birds are found all over India (Salim Ali, 1941), they are available in Delhi market mostly during the pre-monsoon months.

The infection in the two weaver birds was mild and the density of parasitaemia was 8 to 10 parasites per 200 microscopic fields. All stages of the parasites were observed. The parasites were patent in the peripheral blood in one bird for six consecutive days and reappeared on the 13th day after being sub-patent for 6 days.

The morphology of the parasites in the weaver birds was identical in all stages of growth with that of *P. relictum*. The number of merozoites per schizont varied from 7 to 12. Pigment in gametocytes was fine and nuclei of the host cells were displaced and in some cases expelled.

Experiments were carried out to determine whether *P. relictum* found in weaver birds was identical or distinct from the one commonly found in house sparrows of Delhi.

Sub-inoculations from the naturally infected weaver birds were given to five house sparrows, four red munias and four pigeons (all were normal birds). The results are shown in Table I.

TABLE I.

Results of sub-inoculations from naturally infected weaver bird.

Host	Numbers inoculated	Number positives
House sparrows	5	3 (60 per cent)
Red Munias	4	0
Pigeons	4	0

While no infection resulted in red munias and pigeons, 3 (60 per cent) house sparrows were susceptible.

Batches of laboratory bred *Culex fatigans* were fed on the naturally infected weaver birds. The results are given in Table II.

TABLE II.

Results of infections in Culex fatigans fed on naturally infected weaver bird.

Host	Number of <i>Culex fatigans</i> fed	Number dissected		Number positive		Per cent positive
		Gut	Gland	Gut	Gland	
Weaver bird	153	50	50	50	25	50.0

Culex fatigans was found highly susceptible to infections of *P. relictum* from the weaver bird. The resulting infection in mosquitoes was not heavy and was undoubtedly due to the very few gametocytes present in the host bird (1 per 200 oil immersion fields) at the time of feeding.

DISCUSSION.

This appears to be the first record from India of *P. relictum* in the weaver bird. Hamerton (1937) reported infection of plasmodium (sp. ?) in the weaver bird in the London Zoological Gardens. A number of strains of *P. relictum* have been described by several workers. The strain designation is generally given to "any malaria parasite which is isolated from a wild bird and sub-inoculated into experimental host a number of generations while its characteristics are studied" (Hewitt, 1940). On this basis, he has listed 11 strains of *P. relictum* occurring in sparrows, robin, quail, finch, pigeon and wood thrust. From the morphology of the parasite, from the high infection obtained in house sparrows by sub-inoculation and also from high susceptibility of *Culex fatigans* to the plasmodium it is evident that the parasite found in the weaver bird was *P. relictum*.

The occurrence of plasmodial infections is prevalent in many of the common birds. In nature culicine mosquitoes are mainly responsible for transmitting avian malaria. Hunnien (1951) carried out laboratory experiments and found anopheline mosquitoes *A. albimanus* and *A. crucians* highly susceptible to *P. relictum* infections. It is likely that in nature anophelines are reluctant to feed on birds. But if they do feed there is a chance of some of them transmitting avian malaria. This possibility has an important bearing in the epidemiological studies in human malaria, particularly in an area like Delhi State where the malaria control measures have reduced the incidence of human malaria to 2.3 per 1,000 per year (Jaswant Singh, 1952). It should be remembered that there is no method of readily identifying the species from an examination of sporozoites alone.

SUMMARY.

1. Natural infection of *P. relictum* in weaver bird has been described.
2. *P. relictum* in weaver birds appeared to be identical to one commonly encountered in house sparrows.

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ANOPHELINE MOSQUITOES RECORDED IN THE
DISTRICTS OF SHIMOGA AND HASSAN,
MYSORE STATE*.

BY

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In the various check lists of Indian mosquitoes of the genus *Anopheles* by Covell and Puri, including the most recent additions to the known geographical distribution, no record is available from the districts of Shimoga or Hassan in Mysore State (Puri, 1936 : 1948). The purpose of this paper is to tabulate available information from these regions and to comment briefly on some of the species.

Shimoga and Hassan districts occupy somewhat similar positions with respect to the Western Ghats and the resultant rain fall zones. In their western portions—the so-called 'Malnad'—they receive heavy southwest monsoon precipitation, in some places approaching 200 inches annually. These

*The work in Shimoga District in 1946-47 was performed by the Bureau of Malariology of the Department of Public Health, Mysore State; and in 1949-51 by the same Bureau in cooperation with the Malaria Control Demonstration Project of the World Health Organization under the direction of Mr. Paul Bierstein and Dr. S. R. Bhambore.

