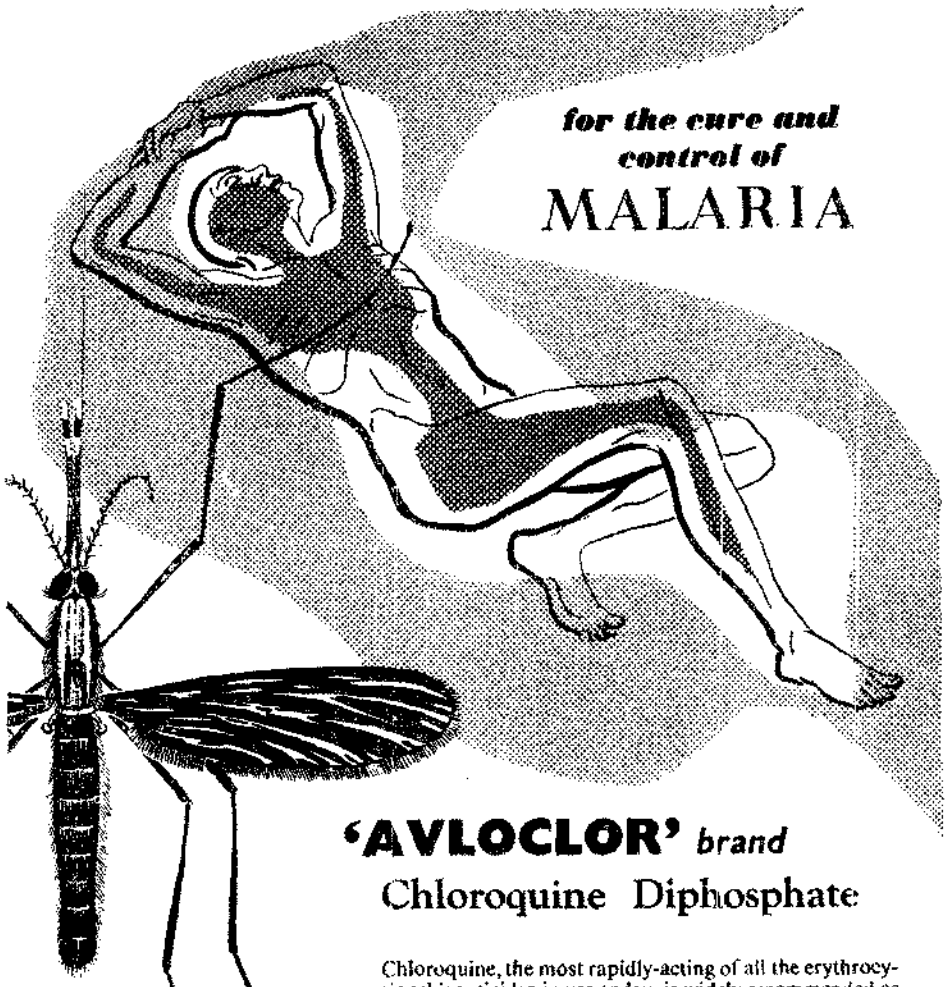
Vol. VIII, No. 1. *March 1954.*

INDIAN JOURNAL OF MALARIOLOGY.

PUBLISHED UNDER THE AUTHORITY OF
THE INDIAN COUNCIL OF MEDICAL RESEARCH.

Editor:—Lieut.-Colonel JASWANT SINGH, M.B., Ch.B., D.P.H., D.T.M. & H.,
Director, Malaria Institute of India.

PUBLISHED FOR
THE INDIAN COUNCIL OF MEDICAL RESEARCH
By
JOB PRESS LTD., KANPUR.



for the cure and control of

MALARIA

'AVLOCLOR' *brand*

Chloroquine Diphosphate

Chloroquine, the most rapidly-acting of all the erythrocytic schizonticides in use today, is widely recommended as the drug of choice for the control of an acute attack of malaria, especially falciparum malaria in which it not only controls the attack but achieves radical cure by a short course of treatment. In semi-immune subjects, clinical cure can be achieved by a single dose of 4 tablets of 'Avloclor'.

The drug does not produce any serious toxic effects when given in therapeutic dosage.

Chloroquine has also established itself as a powerful remedy in hepatic amoebiasis, for the treatment of which it rivals emetine.

Available in tablet form (0.25 gramme) for oral administration, in packs of 10 and 500.

ICI

IMPERIAL CHEMICAL INDUSTRIES (INDIA) LIMITED

Calcutta Bombay Madras Kanpur New Delhi
Ahmedabad Amritsar Bangalore Cochin

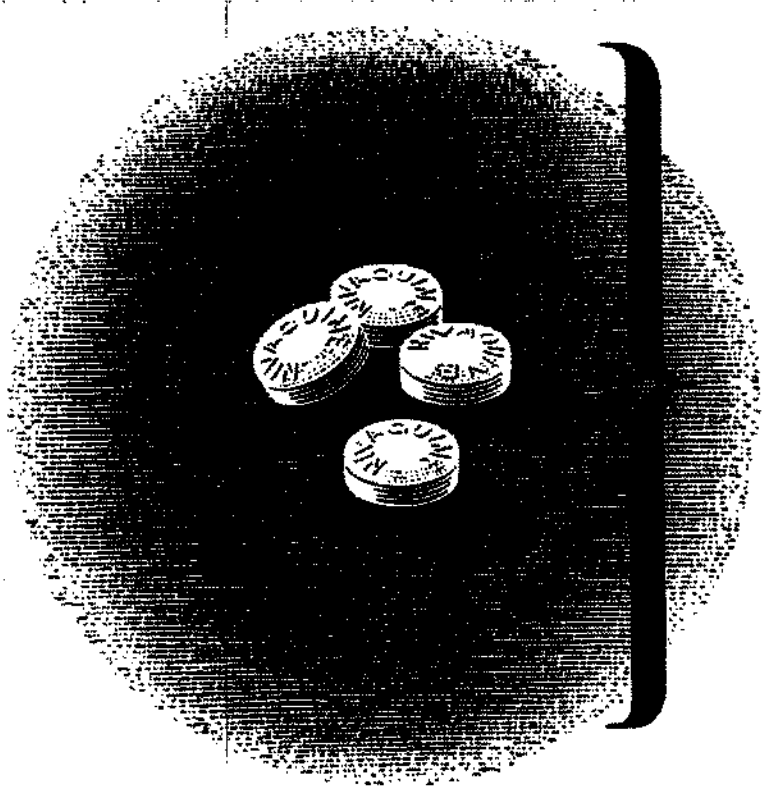
ICP 253

Indian Journal of Malariology, **8**, 1, *March* 1954.

CONTENTS.

Indian Journal of Malariology, **8**, 2, *June* 1954.

CONTENTS.



SINGLE DOSE Technique

in the treatment of the acute attack of malaria

Reports on the use of Chloroquine as the schizontocide in acute attacks of malaria due to *P. falciparum*, *P. vivax* and *P. malariae* demonstrate the early disappearance of fever and parasitaemia, the speed with which symptoms are relieved and the absence of side-effects. The short period over which administration is necessary is an immediate advantage and therapy with one dose of 600 mgm. Chloroquine base has now been shown to be a practical proposition for semi-immune populations in rural areas.

'NIVAQUINE'

trade mark brand

Chloroquine Sulphate

'NIVAQUINE' is supplied as follows:

TABLETS : Containers of 10 x 200 mgm. (each tablet contains 150 mgm. Chloroquine base)

SOLUTION : Boxes of 10 x 5 c.c. ampoules.

M&B

manufactured by : **MAY & BAKER LTD**

distributors

MAY & BAKER (INDIA) LTD. BOMBAY . CALCUTTA . MADRAS . NEW DELHI . GAUHATI

MA309-19

Indian Journal of Malariology, **8**, 3, *September* 1954.

CONTENTS.

Indian Journal of Malariology, **8**, 4, December 1954.

CONTENTS.

Indian Journal of Malariology, 8, 1, *March* 1954.

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

Part IV.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B.(Edin.), D.P.H.(Eng.), D.T.M. & H. (London.),

G. R. CHANDRASEKHAR, M.B.B.S.,†

H. L. BAMI, Ph.D., A.I.I.SC.

AND

A. P. RAY, M.B.B.S.

(*Malaria Institute of India, Delhi.*)

(November 24, 1953.)

In earlier papers, Jaswant Singh, Basu and Ray (1952) and Jaswant Singh, Ray and Chandrasekhar (1953) have discussed in detail procedures and techniques employed for the screening of antimalarials. Based upon these procedures, forty-five new synthetic antimalarials (Tables I to VI) have been screened for antimalarial activity. The minimum effective dose (M.E.D.) of some of the standard antimalarials had been determined previously, out of which M.E.D. of quinine was selected as a basis for comparison in the present screening. The new doses tried here were eight times, two times, half of, and one-eighth of the M.E.D. of quinine. The dilution of drug was so adjusted that under each regime the required quantity of drug calculated in terms of base content needed for a 50 gm. chick was contained in one ml. of aqueous solution or suspension. The number of white leghorn chicks used were four to six for each dosage regime. For each series, an untreated group of 6 to 12 chicks was maintained for comparison purposes.

*This work was carried out under the scheme "Screening of antimalarial drugs" which is financed by the Council of Scientific and Industrial Research. Parts I, II and III of this series have already appeared in *Indian Journal of Malariology*.

†Appointed on the staff of Screening of Antimalarial Drugs Scheme, Council of Scientific and Industrial Research at the Malaria Institute of India.

The compounds were supplied by workers from different institutions† and those compounds which were insoluble, were made into aqueous suspensions with gum acacia for ease of administration.

The compounds screened, have been divided into six chemical groups, viz. substituted dihydro-triazines ; substituted biguanides ; substituted sulphides, thioureas and biurets ; quinozolines ; quinindenes ; and miscellaneous compounds (Tables I to VI). While tabulating the results, activity if present, is indicated against the dosage, expressed in terms of multiples of M.E.D. of quinine. For instance, a compound showing activity at one-fourth M.E.D. of quinine, has a quinine equivalent of four, while a comopund active at 16 times M.E.D. of quinine, has a quinine equivalent of 0·06 only.

TABLE I.

Substituted dihydro-triazines.

Number.	Chemical formula.	Source.	Dose (Multiple of M.E.D. of quinine)	Remarks.
M.I.S. 10	1-p-chlorophenyl-6 : 6-dimethyl-2 : 4-diamino 1 : 6-dihydro-1 : 3 : 5-triazine hydro-chloride.	Malaria Institute of India.	8	Active.
			2	,,
			1/2	,,
			1/8	,,
			1/32	,,
			1/128	,,
			1/256	,,
M.I.S. 11	1-p-bromophenyl-6 : 6 dimethyl-1 : 6-dihydro-2 : 4-diamino-1 : 3 : 5-triazine-hydrochloride.	,,	2	,,
			1/2	,,
			1/8	,,
			1/32	,,
			1/128	,,
			1/256	,,
			1/300	,,
			1/400	,,
			1/512	,,
			1/1024	Inactive.
M.I.S. 12	1-(2 : 4-dichlorophenyl)-1 : 6-dihydro-6 : 6-dimethyl 2 : 4-diamino-1 : 3 : 5-triazine hydrochloride.	,,	8	Toxic
			2	,,
			1/2	Active.
			1/8	Inactive.
M.I.S. 15	1-p-methoxyphenyl-6 : 6 dimethyl 1 : 6-dihydro-2 : 4-diamino-1 : 3 : 5-triazine hydrochloride.	,,	8	Active
			2	,,
			1/2	,,
			1/8	Inactive.
M.I.S. 19	1-p-bromophenyl-6 : 6 diethyl-1 : 6-dihydro-2 : 4-diamino-1 : 3 : 5-triazine hydrochloride.	,,	1/8	Inactive.

†Malaria Institute Survey (M.I.S.) numbers have been given to each compound and the source of supply is acknowledged.

A number of triazine derivatives have been prepared as potential antimalarials but their activity has been reported to be very feeble (Curd *et al.*, 1947) ; Wiselogle, 1946). Recently, Carrington *et al.* (1951) and Crowther and Levi (1953) have discovered an active metabolite of the well-known drug proguanil (paludrine) which belongs to dihydrotriazine group (M.I.S. 10, Table I). A number of other dihydrotriazines akin to proguanil metabolite have been prepared in the Malaria Institute laboratory (Bami, 1954), and out of these, p-bromophenyl analogue of proguanil metabolite (M.I.S. 11) has been found to be four times as active as the proguanil metabolite. While 2 : 4-dichloro-phenyl-analogue (M.I.S. 12) proved to be only slightly active, it may be interesting to mention that 3 : 4-dichloro analogue of M.I.S. 10 has been found to be several times more active than the parent compound (Carrington *et al.*, 1951). Replacement of methyl groups in M.I.S. 11 with longer alkyl groups, such as, in the case of M.I.S. 19, resulted in considerable loss of activity. Similarly M.I.S. 15 (p-methoxyphenyl analogue of M.I.S. 11) was also devoid of activity.

Structural similarities between these dihydro-triazines and their corresponding biguanide derivatives appeared to be responsible for similar biological responses observed in both these cases.

TABLE II.

Substituted biguanides.

Number.	Chemical formula.	Source.	Dose (multiple of M.E.D. of quinine).	Remarks.
M.I.S. 8	N^1-p-(4-methyl-2-pyrimidyl) phenyl-sulphonamide-N^5-isopropyl-biguanide hydrochloride.	Malaria Institute of India.	2	Active.
			1/2	,,
			1/8	Inactive.
M.I.S. 22	N^1-p-(2-diazyl)-phenyl sulphonamido-N^5-isopropyl biguanide-hydrochloride	,,	2	Active.
			$\frac{1}{2}$	,,
			1/8	Inactive.
M.I.S. 25	N^1-p-sulphonamidophenyl-N^5-isopropyl-biguanide hydrochloride.	,,	8	Inactive.
M.I.S. 20	N^1-m-(5-chloro-2-pyrimidyl) phenylsulphonamide-N^5-isopropyl-biguanide hydrochloride.	,,	8	Active.
			2	,,
			1/2	,,
M.I.S. 17	N^1-p-sulphonic phenyl-N^5-p-chlorophenyl-biguanide, hydrochloride.	Indian Institute of Science, Bangalore.	8	Active.
			2	,,
			1/2	,,
			1/8	,,

TABLE II—(*Concld.*)

Number.	Chemical formula.	Source.	Dose (multiple of M.E.D. of quinine)	Remark
M.I.S. 21	N^1-p-chlorophenyl-sulphonyl-N^5-p-bromophenyl-biguanide.	Malaria Institute of India.	8	Inactive.
M.I.S. 23	N^1-phenylsulphonyl-N^5-p-bromophenyl-biguanide.	,,	8	Inactive.
M.I.S. 24	N^1-p-chlorophenyl-sulphonyl-N^5 p-chlorophenyl-biguanide.	,,	8	Inactive.
M.I.S. 34	N^1-p-sulphonic phenyl-N^5-phenyl-biguanide.	Indian Institute of Science, Bangalore.	8	Inactive.
M.I.S. 9	N^1-(6-hydroxy-8-quinolyl)-N^5-isopropyl-biguanide hydrochloride.	Malaria Institute of India.	2	Inactive.
M.I.S. 44	Phenyl-biguanide hydrochloride.	Medical College Patna.	8	Inactive.
M.I.S. 53	2 :6-di(p-chloro-phenyl-biguanido-formyl)-4(p-phenyl-biguanidino)-3-phenyl-pyridine.	Indian Institute of Science, Bangalore.	8	Inactive.

A number of sulpha-biguanides have been previously reported to be feebly active against avian (Jaswant Singh *et al.*, 1949; Bami *et al.*, 1949) and simian malarias (Jaswant Singh *et al.*, 1949). A further variation of the above compounds included introduction of an isopropyl group which is considered essential for activity in this class of compounds (Rose, 1951). However, these new sulpha-biguanides (M.I.S. 8, M.I.S., 22, M.I.S. 25, M.I.S. 20) were only twice as active as quinine and considerably less active than proguanil. Extension of this work to include certain arylsulphonyl biguanides (Bami, 1953*a*) (M.I.S. 17, M.I.S. 21, M.I.S. 23, M.I.S. 24, M.I.S. 34) resulted in inactive compounds except for M.I.S. 17 which was eight times as active as quinine.

Replacement of p-chlorophenyl group of proguanil (N^1-p-chlorophenyl-N^5-isopropyl-biguanide) with potential 6-hydroxy-8-quinolyl nucleus (M.I.S. 9) resulted in complete loss of activity. Similarly M.I.S. 53 and M.I.S. 44 also proved to be inactive.

TABLE III.

Substituted sulphides, thioureas and biurets.