The work in Hassan District in 1950-52 was performed by the same Bureau in cooperation with the Division of Medicine and Public Health of the Rockefeller Foundation. The assistance of Dr. K. S. Nanjundiah in the latter studies is gratefully acknowledged.

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areas are characterized by hilly country supporting thick forests where valleys are steep and narrow and afford the only places where paddy can be grown. To the eastward there is a fairly abrupt transition to relatively dry terrain with 40 inches or less of rainfall per year. Forests are thinner, valleys are shallow and broad, and paddy is more extensively cultivated. This disparity in meteorologic, vegetative and geographic conditions results in a diversity of mosquito breeding places, and the anopheline fauna is consequently rich (Table I).

TABLE I
*Anopheline species recorded in Shimoga and Hassan districts,
Mysore State, India.*

Species	Shimoga District		Hassan District		
			Sakleshpur area		Channarayana area
	Adults	Larvæ	Adults	Larvæ	Adults
1. <i>A. aconitus</i> ..	×	×	×	×	×
2. <i>A. aitheni</i> ..	—	×	×	×	×
3. <i>A. annandalei</i> ..	×	—	×	—	—
4. <i>A. annularis</i> ..	×	×	×	×	×
5. <i>A. barbirostris</i> ..	×	×	×	×	×
6. <i>A. culicifacies</i> ..	×	×	×	×	×
7. <i>A. fluviatilis</i> ..	×	×	×	×	×
8. <i>A. hyrcanus</i> ..	×	×	×	×	×
9. <i>A. insulæflorum</i> ..	—	×	—	×	—
10. <i>A. jamesi</i> ..	×	×	×	×	×
11. <i>A. jeyporiensis</i> ..	×	×	×	×	×
11a. <i>A. jeyporiensis</i> Var. <i>candidensis</i> ..	×	—	×	×	—
12. <i>A. karwari</i>	×	×	×	×	×
13. <i>A. leucosphyrus</i> ..	×	×	×	×	—
14. <i>A. maculatus</i> ..	×	×	×	×	—
15. <i>A. majidi</i> ..	—	—	×	×	—
16. <i>A. pallidus</i> ..	×	×	×	×	×
17. <i>A. philippinensis</i> ..	×	×	×	×	—

TABLE 1 (Concd).

Species	Shimoga District		Hassan District		
	Adults	Larvæ	Sakleshpur area		Channarayapatna area
			Adults	Larvæ	Adults
18. <i>A. splendidus</i> ..	×	×	×	×	×
19. <i>A. stephensi</i> ..	×	×	—	—	×
20. <i>A. subpictus</i> ..	×	×	×	×	×
21. <i>A. tessellatus</i> ..	×	×	×	×	×
22. <i>A. turkhudi</i> ..	×	—	×	—	×
23. <i>A. vagus</i> ..	×	×	×	×	×
24. <i>A. varuna</i> ..	×	×	×	×	×

The first collections made in the areas referred to in this paper were those in Anandapuram and Sorab of Sagar and Sorab taluks respectively in Shimoga District. These areas are in a region of sandalwood and teak forests and have rainfall of rather low intensity, about 40 to 60 inches per year. Following the collections in 1946-47 a second series of studies was made during 1949-51 in a fairly extensive area surrounding Sagar. The latter investigations brought a region of heavier rainfall into consideration, at some points approaching zones of maximum precipitation. Thus the Malnad portion of the district may be considered as fairly thoroughly surveyed from the anopheline standpoint.

Entomological collections in Hassan District were conducted during 1950-52 on a rather wide scale with the town of Sakleshpur as a centre. Special collecting stations were established in villages occupying areas of high intermediate and low rainfall. In addition, adult collections were made during the same period in an irrigated area near Channarayapatna, a typical maidan locality with relatively light rainfall, not quite 30 inches annually. Hence the anopheline fauna of Hassan District has probably been almost completely sampled.

The Bureau of Malariology of the Mysore State Health Department for many years maintained an antimalaria unit at Mudigere in Chikmagalur (formerly Kadir) District. This district lies between those of Shimoga and Hassan and has essentially the same physiographic facies. Mudigere is in the region verging on heavy rainfall. A total of twenty four species of *Anopheles* has been recorded from this region (Sweet, 1933 : 1934 ; Sweet and Ra 1933 : 1934 ; Nursing *et al.*, 1934). As might be expected, lists for the three districts almost duplicate one another. The only discrepancies are :

A. Annandalei, Shimoga and Hassan only ;

A. majidi, Chikmagalur and Hassan only ;

A. minimus, Chikmagalur only.

Identifications of anopheline specimens were made by experienced entomologists. During these investigations, some consistent departures from conventional morphology or habitat of larvæ of different species were noted. These aberrations and other notes on the species are discussed below.

A. aitheni.—In Hassan District, branching of the inner clypeal hairs was found to be variable from several stand-points: number of branches, point where branching took place, and symmetry of right and left hairs of a given larva. However, no regularity could be found in these departures from normal, and typical morphology was encountered more frequently than atypical. Although some specimens were indistinguishable from var. *bengalensis*, the occurrence of intermediate forms with all degrees of variability made it inadvisable to include the latter as a Mysore record.

A. annandalei var. *interruptus*.—The finding of a single adult in a cattle-shed in Shimoga District during the first week of September 1949, constitutes the first record of this species in Mysore State and brings the total number of species of *Anopheles* known from the State to twenty-five. The only other specimen of this species from Mysore State was found in a cattle-shed in Hassan District in September, 1952. Both the specimens were collected at about the same time of the year from areas where annual rainfall varied between 80 and 100 inches. A careful search of tree-holes in both areas failed to reveal larvæ of the species. It seems strange that *A. annandalei* was not found in the intervening Mudigere area during the many years that collections were made there.

A. barbirostris.—In Hassan District larvæ often showed inner clypeal hairs distinctly branched into two in the middle as in *A. aitheni* larvæ. Outer clypeal hairs were found to be normal.

A. fluviatilis.—Before and soon after the onset of the south-west monsoon in Hassan District, when this species had reached its peak of abundance, larvæ could be found in numerous habitats in addition to the "clear water pools along edges of running streams and irrigation channels", given by Puri (1949). They were taken frequently in warm—almost stagnant—side-pickets of streams, sometimes with extremely muddy water and no appreciable current. Marginal vegetation seemed usually to be required for their shelter, but at times a mass of half-submerged rootlets accomplished the same purpose.

A. insulæflorum.—In Hassan District, larval habitats included streams in the open when well flanked by low vegetation in addition to "shady pools along forest streams", as given by Puri (1949). Larvæ were found frequently in locations where branches drooping into the water caused an accumulation of floating debris.

A. jeyporiensis var *candidiensis*.—This variety has not been previously recorded in published lists of anophelines from Mysore State. It was first recognised in Shimoga District in a batch of mosquitoes collected on a night in May, 1946, and thereafter several more specimens were taken. Subsequently, it was identified from collections made at Mudigere in Chikmagalur District and finally in the Sakleshpur region of Hassan District. In all the three districts, it is distinctly rare, being greatly outnumbered by the type form. Careful studies of many *jeyporiensis* larvæ at Sakleshpur have revealed a few that displayed the characteristics of var. *candidiensis*. Unfortunately these larvæ were not reared in order to study the adult characteristics.

A. karivari.—This species is very locally distributed in the Sakleshpur area of Hassan District. However, at one point in a stream where there was an abundance of submerged and emergent vegetation, larvæ were taken repeatedly.

A. maculatus.—The identification of *maculatus* larvæ in Mysore State is predicated on the fact that *theobaldi* is not recorded from the region. According to Christophers (1933), the range of *theobaldi* does not extend south to Mysore. Therefore since *maculatus* adults were often captured in the surveys under discussion, larvae resembling *maculatus-theobaldi* were ascribed to *maculatus*.

A. minimus.—Despite the records of *minimus* from Mudigere in Chikmagalur District, that species was not recorded in either neighbouring District. However, the elapsed time since *minimus* was recorded at Mudigere is sufficient to have permitted a spontaneous disappearance of the species from the region. Perhaps it still exists at Mudigere as an isolated population.

A. pallidus and *philippinensis*.—In Hassan District, the chief character for distinguishing between the larvæ of these species, as given by Puri (1949), was found to be unsatisfactory since branching of the posterior clypeal hairs (i.e. 2-5 in *pallidus* and 7-10 in *philippinensis*) included the zone of supposed numerical demarcation (i.e. 5-7) with considerable frequency.

A. splendidus.—Puri (1949) describes larvæ of this species as 'dirty yellow to greyish or greenish yellow in colour, without any conspicuous spots on the body'. Larvæ from the Sakleshpur area, Hassan District, examined in the living state, corresponded to this gross picture, but under the microscope they usually appeared to be peppered profusely with small dark spots in a striking fashion.