Number.	Chemical formula.	Source.	Dose (multiple of M.E.D. of quinine).	Remarks.
M.I.S. 18	Bis(p-sulphonic phenyl guanyl) sulphide, hydrochloride.	Indian Institute of Science, Bangalore.	8	Active.
			2	,,
			1/2	,,
			1/8	Inactive.
M.I.S. 33	Bis(p-tolyl-guanyl) sulphide hydrochloride.	,,	8	Toxic.
			2	Inactive.
M.I.S. 41	p-chlorophenyl-guanyl thiourea.	,,	8	Toxic.
			2	,,
			1/2	,,
			1/8	Inactive.
M.I.S. 42	N-methylguanidino-N-phenyl thiourea.	,,	8	Toxic.
			2	Inactive.
M.I.S. 40	p-tolyl-4-ethyl-thiol-pseudo-dithiobiuret.	,,	8	Active.
M.I.S. 39	p-chlorophenyl-thiuret.	,,	2	Active.
			1/2	,,
			1/8	,,
M.I.S. 43	p-chlorophenyl-di-ethylthiol pseudo-dithiobiuret.	,,	8	Inactive.

Arylguanyl sulphides (M.I.S. 18, M.I.S. 33) and thioureas (M.I.S. 41 and M.I.S. 42) were found to be toxic and/or inactive except for M.I.S. 18 which was twice as active as quinine. Amongst thiurets (M.I.S. 39) and dithiobiurets (M.I.S. 40 and M.I.S. 43), only M.I.S. 39 showed fair antimalarial activity. This group of compounds even previously had shown limited antimalarial activity (Wiselogle, 1946 ; Rose, 1951 ; Bami, 1953*b*).

TABLE IV.

Quinazolones.

Number.	Chemical formula.	Source.	Dose (multiple of M.E.D. of quinine).	Remarks.
M.I.S. 50	2-methyl-3-(p-chlorophenyl) quinazolone-4-hydrochloride.	Punjab University, Hoshiarpur.	8 2	Toxic. Inactive.
M.I.S. 16	2-methyl-3-(p-methoxy-phenyl) quinazolone-4-hydrochloride.	,,	8 2 1/2 1/8	Active. ,, ,, Inactive.
M.I.S. 46	2-methyl-3(p-β-chloropropionyl-amino-phenyl)-quinazolone-4, hydrochloride.	,,	8 2	Toxic. Inactive.
M.I.S. 47	2-methyl-3(4′-p-chlorophenyl-thiazolyl) quinazolone-4.	,,	8	Inactive.
M.I.S. 48	2-methyl-3-(4′-phenyl-thiazolyl)-quinazolone-4.	,,	8 2 1/2	Toxic.
M.I.S. 49	2-methyl-3(4-p-anisylthiazolyl) quinazolone-4.	,,	8	Inactive.

This series of quinazolone derivatives were devoid of antimalarial activity excepting M.I.S. 16 which was twice as active as quinine. These results support the reported inactivity of substituted quinazolone derivatives (Rose, 1951 ; Bami, 1953*b*).

TABLE V.

Quinindenes.

Number.	Chemical formula.	Source.	Dose (multiple of M.E.D. of quinine).	Remarks.
M.I.S. 27	12-hydroxy-7 ; 9-dichloro-2 : 4-dihydro-β-quinindene.	Malaria Institute of India.	8 2	Inactive. ,,
M.I.S. 28	8-or-10-chloro-12-hydroxy-2 : 4-dihydro-β-quinindene.	,,	8 2	Active. Inactive.
M.I.S. 29	12-hydroxy-7-chloro-2 : 4-dihydro-β-quinindene.	,,	8	Inactive.
M.I.S. 35	12-hydroxy-9-bromo-2 : 4 dihydro-β-quinindene.	,,	8	Inactive.
M.I.S. 36	12-hydroxy-9-methyl-2 : 4-dihydro-β-quinindene.	,,	8 2	Active. Inactive.
M.I.S. 37	12-hydroxy-7-methyl-2 : 4 dihydro-β-quinindene.	,,	8	Inactive.
M.I.S. 38	12-hydroxy-9-ethoxy-2 : 4 dihydro-β-quinindene.	,,	8	Inactive.

Chadha *et al.* (1951*a* : 1951*b*) have reported a number of β-quinindene derivatives out of which a few were tested, but all of them were found to be inactive.

TABLE VI.

Miscellaneous compounds.

Number.	Chemical formula.	Source.	Dose (Multiple of M.E.D. of quinine).	Remarks.
M.I.S. 26	Bis-(2-carboxy phenyl) Xanthogen.	Indian Institute, of Science, Bangalore.	8	Active.
			2	Inactive.
M.I.S. 31	Bis-(4-carboxy phenyl) Xanthogen	,,	8	Inactive.
M.I.S. 13	Thiopegene-9-4-one hydrobromide	Punjab University, Hoshiarpur.	8	Toxic.
			2	,,
			1/2	Active.
			1/8	Inactive.
M.I.S. 45	2 : 3-dimethyl-thiopega-2 : 9-diene-4-one-hydrochloride.	,,	8	Inactive.
M.I.S. 30	N-N'-di(p-sulphonic phenyl)-thiuram-disulphide.	Indian Institute of Science, Bangalore.	4	Inactive.
M.I.S. 32	N-N'-di-(p-chlorophenyl) thiuram disulphide.	,,	16	Toxic.
			4	Inactive.
M.I.S. 14	2-methyl-4-(p-anisidino) 6-chloro-quinoline hydrochloride.	Punjab University, Hoshiarpur.	8	Toxic.
			2	Active.
			1/2	,,
			1/8	Inactive.
M.I.S. 51	N-{p-(4-methyl-2-pyrimidyl)-sulphonamido-phenyl}-3-phenyl chelidamic acid.	Indian Institute, of Science, Bangalore.	8	Toxic.
			2	Active.
			1/2	Inactive.

In this group of miscellaneous compounds, xenthogens (M.I.S. 26 and M.I.S. 31) and disulphides (M.I.S. 30 and M.I.S. 32) did not show any activity. Thiopegene-9-4-one (Khosla *et al.*, 1953) (M.I.S. 13) was twice active while its substituted derivative (Khosla *et al.*, 1953) (M.I.S. 45) was completely inactive. A substituted 4-aminoquinoline (M.I.S. 14) has also shown some activity.

SUMMARY.

Forty-five compounds have been screened for their antimalarial activities. Only certain dihydrotriazines have displayed appreciable activity while the sulpha-biguanides were only slightly active. Remaining compounds were either inactive or only feebly active.

REFERENCES.

Bami, H. L. (1954) ... ... *Curr. Sci.*, **23**, (In press).
Idem (1953*a*) ... ... *Ibid.*, **22**, p. 80.
Idem (1953*b*) ... ... *Ind. J. Mal.*, **7**, p. 183.
Bami, H. L., Natrajan, S., Ramaswamy, A. S., De, N. N., Iyer, B. H. and Guha, P. C. (1949) ... ... *Curr. Sci.*, **18**, p. 59.
Carrington, H. C., Crowther, A. F., Davey, D. G., Levi, A. A. and Rose, F. L. (1951) ... ... *Nature*, **168**, p. 1080.
Chadha, M. S., Chakravarti, K. K. and Siddiqui, S. (1951*a*) ... ... *J. Sci. Ind. Res.*, **10B**, p. 1.
Idem (1951*b*) ... ... *Ibid.*, **10B**, p. 30.
Crowther, A. A. and Levi, A. A. (1953) ... *Brit. J. Pharm.*, **8**, p. 93.
Curd, F. H. S., Landquist, J. K. and Rose, F. L. (1947) ... ... *J. Chem. Soc.*, p. 154.
Jaswant Singh, Basu, P. C. and Ray, A. P. (1952) ... ... *Ind. J. Mal.*, **6**, p. 145.
Jaswant Singh, Ray, A. P. and Chandrasekhar, G. R. (1953) ... ... *Ibid.*, **7**, p. 117.
Jaswant Singh, Ray, A. P., Nair, C. P. and Basu, P. C. (1949) ... ... *Ind. J. Mal.*, **3**, p. 405.
Khosla, M. C., Vig, O. P., Gupta, I. S. and Narang, K. S. (1953) ... ... *J. Sci. Ind. Res.*, **12B**, p. 466.
Rose, F. L. (1951) ... ... *J. Chem. Soc.*, p. 2770.
Wiselogle, F. Y. (1946) ... ... *A survey of antimalarial drugs* 1941-45. Vol. I and II. J. N. Edwards, Ann. Arbor., Michigan.

Indian Journal of Malariology, **8**, 1, *March* 1954.

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

*XV. Acquired resistance to sulphadiazine.

BY

A. K. KRISHNASWAMI,

SATYA PRAKASH

AND

S. P. RAMAKRISHNAN.

(*Malaria Institute of India, Delhi.*)

(November 21, 1953.)

RECENT advances in the chemotherapy of malaria and the extensive use of synthetic antimalarials have led to studies on the possibility of the development of drug resistance by the parasites. Alteration in the sensitivity of plasmodia to drugs brought about by prolonged administration of subminimal doses, has been reported by various workers, and a large number of observations both in the laboratory and in the field, have been recorded during the last decade (Thurston, 1953†). The available references are summarized in Table I. While only a low grade of resistance against antimalarials like quinine and pamaquin, has been reported, resistance to the more recent synthetic antimalarials has been demonstrated to be of a much higher degree. Largest number of observations are on resistance to proguanil.

This paper records studies on a sulphadiazine resistant strain of *Plasmodium berghei* developed in the laboratory from a strain maintained in rats at the Malaria Institute of India. The latter was observed by Ramakrishnan *et al.* (1951) to be highly sensitive to sulphadiazine, a total dose‡ of 6·0 mg. per kg. producing a clearance of parasitæmia from the peripheral blood for a period of three days.

*The studies were partly financed by the Indian Council of Medical Research.

†This publication was received when the present report was ready for publication.

‡The dosages of sulphadiazine given in this paper are in terms of mg./kg. given in six divided doses, one dose twice daily on three consecutive days.

TABLE I.

Record of Plasmodia resistant to Antimalarials.

Drug to which resistance reported.	Species Plasmodium.	Degree of resistance.	Laboratory or field.	Antimalarials to which cross resistance was observed, if any.	Observers.
Quinine	*P. gallinaceum*	2	Laboratory	...	Knoppers (1947).
	P. præcox	...	,,	...	Kritschewski and Halperin (1933).
	P. knowlesi	...	,,	...	Nauck (1934).
Pamaquin	*P. gallinaceum*	4	,,	...	Bishop and Birkett (1948).
	P. knowlesi	4	,,	...	Fulton and Yorke (1941).
	P. knowlesi	...	,,	...	Nauck (1934).
Sulphadiazine	*P. gallinaceum*	32	,,	Paludrine, its methyl homologue (4430).	Bishop and McConnachie (1950*a*)
	P. gallinaceum	...	,,	Other sulpha drugs.	*Ibid.* (1950*b*).
	P. berghei	...	,,	Paludrine.	Rollo (1951).
Paludrine	*P. gallinaceum*	40	,,	Methyl homologue of Paludrine (4430).	Bishop and Birkett (1947).
	P. gallinaceum	20–40	,,	Methyl homologue of Paludrine (4430)	Williamson, Bertram and Lourie (1947).
	P. gallinaceum	...	,,	...	Williamson and Lourie (1947).
	P. gallinaceum	...	,,	Sulphadiazine.	Bishop and McConnachie (1948).
	P. gallinaceum	...	,,	,,	Greenberg (1949).
	P. gallinaceum	40	,,	...	Bishop and McConnachie (1950*a*).
	P. lophuræ	10	,,	...	Thomson (1948).
	P. cynomolgi	1,000	,,	...	Hawking and Perry (1948).
	P. cynomolgi	2,000	,,	...	Schmidt, Genther, and Fradkin (1949).
	P. cynomolgi	1,000	,,	...	Hawking and Thurston (1951).
	P. knowlesi	2,400	,,	Daraprim, Bromoguanide.	Jaswant Singh, Ray, Basu, and Nair (1952).
	P. falciparum	...	Field	...	Chaudhuri and Rai Chaudhuri (1949).
	P. falciparum	...	,,	...	Edeson and Field (1950).
	P. falciparum	...	,,	...	Gilroy (1952).
	P. falciparum	...	,,	...	MacLeod (1951).
	P. falciparum	...	,,	...	Van Goor and Lodens (1950).

TABLE I.—(*Concld.*)

Drug to which resistance reported.	Species Plasmodium	Degree of resistance.	Laboratory or field.	Antimalarials to which cross resistance was observed, if any.	Observers.
	P. falciparum	...	Laboratory	...	Seaton and Adams (1949).
	P. falciparum	...	,,	...	Adams and Seaton (1949).
	P. vivax (Chesson)	> 1,000	,,	Sulpha.	Cooper, Coatney and Imboden (1950).
	P. vivax	...	Field	...	Wilson, Munro and Richard (1952).
	P. vivax	80	Laboratory	...	Seaton and Lourie (1949).
	P. vivax	...	,,	...	Lourie and Seaton (1949).
	P. malariæ	...	Field.	...	Van Goor and Lodens (1950).
	Human	...	,,	...	Gunther, Fraser and Wright (1950).
Mepacrine	*P. falciparum*	...	,,	...	Mackerras (1947).
Camoquin	*P. lophuræ*	...	Laboratory	...	Thomson (1948).
Daraprim	*P. gallinaceum*	15	,,	...	Jaswant Singh, Ramakrishnan, Krishnaswami, Satya Prakash, Mammen and Ray (1952).
	P. gallinaceum	...	,,	...	Rollo (1951).
	P. cynomolgi	100	,,	Paludrine, bromoguanide, active metabolites of proguanil and bromoguanide.	Jaswant Singh, Nair, Ray and Misra (1953).
	P. vivax (chesson)	> 25	,,	Paludrine.	Hernandez, Myatt Coatney and Jeffery (1953).
	P. knowlesi (Nuri strain)	<100	,,	Proguanil, bromoguanide and their active metabolites.	Nair (1953) Personal communication.*
Metachlorodine	*P. gallinaceum*	> 100	,,	...	Bishop and McConnachie (1953).

* Since sending this article to the press, the degree of resistance is reported to have increased to 2 lakhs.—(*Editor*).

MATERIAL AND METHODS.

The albino mice and rats were obtained from the colony maintained at the Malaria Institute of India. No choice was exercised regarding age or sex of the animals used. The inoculations were always given intraperitoneally and the dose of infection was one million parasites per animal.

Injectable sulphadiazine solution (May and Baker's) containing one gramme in 4 c.c. was diluted in distilled water and given orally, according to the technique described by Ramakrishnan *et al.* (*loc. cit.*). Administration of drug was always commenced from the day when the parasitæmia reached a level of two per cent cell infection.

RESULTS.

Development of sulpha resistant strain.—The procedure adopted for inducing resistance was similar to that of Fulton and Yorke (1941), Jaswant Singh, Ray *et al.* (1952) and Jaswant Singh, Ramakrishnan *et al.* (1952). The parasites were passaged serially through mice, which were given gradually increasing doses of sulphadiazine, commencing with a dose of 15 mg. ; the dosage for class II effect has been observed to be 6·0 mg. (Ramakrishnan *et al.*, *loc. cit.*). The dose of the

TABLE II.