A. varuna.—Puri (1949) describes larvæ of this species as "dark greenish grey in colour". In the Sakleshpur series it was quite common to find them varying from light pink to an almost brick red when viewed alive under the microscope by transmitted light. Furthermore, in both Shimoga and Hassan districts, their usual habitat was not "wells and clear rain-water pools", as given by Puri (1949), but the identical habitat occupied by *fluviatilis*, that is, various sorts of streams with abundant marginal vegetation. *Varuna* larvæ almost always exceeded *fluviatilis*

larvæ in numbers at these sites, and it was actually possible to use *varuna* as a guide to the discovery of *fluviatilis* on many occasions by making a concentrated search for *fluviatilis* where *varuna* had already been found.

SUMMARY.

Twenty-three species and one variety of anophelines are recorded from Shimoga District, Mysore State.

Twenty-four species and one variety of anophelines are recorded from Hassan District, Mysore State.

Anopheles annandalei var. *interruptus* and *A. jeyporiensis* var. *candidiensis* are added to the known fauna of Mysore State.

Departures from usual habitats and morphology are discussed, and general comments on some of the species are made.

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STUDIES ON THE BIONOMICS OF *ANOPHELES FLUVIATILIS* JAMES, 1902, IN MYSORE STATE, INDIA.

I. REVIEW OF THE LITERATURE ON BIONOMICS OF
A. FLUVIATILIS.

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INTRODUCTION.

THE present paper is designed to serve as a background to forthcoming reports on several aspects of *Anopheles fluviatilis* James, 1902, in the region of the Western Ghats of Mysore State, India. It seems advisable to present a review of the literature on the bionomics of this *Anopheles* as a separate entity because of the great variety of conflicting observations that has been made on this malaria vector in different parts of the country. These prior observations are not being questioned; they are discussed here in order to avoid unnecessary confusion in the later papers of this series.

This study represents work performed by the entomological staff of the Malaria Investigation Centre, Sakleshpur, Hassan District, of the Bureau of Malariology, Mysore State Health Department, in co-operation with the Division of Medicine and Public Health of The Rockefeller Foundation. The Centre is under the direction of Dr. S. R. Bhombore, whose constant assistance and guidance are hereby gratefully acknowledged.

REVIEW OF THE LITERATURE.

1. *Distribution of larval populations.*—The studies of Adisubramaniam and Vedamanikkam (1943) in the Wynaad indicated that oiling of channels within 1,000 to 1,500 feet of houses resulted in a reduction of adult *A. fluviatilis* catches in houses. A larval census of two breeding channels yielded decreasing numbers of specimens as the vicinity of houses became more remote.

At Daghora in the Hazaribagh Ranges, and also at a railway bridge in the Jeypore Hills, Venkat Rao and Philip (1947) found no such correlation. *Anopheles fluviatilis*, together with *A. minimus* and *A. varuna*, were found breeding in significant numbers up to at least half a mile (2,640 feet) from human dwellings.

Viswanathan (1950) and Ramachandra Rao (1945) have indicated rice-fields in Bombay State as important larval habitats under physical or climatic conditions that cannot be precisely defined, for censuses in successive years have shown that *A. fluviatilis* utilizes this medium for larval tenancy to variable degrees. Streams and channels with a sluggish flow of water, with marginal or emergent vegetation, and with partial shade are conventionally accepted elsewhere as the only sites for optimal development of *A. fluviatilis* larvæ (Puri, 1941; Christophers, 1933; Covell, 1944).

The larvæ of *A. fluviatilis* have been found to become rare in D.D.T.-sprayed areas by Vedamanikkam (1949). This would indicate a significant reduction of the adult population by D.D.T. and be at variance with observations of Muirhead-Thomson (1951) on *A. gambiae* in Africa. Viswanathan (1950) feels that D.D.T. does not exert an excito-repellant effect on adult *A. fluviatilis* in dwellings, and that it therefore serves to reduce the ovipositing fraction of the population of this species. This should result in a reduction of larvæ in sprayed areas.

Viswanathan (1950) who has probably made the most detailed studies of *A. fluviatilis* within a given area, adds the information that in Bombay State the density of larvæ is very small even in the species' most favoured habitats. This is undoubtedly to be construed in a comparative sense, that is in contrast to species such as *A. vagus* or *A. subpictus* whose larvæ may at times be found in teeming abundance. He states, furthermore, that when *A. fluviatilis* larvæ are found in ricefields, they may be most abundant in that habitat just before harvest. There is a significant difference, therefore, between the ovipositing behaviour of *A. fluviatilis* and *A. culicifacies* as recorded by Russell and Ramachandra Rao (1942) in South-eastern Madras, where growth of young rice plants eventually presented an obstacle to oviposition by the latter species, as judged by disappearance of larvæ from paddy fields in the later stages of cultivation.