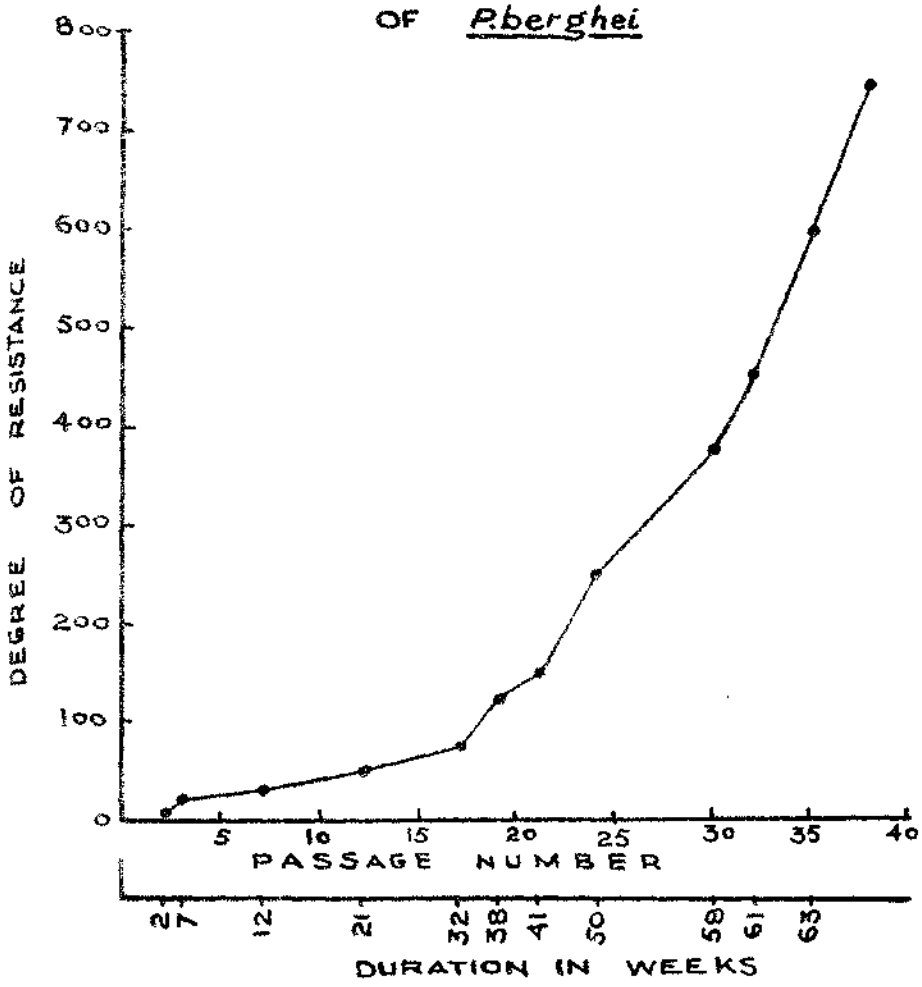
Sulphadiazine resistance of P. berghei: *protocols.*

Serial passage number.	Date of passage.	Details of drug administration.		
		Dose each course mg./20 gm.	Number of courses.	Degree of resistance.
1	June 20, 1951	0·3	1	...
		0·6	6	5
		1·2	2	10
2	September 5, 1951	2·4	2	20
3	September 22, 1951	2·4	1	...
4	October 1, 1951	2·4	2	...
5	October 11, 1951	3·6	1	30
6	October 22, 1951	3·6	2	...
7	November 5, 1951	3·6	2	...
8	November 19, 1951	3·6	3	...
9	December 14, 1951	3·6	1	32
		4·5	1	...
10	December 24, 1951	6·0	1	50
11	January 8, 1952	6·0	3	...
12	February 6, 1952	6·0	1	...
13	February 21, 1952	6·0	3	...
14	March 14, 1952	6·0	3	...
		9·0	1	75
15	April 18, 1952	9·0	1	...
16	April 25, 1952	*Nil*	...	...
17	April 28, 1952	9·0	1	...
18	May 13, 1952	9·0	1	...
19	June 2, 1952	15·0	2	125
20	June 23, 1952	15·0	2	...
21	July 4, 1952	18·0	1	150
22	July 16, 1952	18·0	2	...
		30·0	1	250
23	August 23, 1952	30·0	3	...
24	September 18, 1952	*Nil*	...	...
25	September 20, 1952	30·0	1	...
		45·0	1	375
26	October 4, 1952	*Nil*	...	...
27	October 11, 1952	45.0	1	...
28	October 16, 1952	*Nil*	...	...
29	October 22, 1952	45	1	...
30	November 13, 1952	45	1	...
31	November 29, 1952	*Nil*	...	...
32	December 4, 1952	54	1	450
43	December 11, 1952	*Nil*	...	...
44	December 13, 1952	72	1	...
45	December 19, 1952	72	1	600
36	December 27, 1952	90	2	750
37	January 17, 1953	90	1	...
38	January 24, 1953	90	...	...

drug was so adjusted as not to totally clear the peripheral blood of parasites, and sub-inoculating the strain before such a clearance occurred. Table II and Chart 1 show the serial passages and the progressively increasing dosage schedules adopted during the development of resistance.

CHART 1

PROGRESS OF SULPHADIAZINE RESISTANCE OF *Rberghei*



Estimation of the degree of resistance.—To confirm the development of resistance, and to assess its intensity, comparisons were made on mice inoculated with the parent strain with those inoculated with the resistant strain. The dose of inoculation remained identical. The total quantity of sulphadiazine given was 6·0 mg. per animal to those inoculated with the parent strain and 4,500 mg. per animal for the resistant strain. The course of parasitæmia showed that the

parent strain was temporarily suppressed by the former dose while a similar effect on the resistant strain was observed with 750 times as large a dose.

TABLE III.

Progress of resistance.

Passage number.	Time taken (weeks).	Degree of resistance.
2	2	10
3	7	20
7	12	30
12	21	50
17	32	75
19	38	125
21	41	150
24	50	250
30	58	375
32	61	450
35	63	600
38	68	750

Stability of acquired resistance.—When the degree of resistance was only 75 fold, observations were commenced to study the stability of the acquired resistance, the resistant strain being maintained by blood passage through albino mice without exhibiting the drug. The resistance was tested periodically at intervals of four to six weeks. No change in the resistance was noticed over a period of nine months during which the strain was subjected to 53 serial passages.

Batches of albino mice were subinoculated from rats infected with the resistant strain during the acute phase as also during early latency of infection. The acquired resistance was found to be retained in spite of passage through a different host.

Response to pyrimethamine (daraprim).—Jaswant Singh, Ray *et al.* (1952) observed that a paludrine resistant strain of *P. knowlesi* showed cross resistance to daraprim, while Bishop and McConnachie (1950*b* : 1950*c*) recorded a cross resistance between paludrine and sulphadiazine in *P. gallinaceum*. In view of the structural similarity between proguanil and daraprim (Falco *et al.*, 1951), it was of interest to see if there were any cross resistance to daraprim, a drug to which the parent strain was reported to be highly sensitive (Jaswant Singh, Krishnaswami *et al.*, 1952). Tests carried out at two stages during the development of the resistance, showed that the strain had not lost its sensitivity to daraprim (Table V).

Virulence of the parasites.—The course of infection and pathogenicity of the drug resistant strain were compared with those of the parent strain in batches of albino rats and albino mice inoculated with the two strains (Table IV). Both the strains behaved similarly, running an acute course ending fatally in mice within 6-12 days, while the rats recovered from the acute phase and became chronic. There was no evidence of any change in virulence.

TABLE IV.

Course of parasitæmia of the two strains of P. berghei *in mice and rats.*

Animal number.	Strain of *P. berghei*.	Prepatent period.	Parasite count/10,000 R.B.C. on days following first day of parasitæmia.										Remarks.
			1	2	3	4	5	6	7	8	9	10	
M. 791	R.S.	2	8	720	980	1640	948	1020	1640	1208	...	...	Died 9th day.
M. 792	R.S.	2	68	440	768	1240	680	940	980	1680	1240	2000	Died 12th day.
M. 793	R.S.	2	1	28	92	254	116	880	820	1640	1080	840	Died 11th day.
M. 794	R.S.	5	16	80	320	980	860	...	...	...	...	...	Died 6th day.
M. 1015	P	1	2	16	248	208	138	276	328	680	880	2250	Died 15th day.
M. 1016	P	1	1	8	32	42	220	426	686	880	620	1400	Died 12th day.
M. 1017	P	3	6	18	328	314	560	720	980	690	2640	2400	Died 16th day.
R. 526	R.S.	2	8	24	46	115	256	290	122	168	52	1*	
R. 527	R.S.	1	15	22	56	180	256	140	215	112	18	26*	
R. 487	R.S.	1	8	56	160	144	192	260	210	312	304	256*	
R. 489	P	1	18	96	168	180	196	320	440	520	348	660*	
R. 490	P	1	82	104	112	92	156	234	312	496	505	732	Died 14th day.
R. 488	R.S.	Refractory.											

M=Mouse. R=Rat. P=Parent strain. R.S.=Sulpha resistant strain.
*Became latent after varying periods.

TABLE V.

Course of infection of the two strains of P. berghei *in mice receiving daraprim.*

Mouse number	Strain of *P. berghei*.	Total parasite count per 10,000 R.B.C. on days following infection.											
		1	2	3	4	5	6	7	8	9	10	11	12
458	R	2	80	140	212	320*	112*	8*	0	0	0	10	144
459	R	6	168	180	220	364*	80	2	0	0	6	80	356
951	R	8	12	48	176	280*	260*	198*	6	0	0	0	Pos.
948	P	9	112	280*	360*	20*	0	0	1	68	228	360	660

R=Sulphadiazine resistant strain. P=Parent strain.
*Daraprim 0·0001 mg./20 gm. in 6 doses, given b.d. on these days.

DISCUSSION.

A study of available literature on acquired resistance of plasmodia to antimalarials (Table I) shows that such resistance against sulphadiazine has been recorded by Bishop and McConnachie (1950*b*) in *P. gallinaceum* and by Rollo (1951) in *P. berghei*. Observations recorded in this paper report a 750-fold resistance to sulphadiazine acquired by *P. berghei*, the degree of resistance being reckoned with reference to the dose for class II effect on the parent strain. If, however, this was calculated on the dosages required for the other classes of therapeutic effect, the degree of resistance would be 75-fold for class III effect and 3,000-fold for class I effect (Table VI). In view of the fact that in general practice the physician aims at relief of symptoms often resulting from temporary clearance of parasitæmia, it would appear rational to compare the drug resistance of a parasite to the normal dosage for class II effect.

TABLE VI.

Degree of resistance compared with the different doses for the three classes of effect.

Class of effect on parasite.	Dose of sulphadiazine in mg./kg.		Degree of resistance.
	Parent strain.	Sulpha resistant strain.	
I	1·5	Not done	3000
II	6·0	4500	750
III	60	Not done	75

By repeated exposures to subcurative doses of sulphadiazine, the erythrocytic parasites of *P. berghei* which are normally highly susceptible to that drug, were able to develop a high degree of resistance to the drug. Throughout the observation extending over a period of 18 months, during which the parasites were passaged 39 times, evidence of progressively increasing tolerance to the drug was noticed. A study of the progress of the resistance with successive passages (Table III, Chart 1) shows that the rate of increase in resistance was comparatively slow in the early stages. During the first 24 passages in fifty weeks, the resistance increased 250 fold, while with the next 14 passages during 20 weeks, the degree of resistance rose to 750-fold. It would appear that the process of inducing insensitivity to sulphadiazine in *P. berghei* is slower during the early stages than when a certain degree of tolerance is established ; further increase in the resistance is acquired rapidly.

Acquired drug resistance of plasmodia has been found to be of a stable nature. The sulphadiazine resistant strain of *P. berghei* taken up when the resistance was 75-fold and maintained in mice by serial passage without exhibiting sulphadiazine, showed no decrease in the resistance after 53 passages over a period of nine months.

Morphologically the drug resistant parasites of *P. berghei* appeared identical with those of the parent strain with a marked preference to immature red cells

(Ramakrishnan and Satya Prakash, 1950*b*). The course of parasitæmia both in albino mice and rats (Table IV) also resembled that of the parent strain (Ramakrishnan and Satya Prakash, 1950*a*), the former succumbing to the acute infection, while the latter mostly survived and became latent after varying periods of acute infection.

Change of vertebrate host does not appear to interfere with the acquired resistance. When the sulpha resistant strain was passaged through albino rats, the same degree of tolerance was in evidence, as also in mice subinoculated from such rats at different stages of infection during the acute stage as also latency.

The sensitivity of the sulpha resistant strain of *P. berghei* to daraprim was not dissimilar to that of the parent strain. Observations on cross resistance of proguanil resistant strains of plasmodia to daraprim have also been conflicting. While Rollo (1951) recorded only a slight degree of such cross resistance in a proguanil resistant strain of avian plasmodium, Jaswant Singh, Ray *et al.* (1952) observed a high degree of refractoriness to daraprim in a proguanil resistant strain of simian plasmodium. The concommitant development of resistance to more than one antimalarial thus appears to depend not only on the structural similarity of the drugs and sensitivity of the organisms to the drugs, but also to vary with the individual plasmodium.

SUMMARY.

(1) A 750-fold resistant strain of *P. berghei* to sulphadiazine was developed in the laboratory.

(2) This strain was identical with the parent strain in its morphology and pathogenicity to mice and rats.

(3) The acquired resistance was unaltered during 53 passages without any treatment over a period of nine months.

(4) The sulpha resistant strain was as sensitive to daraprim as the parent strain of *P. berghei.*

REFERENCES.

ADAMS, A. R. D. and SEATON, D. R. (1949) ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **42**, p. 314.
BISHOP, A. and BIRKETT, B. (1947) ... *Nature*, **159**, p. 884.
Idem (1948) ... ... *Parasit.*, **39**, p. 125.
BISHOP, A. and MCCONNACHIE, E. W. (1948) *Nature*, **162**, p. 541.
Idem (1950*a*) ... ... *Parasit.*, **40**, p. 159.
Idem (1950*b*) ... ... *Ibid.*, **40**, p. 163.
Idem (1950*c*) ... ... *Ibid.*, **40**, p. 175.
Idem (1953) ... ... *Ibid.*, **42**, p. 277.
CHAUDHURI, R. N. and RAI CHAUDHURI, M. N. (1949) ... ... *Ind. J. Mal.*, **3**, p. 365.
COOPER, W. C., COATNEY, G. R. and IMBODEN, (JR.), C. H. (1950) ... ... *J. Nat. Mal. Soc.*, **9**, p. 59.
EDESON, J. F. B. and FIELD, J. W. (1950) ... *Brit. Med. J.*, **1**, p. 147.
FALCO, E. A., GOODWIN, L. G., HITCHINGS, G. H., ROLLO, I. M. and RUSSEL, P. B. (1951) ... ... *Brit. J. Pharm.*, **6**, p. 185.
FINDLAY, G. H. (1950) ... ... *Recent advances in chemotherapy*. J. & A. Churchill Ltd., London.
FULTON, J. D. and YORKE, W. (1941) ... *Ann. Trop. Med. Parasit.*, **35**, p. 233.
GILROY, A. (1952) ... ... *Ibid.*, **46**, p. 121.

Greenberg, J. (1949)	*J. Nat. Mal. Soc.*, **8**, p. 80.
Gunther, C. E. M., Fraser, N. M. and Wright, W. G. (1950)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 185.
Hawking, F. and Perry, W. L. M. (1948)	*Lancet*, **2**, p. 850.
Hawking, F. and Thurston, J. P. (1951) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **44**, p. 695.
Hernandez, T., Myatt, A. V., Coatney, G. R. and Jeffery, G. M. (1953) ...	*Amer. J. Trop. Med. Hyg.*, **2**, p. 797.
Jaswant Singh, Krishnaswami, A. K., Satya Prakash, Ray, A. P. and Ramakrishnan, S. P. (1952)	*Ind. J. Mal.*, **6**, p. 183.
Jaswant Singh, Nair, C. P., Ray, A. P. and Misra, B. G. (1953)	*Ibid.*, **7**, p. 1.
Jaswant Singh, Ramakrishnan, S. P., Krishnaswami, A. K., Satya Prakash, Mammen, M. L. and Ray, A. P. (1952)	*Ibid.*, **6**, p. 457.
Jaswant Singh, Ray, A. P., Basu, P. C. and Nair, C. P. (1952)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 639.
Knoppers, A. T. (1947)	*Nature*, **160**, p. 606.
Krishnaswami, A. K., Satya Prakash, Bami, H. L. and Ramakrishnan, S.P. (1953)	*Ind. J. Mal.*, **7**, p. 229.
Kritschewski, I. L., and Halperin, E. P. (1933)	*Trop. Dis. Bull.*, **30**, p. 860.
Lourie, E. M. and Seaton, D. R. (1949) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **42**, p. 315.
Mackerras, I. M. (1947)	*Trop. Dis. Bull.*, **44**, p. 10.
Macleod, R. C. (1951)	*Brit. Med. J.*, **1**, p. 282.
Nair, C. P. (1953)	*Personal communication.*
Nauck, E. G. (1934)	Quoted by Findlay, G. M. (1950) in *Recent Advances in Chemotherapy*, P. 443, J. & A. Churchill Ltd., London.
Ramakrishnan, S. P., Krishnaswami, A. K. and Satya Prakash (1951) ...	*Ind. J. Med.*, **5**, p. 455.
Ramakrishnan, S. P. and Satya Prakash (1950*a*)	*Ind. J. Mal.*, **4**, p. 361.
Idem (1950*b*)	*Ibid.*, **4**, p. 369.
Rollo, I. M. (1951)	*Nature*, **168**, p. 332.
Rollo, I. M., Williamson, J. and Lourie, E. M. (1948)	*Ann. Trop. Med. Parasit.*, **42**, p. 241.
Schmidt, L. H., Gentner, C. S. and Fradkin, R. (1949)	*J. Phar.*, **95**, p. 382.
Seaton, D. R. and Adams, A. R. D.(1949)	*Lancet*, **2**, p. 323.
Seaton, D. R. and Lourie, E. M. (1949) ...	*Lancet*, **1**, p. 394.
Thompson, P. E. (1948)	*J. Inf. Dis.*, **83**, p. 250.
Thurston, J. P. (1953)	*Parasit.*, **43**, p. 246.
Van Goor, W. T. and Lodens, J. G. (1950)	*Trop. Dis. Bull.*, **47**, p. 807.
Williamson, J., Bertram, D. S. and Lourie, E. M. (1947)	*Nature*, **159**, p. 885.
Williamson, J. and Lourie, E. M. (1947) ...	*Ann. Trop. Med. Parasit.*, **41**, p. 279.
Wilson, T., Munro, D. S. and Richard, D. R. (1952)	*Brit. Med. J.*, **1**, p. 564.

Indian Journal of Malariology, **8**, 1, *March* 1954.