Studies in Kanara District, Bombay, further indicate breeding places as usually close to human habitations (Viswanathan, 1950). Scrupulous clean-weeding of streams in the era preceding D.D.T. gave perfect larval control

during two years, although adult prevalence was reduced only partially and an effect on malaria incidence could not be claimed. It was concluded that ricefield breeding was responsible for the continued prevalence of mosquitoes and malaria. At Sirsi Town in Bombay State, a combination of clean-weeding, copper cyanide larviciding of ricefields and pyrethrum spraying of dwellings succeeded in bringing adult *A. fluviatilis* densities below the critical density as regards malaria transmission (accepted as 4.0 adults per 10 man-hours collected by hand in human dwellings in the day time), and malaria rates also dropped sharply. This procedure was felt to be feasible only in situations where the area of ricefields to be treated was small, and where the urban population was sufficiently large to bear the cost of operations.

Finally, Viswanathan (1950) has been unable to distinguish differences in favoured larval habitats in Malnad or Maidan tracts, i.e., in areas of alleged anthropomorphic or zoophilic proclivities respectively, for he is of the opinion that throughout the range of *A. fluviatilis* in Bombay State the larvæ are found principally in waters near human dwellings characterized by sluggish current, marginal vegetation and/or deep-cut banks with a canopy of foliage, and some shade.

2. *Mosquito infection rates.*—Jaswant Singh and Jacob (1944) found an infection rate of 11 per cent in North Kanara. Russell and Jacob (1942) recorded an infection index of 17.3 per cent in the Nilgiris. In other studies in North Kanara, Viswanathan and Ramachandra Rao (1943) found an infection rate of 44.4 per cent.

Viswanathan (1950) observes that the vectorial capacity of *A. fluviatilis* varies from district to district in Bombay State, in some places at certain times reaching 50 per cent and in other places approaching zero. Vectorial capacity varies inversely with the abundance of the species. Another correlation is that vectorial capacity is high in foot-hill regions but low in the Deccan Plateau. He states that *A. fluviatilis* in hilly regions of Peninsular India, whether in the Satpuras, Eastern Ghats, Western Ghats, Nilgiris, Annamalais, or in Travancore, is an extremely efficient vector. It may be significant in the Deccan if it is abundant, but it has been shown repeatedly that it need not be, wherever it occurs, the efficient vector which it is in the Western Ghats. But Christophers (1933) has said that it is an important carrier wherever found.

3. *Anthropophilism and zoophilism.*—Jaswant Singh and Jacob (1944) determined by precipitin tests that in North Kanara sixtyfour per cent of captured *A. fluviatilis* had fed on human blood. These authors stated further that in foothills in the United Provinces the anthropophilic index of *A. fluviatilis* was only five per cent. In the Wynaad, Covell and Harbhagwan (1939) found the species to be anthropophilic to the extent of 97 per cent.

In the Singlibhum Hills, Senior White (1946) made a study of the source of blood meals of *A. fluviatilis* taken from houses, cattlesheds and outdoor resting places. Three-quarters of 187 mosquitoes from houses had fed

on human beings ; none of 49 insects from cattlesheds contained human blood ; while one-third of twelve mosquitoes captured out of doors had fed on man.

Viswanathan (1946) quotes further work by Jaswant Singh at Poona, where 99 per cent of 109 *A. fluviatilis* contained blood of bovine origin. In the course of their work, Viswanathan and Parikh (1946) found that in North Kanara District the D.D.T.-spraying of structures occupied by human beings or by human beings and cattle, but not of structures occupied by cattle only, resulted in a reduction of *A. fluviatilis* mosquitoes but not of purely zoophilic species. This was accepted as evidence of the predominantly anthropophilic nature of *A. fluviatilis* in that area.

Viswanathan (1950) is of the opinion that there is no obvious explanation why *A. fluviatilis* should be primarily anthropophilic in some regions and zoophilic in others. His work in Bombay State indicates, however, that these differences in behaviour are more or less constant geographically, a point that is not brought out in studies that consist of single surveys. This shows either that stimulating forces in a given environment are also constant on the whole, or that given areas are populated by genetically pure strains of mosquito.

The various reports indicate further that anthropophilism is characteristic of *A. fluviatilis* in hill regions where vectorial capacity is high and population density is low, while zoophilism characterizes plains regions where vectorship is poor and densities are high. In view of the irregular distribution of hill tracts in India, one is led to think that the differential behaviour of *A. fluviatilis* can hardly be genetic but must be a reflection of physical attributes of the environment in which the species finds itself. Studies of this question have not yet been made, but it should be possible to investigate the reactions of insectary colonies from plains regions after liberation at a hill station and, reciprocally, the behaviour of Malnad colonies in the Maidan. Since morphologic evidence of geographic strains of *A. fluviatilis* is lacking (see beyond), an experiment such as the one suggested might provide a clue to the existence or non-existence of physiological or genetic geographic strains.

4. *Daytime resting places.*—A mosquito that has fed on a sleeping person may spend the following day in the dwelling where it fed, in a neighbouring cattleshed or in some outdoor shelter. Similarly mosquitoes feeding on cattle may remain in the shed, move to a human dwelling or resort to other shelters. Movements of mosquitoes on succeeding nights before and after oviposition may also be varied, although fairly constant behaviour patterns are followed by some species (Bates, 1949). Information in the case of *A. fluviatilis* has been recorded as follows.

In North Kanara, Jaswant Singh and Jacob (1944) found human habitations to be the common daytime resting places of *A. fluviatilis*. In the Satpura Ranges, Senior White (1941) obtained evidence that a significant

fraction of the population rests out-of-doors during the daytime. His later work in the Singhbhum Hills, already referred to (Senior white, 1946), revealed 12 out of 248 captured specimens, or about five per cent, located out-of-doors in the daytime.

Difficulties in discovering mosquitoes out-of-doors make percentages such as the preceding one misleading. However, it is sometimes possible to use collateral evidence in arriving at an opinion on the question of the outdoor resting population. Studies by Senior White, Ghosh and Venkat Rao (1945) in the Jeypore Hills and the Singhbhum Hills enabled them to compare infection rates and other findings in the two regions. They came to the conclusion that "in the Singhbhum Hills outdoor resting *A. fluviatilis* do not form a main part of the vector population". Furthermore the specimens captured out-of-doors in the Jeypore Hills were apparently females that had just oviposited but had not yet returned to human shelters for their next blood meals. Thus it was still possible that these mosquitoes, when freshly fed, had spent their first two or three post-prandial days within man-made structures which were deserted only on maturation of ova.

Viswanathan and Parikh (1946) found that D.D.T.-spraying of known or conceivable outdoor resting places of *A. fluviatilis* resulted in a detectable reduction in numbers of these mosquitoes in North Kanara. Although the manoeuvre was regarded as an impractical tactic, or an insufficiently investigated one from the standpoint of malaria abatement, the information is of considerable interest since it indicates the extent to which out-of-door resting, even as a transitory act, may be practised in that area by this species of *Anopheles*.

At Sirsi Town, also in North Kanara, Viswanathan and Ramachandra Rao (1943) concluded that newly-fed adults of *A. fluviatilis* may spend the day within houses rather than out-of-doors. But in subsequent studies in the same region these authors (Viswanathan, Ramachandra Rao and Rama Rao, 1944) confirmed their suspicions of outdoor resting *A. fluviatilis* by the capture during the day of one male and sixteen females from deep clefts in rocky banks of a stream margin. All the females had previously fed and were not, therefore, newly-emerged imagines without other choice of their whereabouts. They felt that there was a preponderance of outdoor resting mosquitoes during day time in that region.

Viswanathan (1950) points out the inverse relationship between indoor daytime resting densities and the vectorial capacity of a given local population of *A. fluviatilis*. However he notes also that where this species is a good vector, the fraction of the population that elects to remain indoors during the day is found almost exclusively in human dwellings, with only about five per cent of the indoor population occupying cattlesheds.

Thus two possible groups of *Anopheles fluviatilis* may be visualized: (1) anthropophilic, outdoor-resting, hill-inhabiting, strongly vectorial and low in density and (2) zoophilic, indoor-resting, plains-inhabiting, weakly vectorial and high in density. There may be further groups, in which some of the above characterisations fail to hold true, or in which other traits serve to differentiate populations. But it seems likely that a contrast between the two chief groups may be made in approximately the foregoing way in most cases. When it is remembered that some anopheline species are distinguished morphologically by the mere presence or absence of a small coastal spot or of branching in a larval hair, the above five-fold dichotomy between populations of *A. fluviatilis* must assume some significance in any discussion of geographic races of this species.