DESCRIPTION OF THE EGG OF *ANOPHELES GIGAS GIGAS* GILES, 1901.

BY

M. G. NARAYANDAS, B.Sc. (Hons.).

(*Malaria Institute of India, Delhi.*)

(December 3, 1953.)

According to Christophers (1933), all forms of *Anopheles gigas* are normally found in high altitudes, usually 6,000 ft. or above. The type form occurs, so far as is known, only in the hills in South India, while two varieties—*A. gigas simlensis** James, 1911, and *A. gigas bailey* Edwards, 1929, have so far been recorded from the Western and the Eastern Himalayas, respectively. The other three varieties *A. gigas refutans* Alcock, 1913, *formosus* Ludlow, 1909, and *sumatrana* Swellengrebel, 1920, do not occur in India; the first has been recorded from Ceylon, while the latter two only from outside the Indian region.

The eggs of *A. gigas* and its varieties were not known till recently when the eggs of the variety *refutans* was described in detail from Ceylon (D'Abrera, 1944). A description of the egg of the type form of *A. gigas* is recorded here for the first time.

For the present study, several gravid females of *A. gigas*, type form, were collected from cattlesheds in Ootacamund and made to oviposit individually in the laboratory on moistened filter paper. Besides, viable eggs were also collected from a natural breeding place† during one of the routine collections.

The *form of the egg* is whale-back type, length varies from 615 to 646 microns (Average length 632 microns). Greatest breadth including the floats varies from 240 to 270 microns (average breadth 244·8 microns).

Dorsal surface.—Deck-single moderately broad; full, continuous around the whole margin of the upper surface. The greatest width of the deck about the middle is about 90 microns, about one third of the total width including the floats. Near the poles, the deck surface is much narrower (60 microns).‡ The deck surface

* Russell and Jacob (1942) have recorded a single specimen of *A. gigas simlensis* from the Nilgiris.

† A partially shaded seepage drain near a eudyptus grove. Eggs were found floating in the middle of a small pool.

‡ This measurement was taken in the region of the bosses.

is finely stippled except towards the poles where stippling is coarser and is paler in colour. The bosses at the poles of the eggs are very conspicuous, translucent and light coloured, resembling those in *A. tessellatus*, but are unlike those of other anophelines in which they are black. The number of these bosses vary from seven to nine at each pole. Each of these bosses under a high magnification appears to be made up of a number of smaller ones.

Ventral as well as lateral surfaces are ornamented with pale polygonal markings characteristic of the eggs of the sub-genus *Anopheles*.

Frill fairly broad, 30 microns in width, about one sixth of the depth of the egg. When seen from the side, the frill appears erect, markedly striated, continuous along the margin of the upper surface, passing below the bosses, in which region it becomes narrower and loses its striations.

Floats do not touch the dorsal surface ; length varying from 360 to 450 microns, about two thirds the length of the egg (average 403·2 microns). They are placed about equidistant from the two poles. They are elongated, fusiform, occupying about half the lateral aspect of the egg in the middle portion, the greatest width being about 90 microns. Float ridges fairly well defined, their number varying from 24 to 29 ; terminations of moderate size, more or less rounded, and not broader than the rest of the ridges.

The egg of the type form differs from that of its variety *A. gigas refutans* Alcock, 1913, recorded from Ceylon, only in minor details, which are tabulated below :—

	A. gigas gigas	*A. gigas refutans* (D' Abrera, 1944)
1. Length of egg ...	615 to 645 microns	640 to 680 microns
2. Breadth of egg (including floats)	240 to 270 microns (average for 25 eggs 244·8)	(average for 24 eggs 251·24 microns)
3. Breadth about the middle : Deck at the poles	90 microns 60 microns	96 microns 64 microns
4. Number of float ridges	from 24 to 29	from 25 to 30
5. Bosses at poles	from 7 to 9	9 bosses at each pole
6. Frill (Maximum width)	30 microns	16 microns

REFERENCES.

CHRISTOPHERS, S. R. (1933) ... ... *Fauna of British India including Ceylon and Burma.* Diptera. Vol. IV. Family *culicidae*, tribe *anophelini*. Taylor and Francis, London.

D'ABRERA, V. ST. B. (1944) ... ... *J. Mal. Inst. Ind.*, **5**, p. 344.

RUSSELL, P. F. and JACOB, V. P. (1942) ... *Ibid.*, **4**, p. 355.

Indian Journal of Malariology, **8**, 1, *March* 1954.

THE RELATIONSHIP BETWEEN THE PHYSICAL STATE OF CERTAIN CHLORINATED INSECTICIDES TO THEIR BIOLOGICAL EFFECTIVENESS WHEN APPLIED ON SOLID AND WATER SURFACES*.

BY

RAJINDAR PAL.

(*Malaria Institute of India, Delhi.*)

(March 10, 1954.)

INTRODUCTION.

The physical state of D.D.T. residual spray both on wall and water surfaces greatly affects its biological effectiveness (Pal, 1952 and Pal *et al.*, 1952). Besides D.D.T., other chlorinated insecticides such as D.D.D., B.H.C., methoxychlor, toxaphene, and chlordane are also being commonly used for public health purposes, and it was therefore considered desirable to study the relationship between the physical state of these insecticides and their biological effectiveness. The results are preliminary but are being submitted with the hope that they may be useful to workers in this field.

TECHNIQUE.

The techniques employed both for the adult and larval studies were the same as described by Pal (*loc. cit.*) and Pal *et al.* (*loc. cit.*).

RATE OF CRYSTALLIZATION AND CRYSTAL HABITS.

(*a*) *On solid surfaces.*—Five per cent concentration of D.D.T., B.H.C., methoxychlor, toxaphene and chlordane in deobase, xylene, and acetone solutions and in xylene emulsions were sprayed on glass panels at the rate of 100 mg. of insecticide per sq. ft. The surface deposits were examined at regular intervals to determine the rate of crystallization and type of crystal formation. Like D.D.T., D.D.D., and methoxychlor crystallized from droplets over a period of one to eight weeks after spraying ; but in the case of toxaphene and chlordane, no crystals were found. With B.H.C., crystallization was complete within one week after application (Table I).

* These investigations were carried out during the tenure of a post-doctorate research fellowship of the National Institutes of Health (1948-49) at the Communicable Diseases Centre, Public Health Service, Federal Security Agency, Savannah, Ga., U.S.A.

TABLE I.

Rate of crystallization and crystal habits of D.D.D., methoxychlor, B.H.C., toxaphene, chlordane, and D.D.T. crystals from five per cent solutions of these insecticides in various solvents applied at the rate of 100 *mg. of insecticide per sq. ft. on glass panels by means of a DeVilbiss atomizer.*

Insecticide	Solvent	Condition of insecticide with age of film (weeks)*			Type of crystal habits
		1	4	8	
D.D.D.	Deobase	B	C	C	Mostly spherulitic and masses of both needles and prisms.
	Xylene	A	A	D	
	Acetone	B	B	C	
	Emulsion	A	A	D	
Methoxychlor	Deobase	B	B	C	Mostly spherulitic and masses of both needles and prisms.
	Xylene	B	B	B	
	Acetone	A	A	B	
	Emulsion	B	B	B	
B.H.C.	Deobase	D	D	D	Small fragments of crystals.
	Xylene	D	D	D	
	Acetone	D	D	D	
	Emulsion	D	D	D	
Toxaphene	Deobase	A	A	A	No crystal formation, granular surface deposits.
	Xylene	A	A	A	
	Acetone	A	A	A	
	Emulsion	A	A	A	
Chlordane	Deobase	A	A	A	No crystal formation, granular surface deposits.
	Xylene	A	A	A	
	Acetone	A	A	A	
	Emulsion	A	A	A	
D.D.T.	Deobase	A	D	D	Dendritic needle-like spherulitic interspersed masses of both needles and prisms.
	Xylene	A	B	C	
	Acetone	A	A	D	
	Emulsion	D	D	D	

* The descriptive categories of film condition are 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.

In order to gain some idea of the typical morphology of crystals of D.D.T., B.H.C., methoxychlor, toxaphene and chlordane, the sprayed panels were examined with stereoscopic dissecting and compound microscopes under ordinary, polarised, and ultraviolet light, and photographs taken of typical formations. The size and crystal habits are as follows :—

D.D.T.	7–538 μ	Dendritic, needle-like spherulitic, interspersed masses of both needles and prisms (Plate I, Fig. 1).
Methoxychlor	3–586 μ	Mostly spherulitic and masses of both needles and prisms (Plate I, Fig. 2).
B.H.C.	3–139 μ	Small individual crystal fragments in aggregates (Plate I, Fig. 3).
D.D.D.	3–430 μ	Mostly spherulitic and masses of both needles and prisms (Plate I, Fig. 4).
Chlordane	...	No crystal formation, viscous film (Plate I, Fig. 5).
Toxaphene	...	No crystal formation, viscous film (Plate I, Fig. 6).

(*b*) *On liquid surfaces.*—Five per cent solutions of these insecticides in acetone were applied to the surface of water in test pans at a rate equivalent to one gallon per acre (0·4 lbs. of insecticide per acre). Water surfaces were periodically examined for crystals. These examinations were made both under direct and ultraviolet light. Photographs were taken of portions of the different surface films transferrred to glass slides (Plate II, Figs. 1-6).

As in the case of solid surfaces, toxaphene and chlordane did not crystallize and remained on the surface of water in the form of small lens aggregates ; whereas D.D.T., D.D.D., and B.H.C. crystalized soon after application. Methoxychlor crystallized three days after application, although a few lenses were observed even after two weeks. None of the insecticides tested showed any marked difference in crystal clusters when applied on water surfaces, although differences in cluster sizes were apparent.

THE TOXICITY OF RESIDUAL DEPOSITS OF THESE INSECTICIDES.

(*a*) *On solid surfaces.*—The toxicity of these insecticides was tested against houseflies. A series of glass panels was sprayed with a five per cent solution of each in deobase, xylene, acetone and in xylene emulsion at the rate of 100 mg. of the insecticide per sq. ft. The surface deposits were tested for their biological effectiveness four to six weeks after spray application, regardless of the phases of crystallization.

The 50 per cent knock-down time (Table II) indicated slow activity of B.H.C., toxaphene and chlordane deposits, whereas D.D.T., D.D.D., and methoxychlor deposits were comparatively rapid in knock-down effectiveness. The 24-hour mortality (per cent) of houseflies after 15-minutes' exposure to these deposits indicated that this exposure was not enough to obtain 100 per cent mortality (Table II).

TABLE II.

Average time (minutes) and the 24-hour mortality (per cent) required for 50 per cent knock-down of houseflies after exposure to four to six weeks old D.D.D., methoxychlor, B.H.C., toxaphene, chlordane, and D.D.T. deposits from various solvents. All formulations contained five per cent of the insecticides and were applied at the rate of 100 mg. of insecticide per square foot.

Insecticide	Solvent	50-per cent knock-down time (Minutes)	24-hour mortality from a 15-minute exposure (per cent)
D. D. D.	Deobase	120	0·0
	Xylene	45	10·0
	Acetone	60	0·6
	Emulsion	45	9·4
Methoxychlor	Deobase	30	37·2
	Xylene	15	27·0
	Acetone	15	20·4
	Emulsion	15	40·6
B. H. C.	Deobase	60	0
	Xylene	120+	1·4
	Acetone	120+	0
	Emulsion	120	1·1
Toxaphene	Deobase	120	2·0
	Xylene	60	23·7
	Acetone	120	4·1
	Emulsion	120	88·8
Chlordane	Deobase	120	0·0
	Xylene	120+	1·7
	Acetone	120+	0·0
	Emulsion	120+	0·0
D. D. T.	Deobase	20	99·8
	Xylene	20	99·9
	Acetone	25	87·7
	Emulsion	20	99·9

PLATE I.

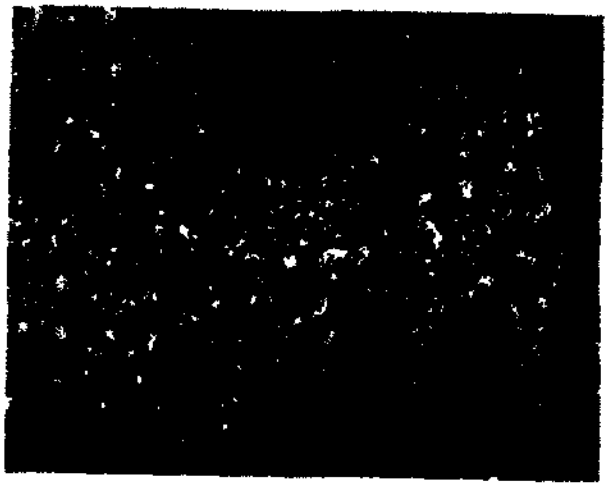


FIG. 1. Dynamic unreadable splendidus, irregular masses of both needles and prisms of DDT.

FIG. 2. Spherulitic crystals and masses of both needles and prisms of methoxychlor.

FIG. 3. Small dense crystal fragments and loose aggregates of DDT.

(Contd.)

PLATE 1. *Garcia.*

FIG. 4. Spherulite, crystals and masses of both needles and prisms of DDD.

FIG. 5. Globular non-crystalline viscous droplets of Bordane.

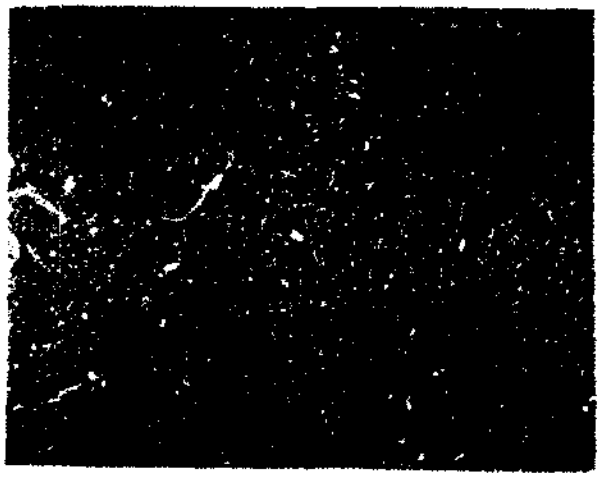


FIG. 6. Globular non-crystalline viscous droplets of toxaphene.

Plate II.

FIG. 1. Crystals of methoxychlor on water surface.

FIG. 2. Non-crystalline masses of toxaphene on water surface.

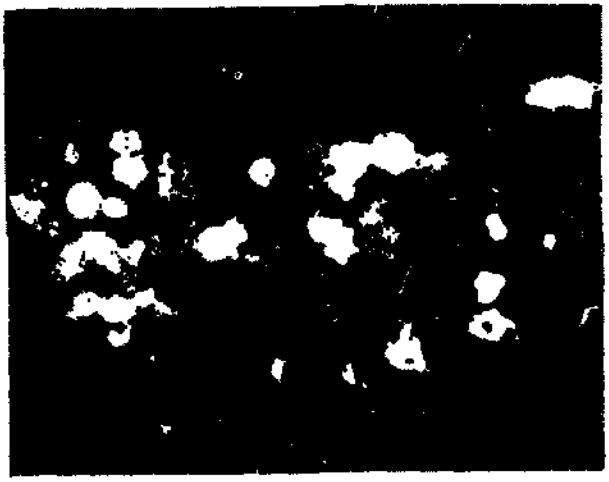


FIG. 3. Crystalline BHC on water surface.

Lamini,

[illegible]

[illegible]

Fig. 1. DDT crystals on water surface.

[illegible] water surface

Fig. 4. Crystals [illegible] water surface.

(*b*) *On liquid surfaces.*—Solutions of 0·6 per cent of these insecticides both in fuel oil and cyclohexanone* were applied at the rate of one gallon per acre (0·05 lb. of insecticides per acre) to the surface of water in test pans (Table III).

TABLE III.

Average 24-hour mortality (per cent) of fourth-instar A. quadrimaculatus *larvæ after 35-minute exposure to larvicidal films of 0·6 per cent solution of various insecticides both in fuel oil and cyclohexanone, applied at the rate of one gallon of solution per acre.*

	Insecticide	Age of treatment in days										
		0	1	4	5	6	7	8	11	13	14	15
Solution in fuel oil	B.H.C. ...	10	90	50	40	20	..	...	...	...	...	...
	Toxaphene ...	10	80	90	50	10	...	...	...	...	...	...
	Methoxychlor ...	20	100	100	100	20	...	...	...	...	...	...
	Chlordane ...	55	60	90	10	0	...	...	...	...	...	...
	D.D.D. ...	65	80	100	100	90	70	100	90	90	25	...
	D.D.T. ...	20	90	100	100	95	100	95	90	95	95	45
Solution in cyclohexanone	B.H.C. ...	60	...	50	...	0	...	...	...	...	...	...
	Toxaphene ...	100	...	30	...	0	...	...	...	...	...	...
	Methoxychlor ...	10	...	60	...	0	...	...	...	...	...	...
	Chlordane ...	100	...	80	...	0	...	...	...	...	...	...
	D.D.D.	100	...	100	...	80	80	...	80	0	...	...
	D.D.T. ...	100	100	100	100	100	100	100	100	Over 35 days		

In another series of tests, the insecticides were combined in various proportions for possible synergistic effects and tested against *A. quadrimaculatus* larvæ. It is concluded that :

1. B.H.C., methoxychlor, toxaphene and chlordane did not compare favourably with D.D.T., whereas D.D.D. did.
2. Combination of insecticides did not give any prolonged residual effect. There appears to be no synergistic effect when these insecticides are mixed together and applied as larvicides.† (Table IV).