5. *Flight dispersal and nocturnal activity*.—Evidence of short flight range of *A. fluviatilis* in the Wynaad has already been presented in connection with the report of reduced adult catches following the oiling of breeding channels within 1,000 to 1,500 feet of houses (Adisubramaniam and Vedama-
uikkam, 1943). Further studies of flight range are apparently lacking.

Conflicting records exist regarding nocturnal activity of *Anopheles fluviatilis*, although the total of information is meagre. In Mysore State, Nursing, Rao and Sweet (1934) found nearly as many *A. fluviatilis* entering houses after midnight as before. But Viswanathan and Ramachandra Rao (1943) found in North Kanara that females entered a human shelter shortly after dusk and completed feeding before midnight in the main. These workers with Rama Rao (Viswanathan, Ramachandra Rao and Rama Rao, 1944) later captured, marked and released entering mosquitoes and found that while some of the insects moved about randomly in the shelters during the night, by morning a large proportion of them had departed, even those that had fed on the same night. The inherent mobility of mosquitoes led some of the marked specimens to re-enter the same or nearby structures on subsequent nights before completion of the gonotrophic cycle.

In experiments with marked gravid *A. fluviatilis*, the same workers (Viswanathan, Ramachandra Rao and Rama Rao, 1945) learned through subsequent collections by spray-killing that specimens released in huts left those shelters at dusk, deposited eggs in near-by waters, and re-entered huts in search of a blood meal within a few hours' time. A few females deferred oviposition until the second night.

Jaswant Singh and Mohan (1951) also used marked specimens and noted irregular movements of *A. fluviatilis* into and out of houses during the night in a hyperendemic village in the foothills of the Nilgiris. Their data show that both fed and unfed specimens participated in these movements, and also the remarkable fact that some females fed on two successive nights, that is, within the period of one gonotrophic cycle. While feeding took place in all four quarters of the night, most of this activity was confined to the second and third quarters, indicating a difference in behaviour between this population and the early-feeding mosquitoes in Bombay.

Experiments to determine directly the range of flight of emerged *A. fluviatilis* from natural larval habitats have not been made.

6. *Morphology: alleged races.*—The well-known morphological distinctness of mosquito eggs in the *maculipennis* group in Europe (Hackett and Missiroli, 1935) has led naturally to a search for morphological variations among strains of important malaria vectors in India. In the case of *A. culicifacies* these studies include those of Rajindar Pal (1945) and of Sweet and Rao (1938), neither of which was productive of results in any stage of the life cycle. Two races of *A. stephensi*, distinguishable by egg measurements and colonization behaviour were detected following the work of Sweet and Rao (1937), Subba Rao, Sweet and Rao (1938) and Rao, Sweet and Subba Rao (1938). To date, however, no morphological distinctions in *A. fluviatilis* populations from various areas have been detected.

Several workers have concluded nevertheless that biological races of *A. fluviatilis* exist in sufficiently clear-cut fashion to encourage attack on the anthropophilic fraction of the population. Senior White (1946) for example was not concerned with the pyrethrum spraying of cattlesheds in the Singhbhum Hills, since none of the *A. fluviatilis* from these sites had fed on human blood. He suggested that the cattleshed group represented a biological race with no attraction towards man. Later (Senior White, 1947) summarised work on anthropophilic indices of *Anopheles* species in East Central India and concluded tentatively that biological races, with respect to feeding habits, exist in the cases of *A. varuna*, *A. culicifacies*, *A. fluviatilis* and *A. minimus*, although only in the case of *A. varuna* did he accept evidence for distinguishing such races by measurement of specimens.

Viswanathan (1950) has presented abundant evidence on the geographic variability of feeding behaviour of *A. fluviatilis* in Bombay State, but in the course of preliminary malaria surveys during which 72,086 adult *A. fluviatilis* were examined, no hint of consistent morphological variation was discovered. Subsequent examination of many larvæ and adults has still remained fruitless in this regard. He believes in the possibility of biological races, however, judging from different vectorial capacities and host predilections in different parts of the range of this mosquito.

Winter larvæ of *A. fluviatilis* from North India and Bombay were found by de Burca and Forshaw (1947) to exhibit structural variations. The commonest anomaly was division of the outer clypeal hair into two equal branches. This finding was often unilateral only. Such natural irregularities serve only to complicate the search for consistent departures from conventional morphology in different regions.

7. *Larval habitats.*—Puri (1941) lists the larval habitats of *A. fluviatilis* as "Clear water pools along edges of running streams and irrigation channels". He further states that the same larval habitats are occupied by *A. minimus*, although the latter breeds in ricefields also. Thus he implies that *A. fluviatilis* does not breed in ricefields. Christophers (1933) also fails to mention ricefields as a larval habitat of this species.

There has been ample confirmation of the stream-breeding proclivity of this species, but occasional exceptions have been recorded from more than one standpoint. Ramachandra Rao (1945) made the important observation that data for two successive years may be at variance for unknown reasons. At all times streams and channels remained the most important breeding habitats in North Kanara District, but in 1944 *A. fluviatilis* larvæ were found in ricefields sufficiently often to make this habitat a significant one in the ecology of the species. Larvæ were obtained from fields both fallow and cultivated.

Viswanathan (1946) has supported this extension in the concept of larval *A. fluviatilis* habitats by listing optimal breeding places in Bombay State as: streams, channels, shallow earthen wells, sugarcane and betelnut garden trenches, uncultivable swampy fields and ricefields in all stages of paddy culture. He says elsewhere (Viswanathan, 1946a) that clean-weeding of streams and channels in North Kanara District is not sufficient as a sole control measure owing to the degree of additional *A. fluviatilis* production in ricefields.

8. *Critical densities for effective transmission.*—The concept embracing density of the vector population and efficiency of disease transmission has been treated by most malariologists who have dealt with *A. fluviatilis*, since where this species is important it has usually exhibited a strikingly high infection rate but sparse populations (*vide supra*). However, Viswanathan (1945: 1946a: 1950) appears to have been the only one to reduce his findings to a numerical formula. In his opinion the capture of four anthropophilic *A. fluviatilis* per ten man-hours (0.4 per man-hour) is the threshold density for effective malaria transmission.

SUMMARY.

1. A review of the literature on the bionomics of *Anopheles fluviatilis* has been presented in order to provide a background against which to project findings in the case of recent and current studies of this mosquito in the Western Ghats of Mysore State.

2. There seems to be sufficient evidence to conclude that in Peninsular India, *A. fluviatilis* populations exhibit different traits. The species is usually either hill-inhabiting, anthropophilic, outdoor-resting, highly vectorial and relatively uncommon; or plains-inhabiting, zoophilic, indoor-resting, weakly vectorial and relatively abundant. But as yet no morphological evidence supporting the belief in such geographic races has been detected.

3. Many reports on the biology and ecology of *A. fluviatilis* are in conflict with one another. Since the conflicting reports were made in different regions, there is no reason to suspect that any of them are incorrect. Variation from year to year in a given region are noted only uncommonly, because most of the regional studies have been of short duration. Some of the discrepant reports may be listed as follows.

(a) *Larvæ*.—In some regions larvæ are found primarily in streams or channels; in others they are found in a variety of habitats including rice-fields. Where larvæ are found principally in streams, their abundance is sometimes related to the proximity of human dwellings but in other cases not.

(b) *Adults*.—Adult prevalence seems to vary with feeding habits. Zoophilic *A. fluviatilis* have been found at the rate of 34 per man-hour in the Nira Canal zone of Sholapur District in the Bombay Deccan but with an infection rate of only 0.11 per cent. Where it is anthropophilic in nearby regions, a density of only 0.4 per man-hour is more greatly to be feared since the infection rate may be as high as 11 per cent. An infection rate of 44.4 per cent or more is recorded for *A. fluviatilis* in other parts of its range.

Controversies exist both as to the time of feeding and as to daytime resting places. In North Kanara, *A. fluviatilis* was found to enter structures shortly after dusk and to feed chiefly before midnight, but in Mysore and in the Nilgiris almost as many entered after midnight as before. Further studies in North Kanara indicated that a large proportion of fed *A. fluviatilis* left the shelters before dawn and took up outdoor resting places during the subsequent completion of their gonotrophic cycle. In the Jeypore Hills, however, *A. fluviatilis* captured out-of-doors were found to be mosquitoes that had already completed oviposition but had not yet returned indoors for their next blood meal. This is at further variance with work in Bombay State, which disclosed that fully gravid females there, when released in huts, almost invariably left such shelters at dusk for oviposition but returned in search of blood meals on the same night.

The disparity reflected in the foregoing reports by outstanding workers makes it evident that *Anopheles fluviatilis* can scarcely be a homogeneous species in the parts of its range in India where it has received attention.

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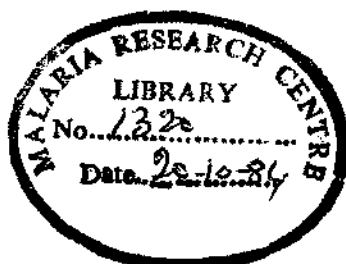


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