A series of experiments was also set up to determine the effectiveness of these insecticides when applied as emulsions. For this purpose five per cent xylene-Triton X-100 emulsions of D.D.D., B.H.C., methoxychlor, toxaphene, chlordane,

*These two solvents were selected because D.D.T. solution in these solvents gave extremes of biological effectiveness (Pal *et al.*, *loc. cit.*).

†This is interesting as D.D.T. and B.H.C. combined sprays have been found to be comparatively more effective against adult mosquitoes when applied as a residual spray, particularly on mud surfaces (Pal, 1951 ; Jaswant Singh *et al.*, 1951 ; Dicke and Paul, 1951 ; Van Tiel, 1952 ; Sharma and Pal, 1952 and Davidson, 1953.)

and D.D.T. were applied at a rate equivalent to one gallon per acre (0·4 lb. of insecticide per acre). Of these insecticides, only D.D.D. and methoxychlor compared favourably with D.D.T. (Table V). It seems that methoxychlor is more effective as a larvicide when applied in the form of emulsion than as a solution in Number 2 fuel oil or cyclohexanone.

TABLE IV.

Average 24-hour mortality (per cent) of fourth-instar A. quadrimaculatus *larvae after 35-minute exposure to larvicidal films of* 0·6 *per cent solutions of various combinations of synthetic insecticides in fuel oil applied at the rate of one gallon of solution per acre.*

Insecticides	Age of treatment in days				
	0	1	5	6	7
D.D.T., B.H.C., toxaphene, methoxychlor, chlordane, and D.D.D., 0·1 per cent each	60	40	100	100	10
D.D.T. 0·4 per cent and chlordane 2 per cent	50	70	100	80	10
D.D.T. 0·3 per cent and D.D.D. 0·3 per cent	0	60	100	90	50
D.D.T. 0·3 per cent and B.H.C. 0·3 per cent	10	60	90	100	10

TABLE V.

Average 24-hour mortality (per cent) of fourth-instar A. quadrimaculatus *larvae after 35-minute exposure to larvicidal films of five per cent emulsions of various insecticides applied at the rate of one gallon of solution per acre.*

Insecticide	Age of treatment in days											
	0	1	2	3	7	8	9	10	14	15	16	17
D.D.D.	90	100	100	100	90	80	70	80	80	90	80	70
Methoxychlor	70	30	70	80	100	60	90	80	70	50	60	50
B.H.C.	10	40	50	100	50	30	10	30	20	0	0	0
Toxaphene	30	60	90	100	90	100	60	70	0	0	0	0
Chlordane	90	90	100	100	100	20	20	10	0	0	0	0
D.D.T.	90	100	100	99	100	90	100	100	100	80	100	100

SUMMARY.

The relationship between the physical state of D.D.D., B.H.C., methoxychlor, toxaphene and chlordane deposits on solid or water surfaces to their biological effectiveness, has been given preliminary study.

Deobase, xylene, and acetone solutions and xylene emulsions of these insecticides were sprayed on glass panels, and the crystal habits and rates of crystallization were determined. D.D.D. and methoxychlor crystallized gradually in one to eight weeks ; no crystalline structures occurred from toxaphene and chlordane deposits, while B.H.C. was completely crystallized within one week after application.

On water surfaces, five per cent solutions of these insecticides in acetone were applied at a rate equivalent to one gallon per acre (0·4 lb. of insecticide per acre). D.D.D. and B.H.C. crystallized soon after application ; methoxychlor after three days, while toxaphene and chlordane remained as small lenses.

Deposits of 100 mg. per sq. ft. of these insecticides in deobase, xylene, acetone solutions and in xylene emulsions were prepared on glass panels. Time required for 50 per cent knock-down of house flies was two hours or more with B.H.C., chlordane, and toxaphene deposits but comparatively rapid with D.D.T. and methoxychlor deposits. Exposure of adult flies for 15 minute periods to these deposits did not give 100 per cent 24-hour mortality in any case.

B.H.C., methoxychlor, toxaphene, and chlordane gave residual effectiveness inferior to D.D.T. when applied as 0·6 per cent solution in fuel oil and in cyclohexanone at the rate of one gallon per acre. Results with D.D.D. were comparable to those with D.D.T., especially in fuel oil films. Various combined applications did not show any synergistic action when applied as larvicides.

Of five per cent of B.H.C., toxaphene, methoxychlor, chlordane, D.D.D., and D.D.T. in xylene emulsions applied at a rate equivalent to one gallon per acre (0·4 lb. of insecticide per acre), only D.D.D. and methoxychlor compared favourably with D.D.T. against *A. quadrimaculatus* larvæ.

REFERENCES.

Davidson, G. (1955)	*Bull. Ent. Res.*, **44**, pp. 231-254.
Dicke, R. J. and Paul, J. J. (1951) ...	*J. Econ. Ent.*, **44**, pp. 896-898.
Jaswant Singh, Pal, Rajindar and Sharma, M. L. D. (1951)	*Ind. J. Mal.*, **5**, pp. 235-248.
Pal, Rajindar 1951	*Nature*, **167**, p. 368.
Idem (1952)	*Ind. J. Mal.*, **6**, pp. 239-250.
Pal, Rajindar, McCauley, R.H. and Fay, R. W. (1952)	*Ibid.*, **6**, pp. 225-238.
Sharma, M. L. D. and Pal, Rajindar (1952)	*Ibid.*, **6**, pp. 317-324.
Van Tiel, N. (1952)	*Bull. Ent. Res.*, **44**, pp. 231-254.

Indian Journal of Malariology, **8**, 1, *March* 1954.

THE RELATIONSHIP BETWEEN THE LIPOID SOLUBILITY OF D.D.T. AND ITS CONTACT TOXICITY TO HOUSEFLIES AND MOSQUITOES.

BY

RAJINDAR PAL,

M. L. D. SHARMA

AND

B. S. KRISHNAMURTHY.

(*Malaria Institute of India, Delhi.*)

(March 10, 1954.)

INTRODUCTION.

Although, as a rule, chlorinated hydrocarbon insecticides are poisonous to all multicellular organisms, their apparent specificity to insects is largely due to their ability to penetrate the cuticle. In fact, the penetrability of a chemical is of more importance than its intrinsic toxicity as a contact insecticide. The first step in the penetration of a contact insecticide is naturally its dissolution in the epicuticular waxes. Pal (1950) studied the wetting of insect cuticle by insecticidal liquids and found that apart from the chemical and physical nature of the epicuticular waxes, irregularities on the body surface of the insect were also important. The case of residual insecticides is, however, completely different. The insecticide is not present in the form of a liquid, but is available as crystals. Under these circumstances the rate and degree of dissolution of crystals in the epicuticular waxes is a factor of primary importance in the toxicity of these insecticides. As has been pointed out by Pradhan *et al.* (1952), there is no experimental evidence of this phenomenon, and the relative solubility of insecticidal chemicals in these epicuticular waxes has not so far been studied. They investigated the solubility of D.D.T. crystals in epicuticular waxes extracted from the larval exuviæ of *Euproctis lunata wlk* (Lymantridae, Lepidoptera) and *Trogoderma granaria Everts*

Dermestidae, Coleoptera) and found a correlation between it and the susceptibility of the insect to D.D.T. residual deposits. *T. granaria* as compared to *Euproctis lunata* was found to be less susceptible to D.D.T. and the solubility of D.D.T. crystals in its epicuticular wax too was poor.

In the control of insects of public health importance, it has been commonly observed that D.D.T. residual deposits are generally more toxic to houseflies and anopheline mosquitoes than to culicine mosquitoes.* This difference may be due to the area of contact which these insects offer to these deposits, or to the comparative solubility of D.D.T. crystals in their epicuticular waxes or that some insects are physiologically more vulnerable than others.

The present studies were undertaken to determine whether the apparent differences in the toxicity of D.D.T. to the normal strains of *Musca nebulo*, *Anopheles stephensi* and *Culex fatigans* was in any way related to the difference in the rate of dissolution of D.D.T. crystals into the epicuticular waxes of these insects.

TECHNIQUE AND RESULTS.

Epicuticular waxes (lipoids) of these insects were obtained separately by immersing a large number of each of three insects under study in chloroform for 24 hours. The lipoid chloroform solution was then filtered in each case through a sintered glass funnel and the chloroform was allowed to evaporate at room temperature. One per cent solution of the lipoid was prepared by redissolving the residue in chloroform. One c.c. of this solution was then uniformly applied to the excavated portion of cavity glass slide (area about 2·27 sq. cm.) which was kept at room temperature for 48 hours to ensure complete evaporation of chloroform leaving a more or less uniform wax film. The cuticular lipoids of the three insects were seen to have distinct characteristic patterns of their own as is evident from their photo-micrographs (Plate III, Figs. 2-4).

Fine crystals of specially pulverised D.D.T. were dusted on to these wax treated surfaces and photo-micrographed at regular intervals of five minutes, 30 minutes, one, three, six and 24 hours without in any way disturbing them. It was observed that in the cuticular lipoids of *Musca nebulo* and *A. stephensi*, D.D.T. crystals started showing signs of dissolution by losing their shape and dwindling in size almost immediately, but in that of *Culex fatigans* they did not dissolve even after 24 hours (Plate VI, Figs. 1-6). In the case of *Musca nebulo*, complete disappearance of crystals was apparent after three to six hours (Plate IV, Figs. 1-6) while in *A. stephensi* most of the crystals disappeared in six hours (Plate V, Figs. 1-6). This is of considerable interest because this observation is in complete conformity with the biological resistance of *C. fatigans* to D.D.T. observed in nature.

SUMMARY.

D.D.T. deposits are generally more toxic to houseflies and anopheline mosquitoes than to culicine mosquitoes. Besides other factors, this may be due

*Standard laboratory bred normal strains of *Musca nebulo*, *A. stephensi* and *Culex fatigans* when exposed to D.D.T. deposits (50 mg./sq. ft.) on glass panels, required 36, 23 and 96 minutes for 75 per cent knockdown and 51, 30 and 144 minutes for 100 per cent knockdown, respectively.

PLATE III

FIG. 1.

DDT CRYSTALS.

FIG. 1. Specially ground DDT crystals on glass slide.

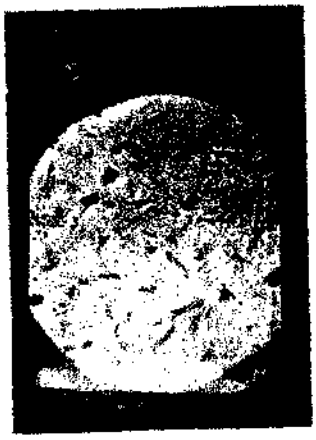


FIG. 2.
M. nebula.

FIG. 3.
A. stephensi.

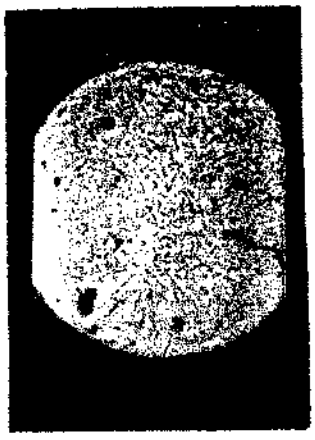


FIG. 4.
C. fatigans.

CUTICULAR LIPOIDS.

FIGS. 2, 3 and 4. Cuticular lipoids of *Musca nebula*, *Anopheles stephensi* and *Culex fatigans*, each showing a distinct pattern.

PLATE IV

FIG. 1
5 minutes

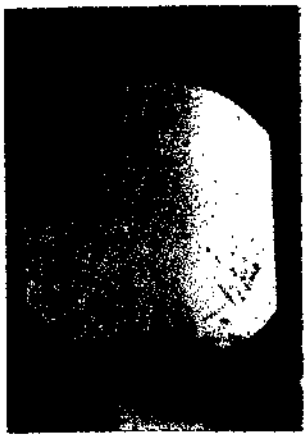


FIG. 2
half hour

FIG. 3
one hour

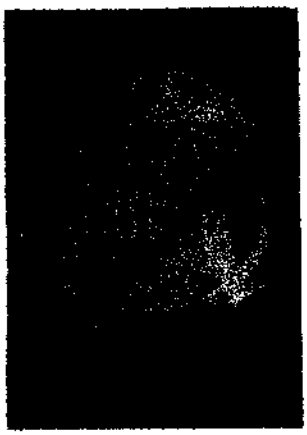


FIG. 4
3 hours

FIG. 5
6 hours

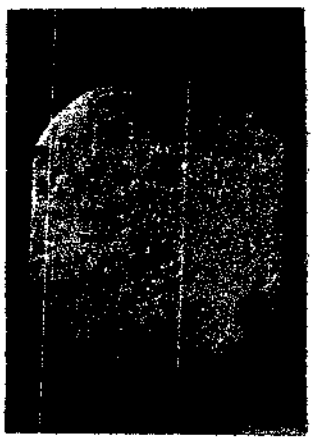


FIG. 6
24 hours

MUSCA VEBULO.

FIGS. 1 to 6. Solubility of DDT crystals in pericuticular lipoids of *M. nebula* 5 minutes to 24 hours after treatment).

PLATE V.

Fig. 1.
After 5 minutes

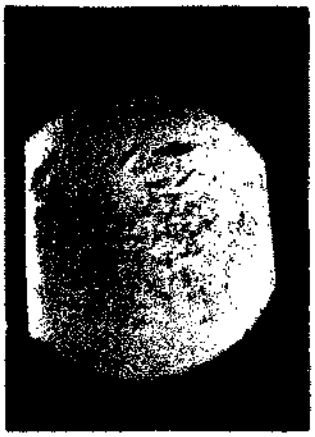


Fig. 2.
half hour

Fig. 3.
one hour

Fig. 4.
3 hours

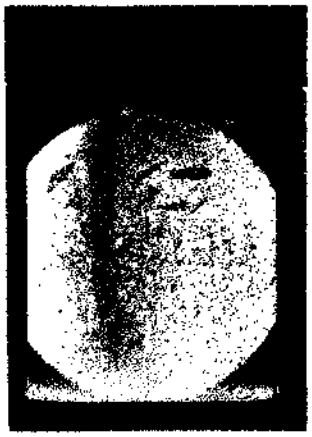


Fig. 5
6 hours

Fig. 6.
24 hours

ANOPHELES STEPHENSI

FIGS. 1 to 6. Solubility of DDT crystals in pericuticular lipoids of *A. stephensi* from 5 minutes to 24 hours after treatment.

PLATE VI.

Fig. 1.
1 minute

Fig. 2.
half hour

Fig. 3.
one hour

Fig. 4.
3 hours

Fig. 5.
6 hours

Fig. 6.
24 hours

CULEX FATIGANS.

Figs. 1 to 6. Selectivity of DDT crystals on the muscular tissues of Culex fatigans. 1 minute to 24 hours after treatment.

to the difference in the rate of solubility of D.D.T. crystals in the cuticular lipoids of various insects. Studies showed that D.D.T. crystals dissolved comparatively more rapidly in the epicuticular waxes of *Musca nebulo* and *A. stephensi* than in that of *C. fatigans* and there appears to be a direct correlation between the lipoid solubility and the insecticidal action.

REFERENCES.

PAL, RAJINDAR (1950) ... ... *Bull. Ent. Res.*, **41**, pp. 121-129.
PRADHAN, S., NAIR, M. R. G. K. and KRISHNASWAMI, S. (1952) ... ... *Nature*, **170**, p. 619.

Indian Journal of Malariology, **8**, 1, *March* 1954.

EFFECT OF ORGANIC MATTER IN THE CONTROL OF *CULEX FATIGANS* BY D.D.T. LARVICIDE.

BY

P. PARTHASARATHY,

(*Madras State Health Service*)

AND

C. W. KRUSE.

(*Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Md.*)

(April 8, 1954.)

INTRODUCTION.

In many parts of the world, filariasis still remains a major problem of public health. Many species of mosquitoes have been discovered to act as intermediate hosts to the filaria *Wuchereria bancrofti*. Of these, *Culex fatigans* appears to be the outstanding vector of filariasis. Although the knowledge of the importance of this mosquito in filariasis transmission has been known since Manson's demonstrations in 1878, not much information has been assembled on the bionomics of the Culex species as compared with the large volume of literature on the Anopheles malaria vectors.

The control of filariasis either by measures against the adult mosquito or against the parasite in man, alone has not given satisfactory results so far. Even residual spraying of dwellings with D.D.T. has not resulted in a dramatic reduction of filariasis. Reports from British Guiana (1950) show that *Culex fatigans* possesses a greater tolerance to D.D.T. treated surfaces than the other mosquito species of public health significance. It is also reported by Kohler (1949) that in St. Croix, Virgin Islands, the residual spraying reduced the Culex population by about 50 per cent only. He further showed that of the *C. quinquifasciatus* collected in the areas treated with D.D.T., the infection rate has been reduced by about 50 per cent. Oil larvicide applied to the typical breeding places of *Culex fatigans* has met with moderate success.

The ideal breeding situation is the sewage polluted puddles in and around urban areas where tremendous numbers of this species may be produced from a relatively small breeding area. The use of D.D.T. has helped considerably in reducing the cost of oil larviciding. When applied to clear water breeding places of *Culex fatigans* at a rate of 0·1 to 0·2 lb. D.D.T. per acre,* effective control has been obtained. Such favourable control has not been obtained on the filthy sewage polluted breeding places. Two or three times the normal dose has not resulted in consistent control of the larvæ of *Culex fatigans*. While this has been a general observation among field workers, no rational explanation of this inconsistency in results has so far been attempted. Since these ideal breeding areas of culicine mosquitoes have a high concentration of organic solids, these solids may have a bearing on the toxicity of D.D.T. as a mosquito larvicide. This has been suggested by Arnold, Ferguson, and Upholt (1945) who were studying the loss of residual effect of D.D.T. by the action of the bottom mud complex of ponds treated with heavy dosages of D.D.T. Upholt (1947) reported that the inactivation of D.D.T. used as residual larvicide for anopheline mosquitoes was due to the physical adsorption of D.D.T. on the oganic particles of mud and salt. He further suggested the use of competitive adsorbents to divert the organic matter from the D.D.T. A practical procedure for overcoming the difficulty of larviciding grossly polluted water by residual D.D.T. application has been described by Shearman (1950). The technique calls for the suspension of D.D.T. plaster of paris saw dust bricks or pellets six inches below the surface of open septic tanks. So suspended, Culex control was obtained for 24 days. No control was obtained when the bricks were allowed to settle to the bottom.

Most of these above-mentioned studies dealt with the heavy dose residual D.D.T. larviciding and the problem of rapid decay of toxicity. The most economical use of D.D.T. calls for much lower application rates applied routinely. It was the aim of this study to determine the effect of organic solids on the initial toxicity of D.D.T. to larvæ of *Culex fatigans* and to suggest modification in routine larviciding techniques for improving effectiveness of this operation. It is further hoped to show the effect of organic solids on the production of *Culex fatigans* and to point to the need of altering the character of ideal breeding places.

METHODS AND MATERIALS.

In view of the need for very closely controlled experiments, all tests were conducted in the laboratory with insectary reared *Culex fatigans*. The larvæ were reared in and fed on the standard canned pea soup solution. The soup, of the strained baby food type, was diluted to 1,000 p.p.m. total solids with distilled water. About one per cent by weight of yeast was added and the whole matured in a closed bottle at room temperature for two days. Adult *Culex fatigans* were fed weekly on live chickens and supplemented with fruit or dilute honey. No difficulty was experienced in maintaining a large, hearty mosquito population.

In all larvicidal tests, only early fourth or late third instar larvae were used. The tests were conducted in single service, water-proof, carbdoard containers.

*Refer.—P. F. Russell's Book on Malariology ; and Health Bulletin No. 11, p. 32 of the Malaria Institute, Delhi.

Three, of different sizes and shapes, were employed in order to obtain the relationship of water volume to surface area to D.D.T. dosage applied. The number of larvæ used in each replicate was varied from 10 to 160. It appeared that larval densities of at least one larva per square inch of water surface gave reasonably consistent results.

The experimental design is summarized as follows :

		Water volume c.c.	Water area sq. cm.	Volume surface	Number of larvæ replicate
Container	A ..	300	286	1·0	50
	B ..	420	78	5·5	25
	C ..	165	53	8·8	25

D.D.T. technical applied expressed as :
Pounds D.D.T. area of water surface
Parts D.D.T./million parts of water (p.p.m.)
Micrograms D.D.T./larva (μg./larva)

Larvicide used : Emulsion concentrate in kerosene containing 100 mg. D.D.T. technical and 100 mg. emulsifier B 1956 per ml. of solution. Final aqueous emulsion made by measuring out concentrate with Number 26 hypo syringe into 5 c.c. distilled water.

Control spray : Same as above but omitting the D.D.T.

Application : Measured quantities of spray were applied uniformly over the water surface with small DeVilbiss atomizer.

Mortality : Recorded at end of 24 hours. All mortality was corrected for controls by the following :

$$\text{Per cent mortality} = \frac{D - K}{100 - K}$$

where D = per cent kill due to D.D.T. larvicide.
K = per cent kill due to kerosene emulsion.

Angular values : Angles in degrees corresponding to percentages, obtained by the equation " angle = Arc Sin V percentage. The values were computed by CI Bliss tables—Refer Snedecor 16-6 and 16-7 for poison distribution.

PROCEDURE.

Before larvicidal tests could be inaugurated, preliminary investigation was required on the effect of organic solids on the growth and production of *Culex fatigans*. To obtain this necessary information, standard white enamel basins 7 inches × 12 inches were filled to a depth of one inch with concentrations of pea soup solutions from 8,000 p.p.m. down to plain tap water. Each basin was planted with 50 freshly hatched first instar larvæ of *Culex fatigans*. The larval growth was watched, keeping the liquid and solid concentration in the basins constant during the period by adding water to compensate for the evaporation loss. The larvae pupating each day were counted and weighed for each concentration of

pollution. Before weighing, the pupæ were dried in two successive dry filter papers for two minutes before transferring to a dry crucible for weighing on an analytical balance. The process of drying was quite uniform and was facilitated by the pupæ frisking about on the filter paper. This procedure did not kill the pupæ.

The results of these preliminary investigations are given in Table I and the average of all trials are shown in Chart I. Since these tests were all done under normal room temperature, the various trials were not strictly comparable with each other, but it is believed the concentrations in each trial were comparable between themselves. The 1,000 p.p.m. of total solids in the growth solution gave in four trials the maximum survival rate and also the maximum average weight per pupa. The survival rate is high for lower pollutions, but the weight of pupa decreases. If survival and weight of pupa are together taken as the index of growth, then 1,000 p.p.m. is the optimum, the growth decreasing with either increased or decreased pollution concentration. Above 2,000 p.p.m. total solids, there appears to be less growth and at 8,000 p.p.m. the growth is definitely inhibited.

TABLE I.

Effect of organic pollution on the growth of Culex fatigans *larvæ.*

Pollution total solids p.p.m.	Per cent pupation (P)					Average weight of pupa in mg.(W)					Growth factor W×P
	1	2	3	4	Average	1	2	3	4	Average	
8,000	0	2	8	0	2·7	...	2·8	3·6	...	3·3	8·9
6,000	40	...	2	0	4·5	...	...	3·4	...	3·4	15·2
4,000	30	69	12	2	43·9	...	3·5	4·3	3·3	3·5	153·7
3,000	...	...	16	16	16·0	...	...	4·2	3·5	3·9	62·4
2,000	10	83	14	54	61·5	...	3·3	3·8	4·1	3·4	209·1
1,500	...	...	36	62	49·0	...	...	4·5	3·9	4·1	200·9
1,000	100	98	44	82	84·6	...	3·6	4·5	4·9	3·7	313·0
750	...	...	42	40	41·0	...	...	4·1	3·7	3·9	159·9
500	70	88	34	38	67·3	...	3·2	3·9	3·4	3·3	222·1
250	90	...	60	48	54·5	...	...	3·1	3·1	3·1	169·0
0	...	...	...	...	...	...	...	...	...	...	...

From the growth-pollution information, it was decided to set all larvicidal test concentrations of pollution of 1,000, 2,000, 4,000, and 8,000 p.p.m. of total solids. Of the two types of containers employed to observe the area-volume relationship to larvicidal efficiency, the deeper cup was considered to be more comparable to the natural breeding places, having an area-volume relationship 1/5 that of the paper plates.

CHART 1.

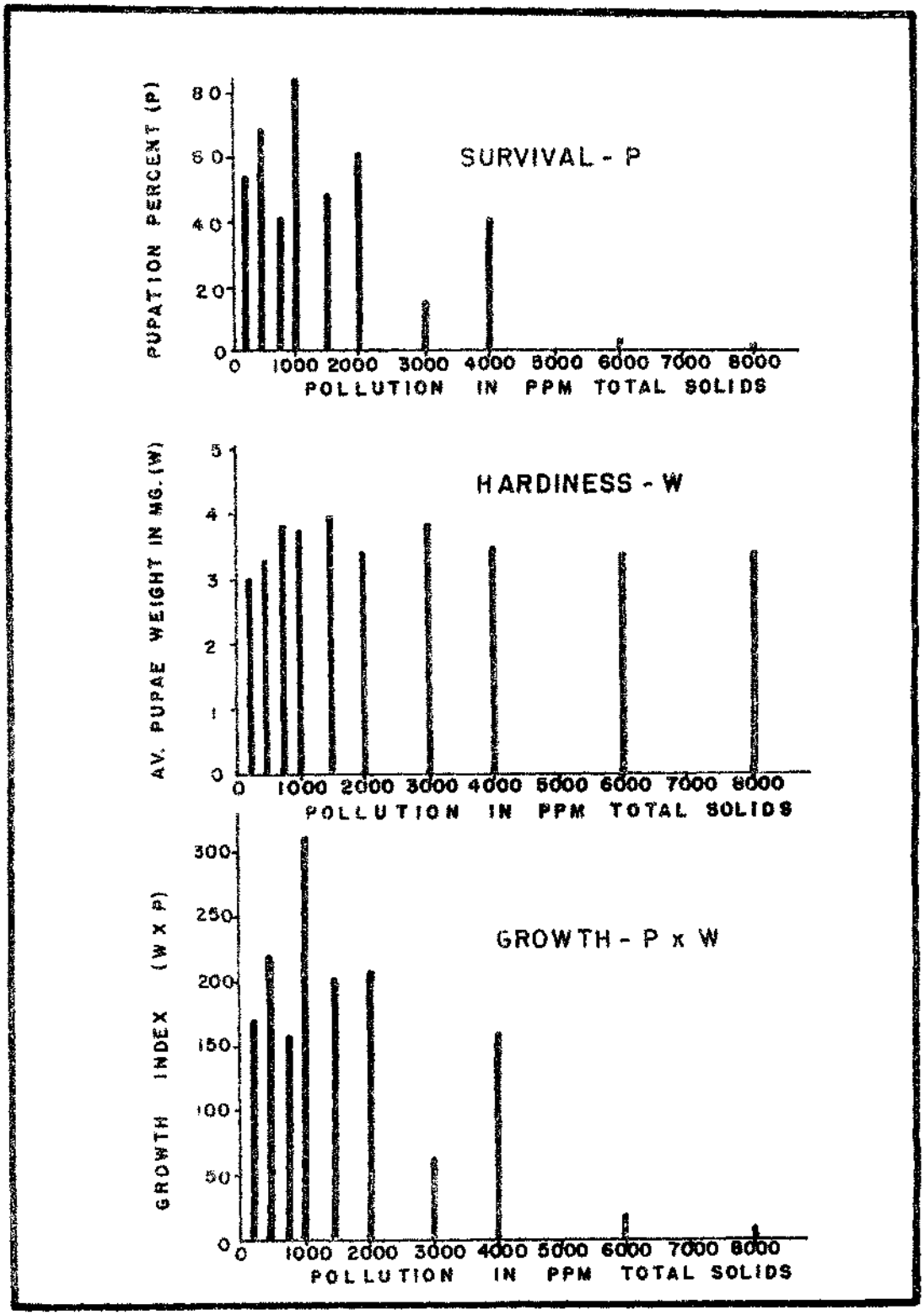

PUPATION PERCENT (P)
80
60
40
20
0
SURVIVAL - P
0 1000 2000 3000 4000 5000 6000 7000 8000
POLLUTION IN PPM TOTAL SOLIDS
AV. PUPAE WEIGHT IN MG. (W)
5
4
3
2
1
0
HARDINESS - W
0 1000 2000 3000 4000 5000 6000 7000 8000
POLLUTION IN PPM TOTAL SOLIDS
GROWTH INDEX (W X P)
300
250
200
150
100
50
0
GROWTH - P x W
0 1000 2000 3000 4000 5000 6000 7000 8000
POLLUTION IN PPM TOTAL SOLIDS

TABLE I (a)

Summary of data obtained on the mortality of early fourth- instar Culex fatigans *larvae in artificially polluted waters with various dosages of D.D.T. expressed as parts per million, micrograms per larvae and pounds per acre.*

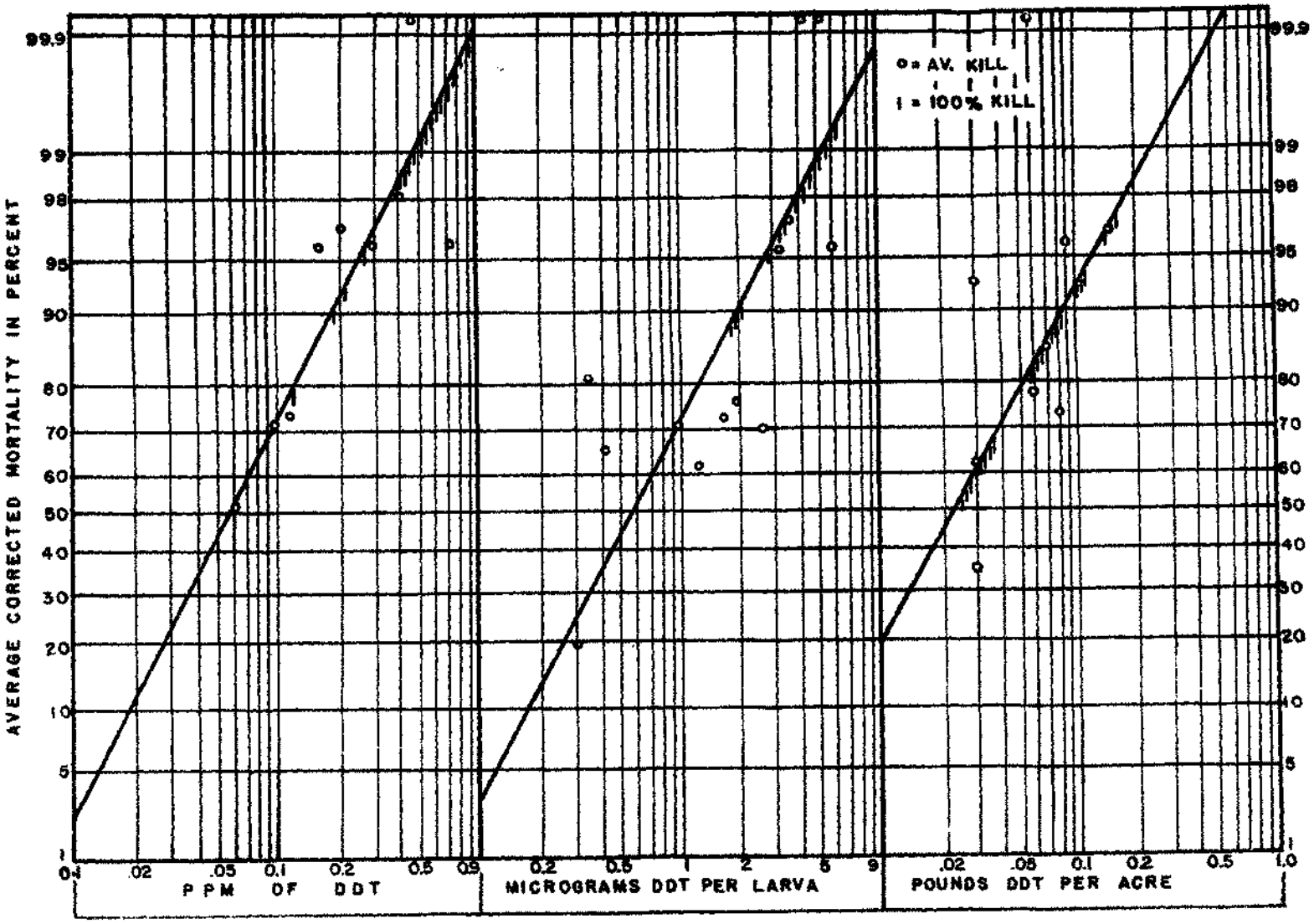
Type of container.	Number of larvæ.	D.D.T. DOSAGE EXPRESSED AS: Pounds per acre. i.	Parts per million. ii.	Microgram per larvæ. ii.	Percentage kill
A	25	·03	·06	1·0	37
A	25	·06	·12	2·0	78
A	25	·09	·18	3·0	96
A	50	·06	·12	1·0	82
B	25	·08	·10	1·8	74
B	50	·15	·20	3·7	97
C	50	·03	·29	1·5	96
C	50	·06	·58	3·5	100
C	50	·09	·87	5·2	96

A and B : Containers depth area not greater than 1.
C : Containers depth area not less than 1.

RESULTS OF LARVICIDAL TESTS.

Several preliminary runs were conducted on the pollution series with dosages of D.D.T. varying from 0·015 to 0·09 lbs. D.D.T./acre or 0·06 to 0·09 p.p.m. or 0·3 to 6·0 micrograms D.D.T. per larva. The results of these tests were averaged disregarding the concentration of total organic solids in the test solution in order that some idea of dosage mortality could be obtained for *Culex fatigans*. As is often the case with pan tests, these dosages gave much higher kill than would be anticipated in field application. The results of these preliminary investigations have been plotted on log probability paper (Chart 2) using the three different D.D.T. dosage parameters, pounds/acre, parts per million and micrograms per larva. Log scale has been adopted for both axes as it will give the same curve, as natural scale, excepting that in the former, the wide range is condensed to a small space. Only an ordinary straight line relationship has been tried to be fitted in, hence the same scale for both axes has been adopted with a view to show the graphs in the same sheet. From these curves, it may be concluded that the LD_{90} for *Culex fatigans* in the bioassay would be about 0·08 pounds D.D.T./acre or 0·2 p.p.m. or 2 micrograms D.D.T./larva. It should be noted that of the parameters of dosage employed, pounds D.D.T./acre seems to give the poorest fit while p.p.m. of D.D.T. gave the best fit and most consistent results. In theory, the micrograms/larva should give the best fit provided the number of larvæ used

CHART 2.



Summary of data obtained on the mortality of early fourth instar *Culex fatigans* larvæ in artificially polluted waters with various dosages of DDT expressed as parts per million, micrograms per larvæ and pounds per acre.

in each test is sufficiently large so as to occupy all of the volume yet not so great as to share available D.D.T. per unit volume among too many individuals.*

TABLE II.

The effect of organic solids on the mortality of Culex fatigans *to D.D.T. emulsion larvicide applied at* 0·06 *pounds D.D.T. acre equivalent to* 0·12 *p.p.m. D.D.T. or* 2·2 *µg. D.D.T. per larva. Ten replicates of* (8 *of* 25 *and* 2 *of* 50) *fourth-stage larvæ.*

Pollution : Total solids p.p.m.	Corrected mortality in per cent											Control mortality			
	1	2	3	4	5	6	7	8	9	10	Average	1	2	3	Average
8,000	56	72	80	43	24	96	69	50	79	72	65	0	16	8	8
4,000	84	84	92	91	43	96	86	45	68	75	76	0	8	12	7
2,000	48	96	96	80	69	96	96	83	100	89	84	0	0	8	3
1,000	80	92	100	96	88	96	100	71	83	89	90	0	4	4	3

The test of the effect of organic pollution on larvicidal efficiency is tabulated in Table II. The dosage of D.D.T. applied was chosen as one expected to give good kill in tap water. The dosage was applied to a series of 10 replicates of 25 larvæ for each organic pollution concentration tested. The values mean/variance were examined and found to be practically constant indicating that the values of the variance in percentages depend to some extent on the mean value. In such cases, where the mean and the variance are not independent, Poissons distribution has to be assumed. For tests of significance, it is desirable to change the variables into a new set of values wherein the mean and variance shall be independent. This transformation is achieved in many ways, of which conversion of percentages to angles is one—*Refer* Snedecor, p. 447. The percentages were therefore converted to angular values. Of the ten trials, two had a sample of 50 instead of the 25 in other tests, and hence were omitted for the analysis of variance. The variance is also compared by Fisher variance rates to control the other varying factors in the experiment, such as temperature, etc. The results of this statistical test are tabulated as follows :—

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total ...	31	7241	...
Pollutions ...	3	2135	712
Trials ...	7	4138	591
Discrepancy ...	21	968	46

*The desirable larvæ density may be determined experimentally as was later reported by Kruse *et al.* (1952).—Factors affecting evaluation of insecticides against Anopheles larvæ—*J. Econ. Ent.*, **45**, (4), p. 598.

Referring to appropriate tables, it may be found that the variation in kills between pollutions is largely significant.

The ten tests 3, 3 and 4 were done on three different days separated by a week or ten days and hence were not under similar atmospheric conditions, as they were all done at room temperature—the idea being to do the tests under normal field conditions as far as possible. The pollutions were also prepared afresh for each day, keeping the same maturing period in the constant room temperature and measuring the pollution by the total solids alone. Even though total solids gives a rough relationship to the other characteristics of the sewage as B.O.D. etc., it could not be said that the other characteristic of the pollution were exactly the same on the three days, after the pollutions were placed on the test pans. It was probably because the three groups were not exactly comparable, that the variation between trials is high. This was the reason why the trials were included as a variable and the analysis has been made for two variables. Normally if all the trials were done on the same day, the analysis should have been for one variable only viz., pollution.

DISCUSSION OF RESULTS.

There was practically no kill in the fourth- instar larvæ introduced in the controls for all tests. This appears to be contradictory to the information supplied by rearing experiments where pollution concentrations in excess of 1,000 p.p.m. exerted a significant inhibition to larvæ development. Moreover, the fourth- instar larvæ introduced to the higher pollutions not only survived, but pupated with as good a weight as those grown in the optimum medium. This would seem to indicate that advanced stages of larvæ floated into highly polluted pools may hatch out into as good an adult as any. Hence, higher organic solid concentrations (artificial pollution) cannot be relied upon for complete control but it may prevent heavy production from deposited ova. In lower pollution concentrations, the hardiness of the larvæ seems to decrease, although the survival rate is quite high. This may account for one of the factors for greater D.D.T. kill in the lower pollution concentration by the lowering of the resistance of the larvæ.

It is felt that inconsistencies in larval kills of *Culex fatigans* in polluted waters may be partly explained by the measuring of the dosage of D.D.T. applied. Much of the information on mosquito control with D.D.T. in the literature is based on Anopheles mosquitoes which are top feeding and generally clean water species. It has been repeatedly shown that they were susceptible to very low dosages of D.D.T. applied as surface droplets in oil solvents. Surface application of D.D.T. at the usually recommended dosage of 0·1 pound D.D.T. per acre may not be expected to give satisfactory kill of *Culex fatigans* in all situations. With emulsion larvicides it may be assumed that the D.D.T. particles diffuse through the body of the water and are in greater contact with the *Culex* larvæ than when deposited on the surface. The application of D.D.T. as p.p.m. of the volume of water in the breeding area bears little or no relationship to the area dosage but rather on the depth of the water in the pool. The bioassays show that D.D.T. dosage on the pound per acre basis is considerably unreliable. It may be seen that the area to volume ratio of the typical breeding area for *Culex fatigans* is so

small that D.D.T. on the area basis will give very little D.D.T. per unit of volume. A much better expression would be on the p.p.m. basis. It is felt that practical difficulties in expressing dosage in p.p.m. may be minimized by preparing suitable application charts. Assuming that a field application rate of 0·5 p.p.m. D.D.T. will be necessary for satisfactory control, a chart may be prepared similar to Table III. Thus, by adjusting the nozzle discharge and the per cent D.D.T. in the larvicide, reliable dosage may be maintained for areas of some known average depth. The manner of applying the larvicide would be the same as normally done with any D.D.T. larvicide, i.e., utilizing good atomization, wind drift dispersion of droplets, etc.

TABLE III.

Nozzle discharge m.l. min.

Average depth of pool (ft.)	Concentration of D.D.T. in emulsion larvicide (per cent).									
	1	2	3	4	5	6	7	8	9	10
0·2	570	...	...	...	...	...	...	...	...	...
0·4	1150	575	...	...	...	...	...	...	...	...
0·6	...	850	570	425	...	...	...	...	...	...
0·8	...	1150	765	575	460	...	...	...	...	...
1·0	...	...	950	715	570	475	...	...	...	...
1·5	...	...	...	1060	850	710	610	530	470	...
2·0	...	...	...	...	...	...	...	...	...	1150

Basis: Slow wedding march pace—20 ft. effective swath = 0·05 acre/min.
Common nozzles—500 to 1,000 m./min. at 40 p.s.i.
Treatment dosage—0·5 p.p.m. D.D.T.

One can only speculate as to the mechanism by which particular organic solids in the breeding medium lowers the efficiency of D.D.T. larvicide on *Culex fatigans*. If it is assumed that the D.D.T. in minute emulsion particles are uniformly distributed throughout the medium, any great increase in organic particles over the number of D.D.T. particles could impose a mechanical barrier between the insect and the insecticide. Also, as the total solids increase, the more abundant becomes the organic food in the breeding medium. This may tend to eliminate the insect's need for extensive foraging and consequently would reduce the chances of contact with D.D.T.

Again, there is the theory of physical adsorption of the D.D.T. on the organic particle. If pools are quiescent, the organic solids tend to settle and much of the D.D.T. would be swept to the bottom where it becomes insecticidally ineffective. It would seem that this thesis is supported by the success of the leeching or slow dissolving type of D.D.T. application in open sewage pools as opposed to the ineffectiveness of a single massive D.D.T. dose for residual larvicidal effect. These factors deserve further study.

SUMMARY AND CONCLUSIONS.

Laboratory studies were conducted to assess the relationship between the organic total solids in the breeding medium to the control of *Culex fatigans* with a D.D.T. emulsion larvicide.

The results of these studies seem to show that the total organic solids act in two ways in the mosquito control problem. First, in creating conditions for mosquito production and secondly, in physically interfering with the contact of the mosquito larvæ with the D.D.T. particle. The optimum pollution concentration for production of *Culex fatigans* was found to be 1,000 p.p.m. total solids. Above this value there was a definite inhibition to mosquito production. One cannot conclude, however, that increasing the solid concentration of sewage pools will result in a practical anti-mosquito measure. It has been shown that as the solid concentration increases, the efficiency of D.D.T. larvicide decreases. It appears best to concentrate effort on the removal of sewage solids from the waste water entering open drainage ditches. This will act in the direction of producing less hardy mosquitoes and conditions which result in efficient control with D.D.T. larvicide.

It becomes quite apparent that the customary practice of applying D.D.T. larvicide on a pound per acre basis is of no value in the control of *Culex fatigans* in sewage polluted pools. The experiments show that the dosage should be recommended on a p.p.m. or volume basis. This will require the field forces to prepare dosage charts for applying the proper amount of larvicide to pools of varying depth. From laboratory experience, it appears that a D.D.T. dosage of 0·2 p.p.m. gave satisfactory control of *Culex fatigans* in all ranges of organic solids up to 8,000 p.p.m. This figure probably will have to be raised to meet existing conditions in the field and probably will be as high as 0·5 p.p.m. for grossly polluted pools.

ACKNOWLEDGMENTS.

The authors are much indebted to Dr. Lloyd E. Rozeboom, of the Department of Parasitology, for his contribution of insectary facilities for laboratory experiments, and to Dr. Charles E. Renn, of the Johns Hopkins University, for his valuable suggestions and criticisms.

REFERENCES.

ARNOLD, EARL H., FERGUSON, FREDERICK F., and UPHOLT, WILLIAM M. (1945) ... The experimental use of D.D.T. sprays as mosquito larvicides. *Public Health Reports, Supplement No.* 186.

GILLETT, GEORGE. (1950) ... ... *Malaria, filariasis and yellow fever in British Guiana.* Publication by Mosquito Control Service, Medical Department, British Guiana.

KOHLER, CHARLES E. (1949) ... ... Filariasis control by D.D.T. residual house spraying in St. Croix, Virgin Islands. *Public Health Reports*, **64**, 27, p. 857.

SHARMAN, G. B. (1950) ... The control of culicine mosquito breeding in septic tanks by means of D.D.T. bricks. *J. Roy. Army Med. Corps.*, **94**, 5, p. 259.

UPHOLT, WILLIAM M. (1947) ... ... The inactivation of D.D.T. used as anopheline mosquito larvicides. *Public Health Reports*, **62**, 9, p. 302.

Indian Journal of Malariology, **8**, 1, *March* 1954.

A FIRST RECORD* OF *ANOPHELES THEOBALDI* GILES, 1901, FROM MYSORE STATE.

BY

S. R. BHOMBORE,

(Bureau of Malariology, Mysore State Health Department, Bangalore),

N. L. SITARAMAN

AND

C. ACHUTHAN.

(Malaria Investigation Centre, Saklaspur.)

(January 21, 1954.)

THE various lists about the geographical distribution of Indian anophelines by Covell and Puri (1936) refer to *A. theobaldi* Giles as a common species in the central and western portions of the Deccan Plateau, though also recorded from scattered localities in other parts of India. Mysore State has not so far been included in the known range of distribution of this species. The species recorded by the Malaria Investigation Centre, Saklaspur, Hassan District, Mysore State (Rao *et al.*, 1952) were the same as those recorded already in the various anopheline surveys conducted at the Malaria Study Station, Mudigere, Chickmagalur District, Mysore State, up to 1933, and this species was not recorded.

In the course of observations on the behaviour of anophelines in relation to structures treated with D.D.T. and B.H.C., opportunities arose to study intensively the anopheline fauna of a village (Kollahalli) near Saklaspur Town. During these studies, nineteen species of anophelines already recorded from Mysore State were taken. In the early hours of the night of February 16, 1953, the first specimen of *A. theobaldi* was collected from a cattleshed. Another specimen was taken a few days later in the same village.

*Published with the permission of the Director of Public Health, Mysore State, Bangalore.

This species closely resembles *A. maculatus* ; but is distinguished from it by the consistent absence of the dark band on segment four of the hind tarsus, last two segments of the hind leg being continuously white (Christophers, 1933). As an individual variation, such a condition may sometimes be found in *A. maculatus* also, though as a rule one leg at least shows the presence of the dark band. As pointed out already by Christophers (*loc. cit.*), there are some minor differences in the wings and the legs of the two species. In *A. theobaldi* the base of the costa is darker than in *A. maculatus* and dark areas on costa are more extended ; fore tarsi are only apically banded and mid tarsi are very narrowly banded. The larvæ of the two species appear to be indistinguishable from each other (Puri, 1949).

A number of larvæ indentified as *A. maculatus* were reared, but in all cases the adults emerging confirmed the larval identifications. Farther studies on the larval and pupal skins are in progress.

ACKNOWLEDGMENTS.

Grateful acknowledgements are due to Dr. M. L. Bhatia, Entomologist, Malaria Institute of India, Delhi, for confirming the specimen as that of *A. theobaldi* Giles, and communicating that this was the first record of this species from Mysore State.

REFERENCES.

Christophers, S. R. (1933) ... ... *The fauna of British India, including Ceylon and Burma. Diptera. Vol. IV. Family Culicide, Tribe Anophelini.* Taylor and Francis, London.

Covell, G. and Puri, I. M. (1936) ... *The distribution of anopheline mosquitoes in India*, by G. Covell. Second Edition. Health Bulletin No. 17. Malaria Bureau No. 8, Manager of Publications, Delhi.

Puri, I. M. (1949) ... ... *Synoptic tables for the identification of the full-grown larvæ of the Indian anopheline mosquitoes.* Sixth Edition. Health Bulletin No. 16. Malaria Bureau No. 7. Manager of Publications, Delhi.

Rao, B. A., Rama Rao, T. S., Sitaraman, N. L. and Brooke Worth, C. (1952) ... Anopheline mosquitoes recorded in the districts of Shimoga and Hassan, Mysore State. *Ind. J. Mal.*, **6**, 4, pp. 475-480.

Indian Journal of Malariology, **8**, 1, *March* 1954.

SEASONAL PREVALENCE OF ANOPHELINES IN WESTERN HILL TRACTS OF MYSORE STATE.*

BY

S. R. BHOMBORE,

(*Bureau of Malariology, Mysore State Department of Public Health, Bangalore*),

N. L. SITARAMAN,

(*Entomologist, Malaria Investigation Centre, Saklespur*)

AND

C. BROOKE WORTH,

(*Staff Member, Division of Medicine & Public Health, The Rockefeller Foundation.*)

[March 30, 1954.]

INTRODUCTION.

PUBLISHED observations about the seasonal prevalence of anophelines in western hill-tracts of Mysore State are meagre, and almost exclusively deal with the records of anopheline surveys conducted at Mudigere, Chickamagalur District, Mysore State (Sweet and Rao, 1933). With the establishment of a Malaria Investigation Centre at Saklespur, Hassan District, Mysore State, in 1950, in the heart of the Malnad, opportunities arose to collect a wealth of information pertaining not merely to malaria vectors but to the anopheline fauna as a whole. This knowledge was gained through routine and standardized methods of population sampling as practised by malariologists, namely, collection of larvæ from their breeding places and of adults from daytime resting places.

*The studies on which this paper is based were conducted by the Bureau of Malariology, Department of Public Health, Government of Mysore, with the support and under the auspices of the Division of Medicine and Public Health of The Rockefeller Foundation.

In this paper, the data accumulated over a period of two years has been analysed. The data are important for a number of reasons. In some instances, the abundance of a non-vectorial species of Anopheles may serve as a rough guide to the presence of an undetected vector, judging by the similarities in larval or adult habitats and behaviour as exhibited by some species of the *myzomyia* group. Some species are important vectors in localized regions, while others are vectors throughout their range of distribution. In the case of two species of approximately equal susceptibility, one exhibiting a marked preference for human blood, the other indifferent as to the source of the meal but occurring in larger numbers than the former, the latter species may become an important vector by virtue of sheer numbers. In other cases, a highly prevalent, although non-vectorial, species of Anopheles may serve as an indicator of the waning of residual toxicity of D.D.T. in sprayed structures. In both cases a knowledge of the seasonal prevalence of the so called "unimportant" anophelines is needed if one wishes to use the collateral evidence which their presence provides.

Though the anopheline fauna in the area was intensively studied from other points of view, the following notes on the seasonal prevalence, as displayed during two years' continuous observations, are presented as such knowledge relating to all mosquitoes is useful, no matter how unimportant some of the species may appear to be, where human disease is concerned.

AREA OF STUDIES.

The areas where these studies were conducted display remarkable variations in rainfall—and consequently in flora and fauna—on an unusually sharp gradient. Mysore State occupies the centre of South India. It lies between 11° 36′ and 15° 2′ north latitude and between 74° 36′ and 78° 36° east longitude. A large part of the State consists of the southernmost extension of the Deccan Plateau, an extremely ancient geological formation, 3,000 feet above the sea level. As one proceeds from west to east, the annual rainfall recorded from the Western Ghats decreases from 230 inches at the western edge at an average rate of ten inches per mile. Thus a region that is virtually drenched with rain, each monsoon may be no more than 25 miles away from another region where there is low rainfall.

For studying the epidemiology of Malnad malaria in typical situations where no residual spraying programmes were in force, three areas were selected in the different rainfall regions, namely, Kadmane, with annual rainfall more than 200 inches ; Yeslur, with 80 to 100 inches and Bikkodu, with 45 inches annual rainfall. There is no irrigation system in any of the three areas. The area of high rainfall consists of dense forests, steep rugged valleys and is sparsely populated. Cardamom is the chief crop, tea is grown in some parts, and in the narrow valleys paddy is also cultivated. The intermediate rainfall area consists of broader and less precipitous valleys, is densely populated, and the cultivation of land is correspondingly more intensive. Cardamom, coffee and pepper are the chief crops and paddy is cultivated in the broad valleys. The main source of water is the south-west monsoon (June-September) although the north-east monsoon (October-December) also provides some. The low rainfall area is almost a plain country,

supporting extensive areas of scrub jungle. This is a gently rolling area, with a low range of hills. Coffee and rice are the main crops.

ANOPHELINE SURVEYS.

Routine collections of larval and adult anophelines were carried out from April 1951 to March 1953 on a weekly basis in the three study areas. For collection of adults in each of the various villages, attempts were made to select catching stations comprising a human dwelling, a mixed dwelling* and a cattleshed.

When mixed dwellings were not available in some villages, only human dwellings and cattlesheds were selected for routine visits. Most dwellings in the three areas were thatched, mud-walled structures, with earthen floors.

Daytime collection of adult mosquitoes were made from the selected catching stations with the help of a suction tube, for thirty minutes, invariably between 09·30 and 12·00 hours. These were supplemented with night collections once a week in the three areas from February, 1952, to January, 1953. Window traps were set up only in the low rainfall area between July, 1952, and January, 1953.

As regards the site for routine larval collection in the three areas, particular importance was attached to running water, as the distribution and abundance of the alleged vector species, *A. fluviatilis*, was the main object in view. Tanks, paddy fields and domestic water collections—in fact all types of water collections were also examined, so as to obtain information about all other anopheline species. As far as possible, standardized technique and dippers of uniform design were used. Thirty minute collections were made at each selected site. Though the larval survey may be comprehensive as regards the different indigenous species, it may lack something of quantitative character and the precision of the adult collections.

TABLE I.

Rainfall record.

January 1951—March 1953.

Year and month.	Bikkodi			Yeslur.			Kadmane.		
	Rainfall (Inches)	Number of rainy days.	Average rainfall per rainy day (Inches).	Rainfall (Inches)	Number of rainy days.	Average rainfall per rainy day (Inches).	Rainfall (Inches)	Number of rainy days.	Average rainfall per rainy day (Inches).
1951									
January ...	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00
February	0·00	0	0·00	0·19	1	0·19	0·00	0	0·00
March ...	0·57	2	0·28	0·25	1	0·25	0·10	1	0·10
April ...	3·96	7	0·56	2·46	6	0·41	2·79	4	0·69
May ...	7·67	12	0·64	4·37	8	0·54	6·40	11	0·59
June ...	5·18	19	0·27	10·25	17	0·60	41·91	29	1·45
July ...	13·48	17	0·79	29·25	15	1·95	73·65	28	2·63
August ...	4·65	18	0·26	11·41	18	0·63	48·75	20	2·44
September	7·40	13	0·57	8·55	11	0·78	4·20	6	0·70
October ...	4·42	14	0·32	4·32	7	0·62	10·42	10	1·04
November	3·12	6	0·52	0·70	2	0·35	1·55	2	0·77
December	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00

*Dwellings in which people and domestic animals were sheltered at night under the same roof, and in which there was free access for mosquitoes to migrate from one section to the other.

TABLE I —(*Contd.*)

Year and month.	Bikkodu.			Yeslur.			Kadmane.		
	Rainfall (Inches)	Number of rainy days.	Average rainfall per rainy day (Inches)	Rainfall (Inches)	Number of rainy days	Average rainfall per rainy day (Inches)	Rainfall (Inches)	Number of rainy days.	Average rainfall per rainy day (Inches)
1952									
January ...	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00
February	0·33	2	0·16	0·85	2	0·42	0·50	1	0·50
March ...	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00
April ...	3·72	11	0·34	1·60	5	0·32	2·90	5	0·58
May ...	1·63	9	0·18	2·45	5	0·49	3·45	6	0·57
June ...	5·81	21	0·28	11·57	22	0·52	41·55	28	1·48
July ...	8·57	20	0·43	26·15	22	1·19	60·30	28	2·15
August ...	5·14	28	0·18	15·95	20	0·79	59·80	29	2·06
September	2·03	9	0·23	2·00	3	0·67	5·80	7	0·83
October ...	6·48	15	0·43	6·25	8	0·78	19·85	13	1·53
November	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00
December	0·00	0	0·00	0·20	1	0·20	1·35	2	0·67
1953									
January ...	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00
February	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00
March ...	0·00	0	0·00	0·00	0	0·00	0·00	0	0·00

The rainfall data for the period of study is tabulated in Table I, and the average rainfall for the previous ten years is given in Table II. Temperature and humidity data for the three study areas are not available, but meteorological data obtained from the observatory at Hassan and from laboratory records maintained at the Malaria Investigation Centre, Saklaspur (corresponding to the intermediate rainfall area), are summarised in Table III.* These may not give precise information but they show the general climatic trend.

TABLE II.

Average monthly rainfall in the three areas for 10 *years* 1941-1951.

Months.	Bikkodu (Inches).	Yeslur (Inches).	Kadmane (Inches).
January ...	0·50	0·50	0·50
February ...	0·50	0·50	0·60
March ...	0·60	0·50	0·60
April	2·00	2·00	1·60
May	4·00	3·50	5·00
June	6·00	11·00	38·00
July	14·25	31·50	93·25
August ...	8·00	18·50	57·00
September ...	4·40	6·00	20·00
October ...	6·00	6·50	7·00
November ...	2·30	3·40	3·00
December ...	1·40	1·00	1·00

* Beside the three Tables published with this paper, a number of others giving details of the mosquitoes collected from various types of resting places, at different times of the year, were also submitted by the authors. These have been placed in the library of the Malaria Institute of India, Delhi, and will be available on loan to workers who may wish to consult them.— Editor.

TABLE III.

Meteorological data.

Months	Hassan. Monthly means. Maximum °F	Minimum °F	Relative humidity per cent 08·00 hours	Rainfall Inches	M.I.C., Saklaspur. Monthly means. Maximum °F	Minimum °F	Relative humidity per cent 08·30 hours	Rainfall Inches
January ...	82·8	57·0	75	0·17	77·5	66·2	68	0·36
February ...	87·0	59·5	71	0·24	81·5	68·0	74	0·48
March ...	91·6	63·2	68	0·37	85·5	66·9	74	0·07
April ...	92·4	67·3	74	2·20	87·5	70·5	78	2·37
May ...	89·4	67·6	78	4·62	84·7	73·7	65	3·62
June ...	81·0	66·4	85	3·61	73·5	70·5	89	16·27
July ...	77·6	65·6	88	5·80	72·8	69·3	89	27·18
August ...	78·7	65·3	87	3·90	71·8	68·8	88	18·68
September ...	81·0	64·8	85	4·33	76·5	72·8	84	6·53
October ...	82·2	64·7	83	6·07	80·5	69·4	69	6·61
November ...	80·8	61·4	79	3·03	74·7	65·5	76	1·17
December ...	80·6	57·6	78	0·66	76·0	66·6	80	0·22
Annual ...	83·8	63·4	79	35·00	81·5	68·9	78	77·56

The data for Hassan are from records maintained at the Meteorological Observatory and are based on 45 years' observations. The data for Saklaspur are from records maintained at the Malaria Investigation Centre Laboratory and are based on two years' observations.

OBSERVATIONS.

Twenty-three anopheline species were encountered in the three areas during these studies. Charts 1 to 3 depict the prevalence of some of these species.

Though the capture of adult mosquitoes from man-made shelters is sharply limited to those species which rest in such structures, and does not provide any clue to the real density of the whole anopheline populations, it, nevertheless, provides an index of the amplitude of population fluctuations. The expression of the actual numbers collected as per man-hour figures seems to give a working idea of relative abundance, at least for those species that prefer dwellings as diurnal resting places.

Data on larval populations do not, as a rule, provide reliable indices to numbers of adult mosquitoes prevalent in an area. Sometimes the overall adult

CHART 1.

SEASONAL PREVALENCE OF ANOPHELINES

BIKKODU AREA

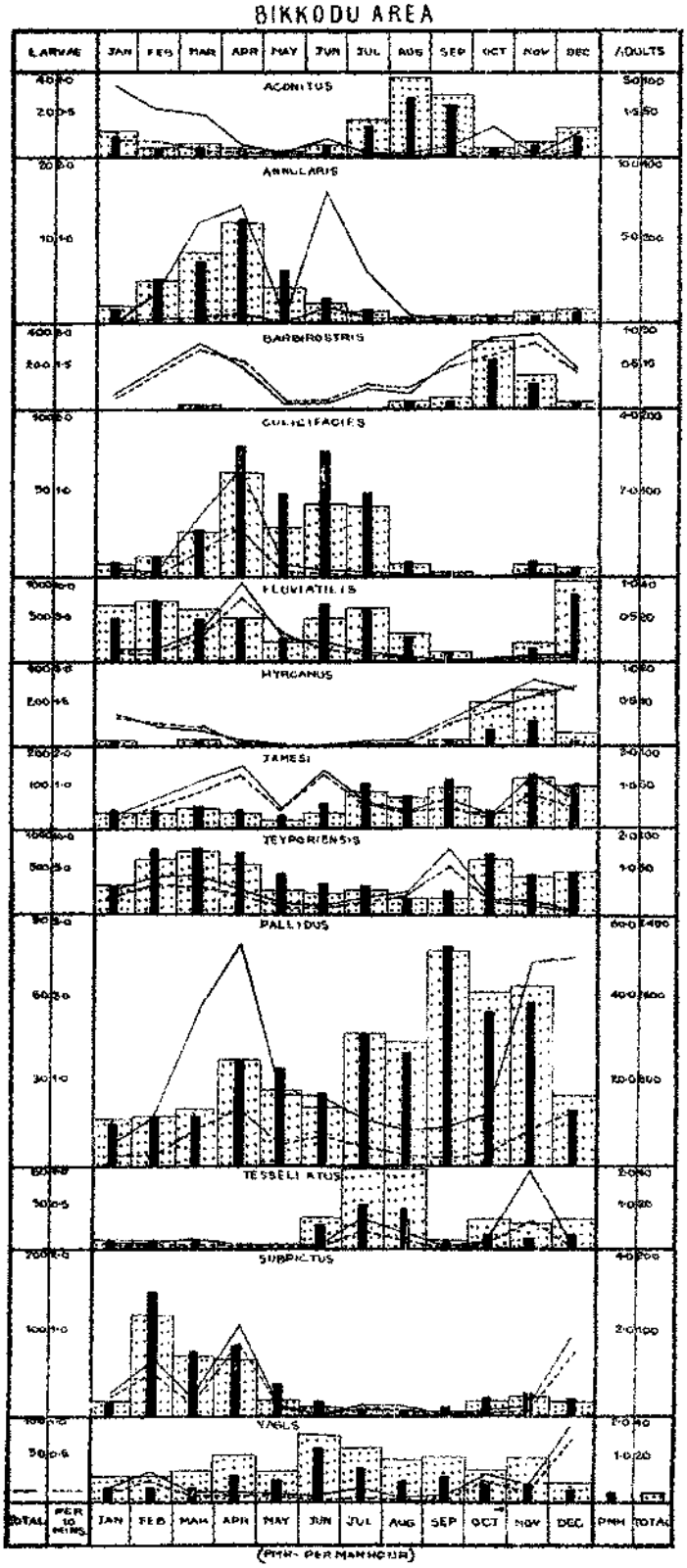


CHART 2.

SEASONAL PREVALENCE OF ANOPHELINES

YESLUR AREA

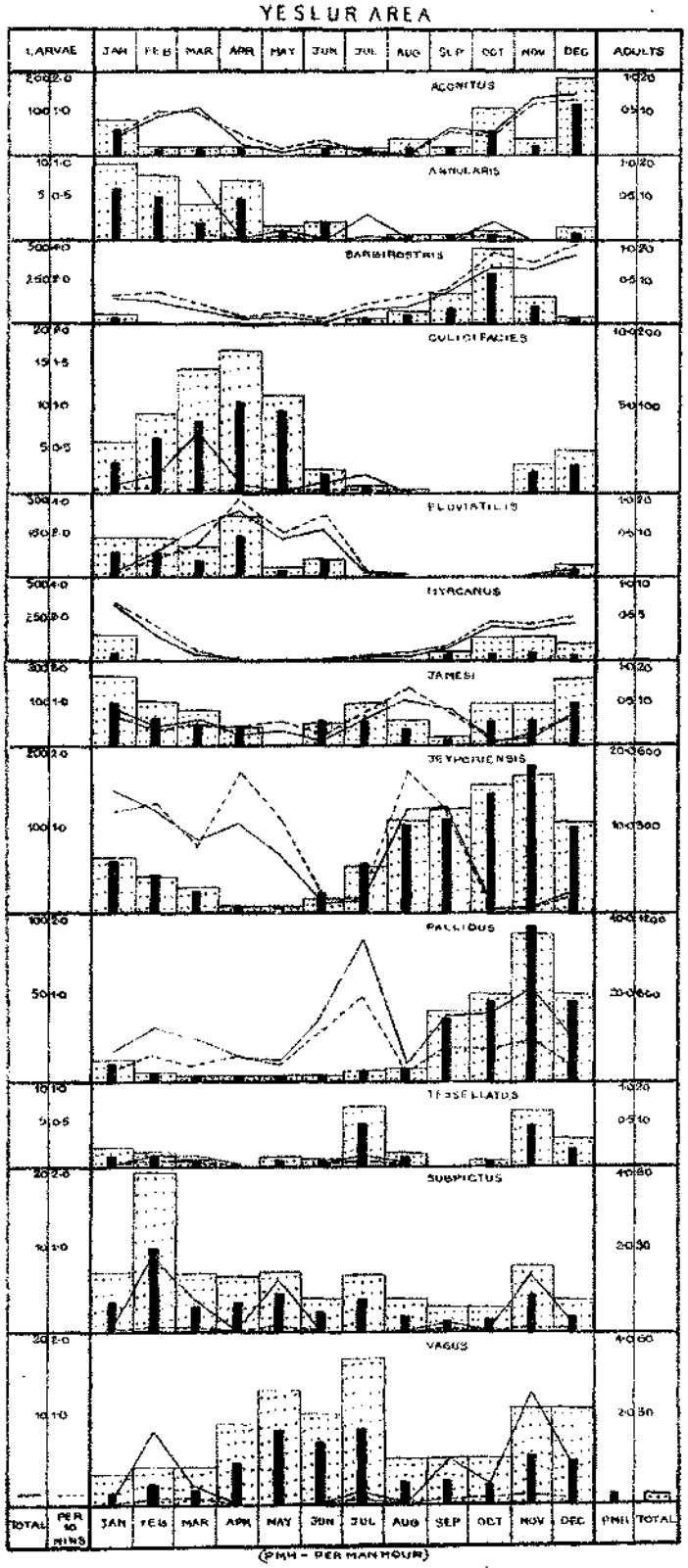
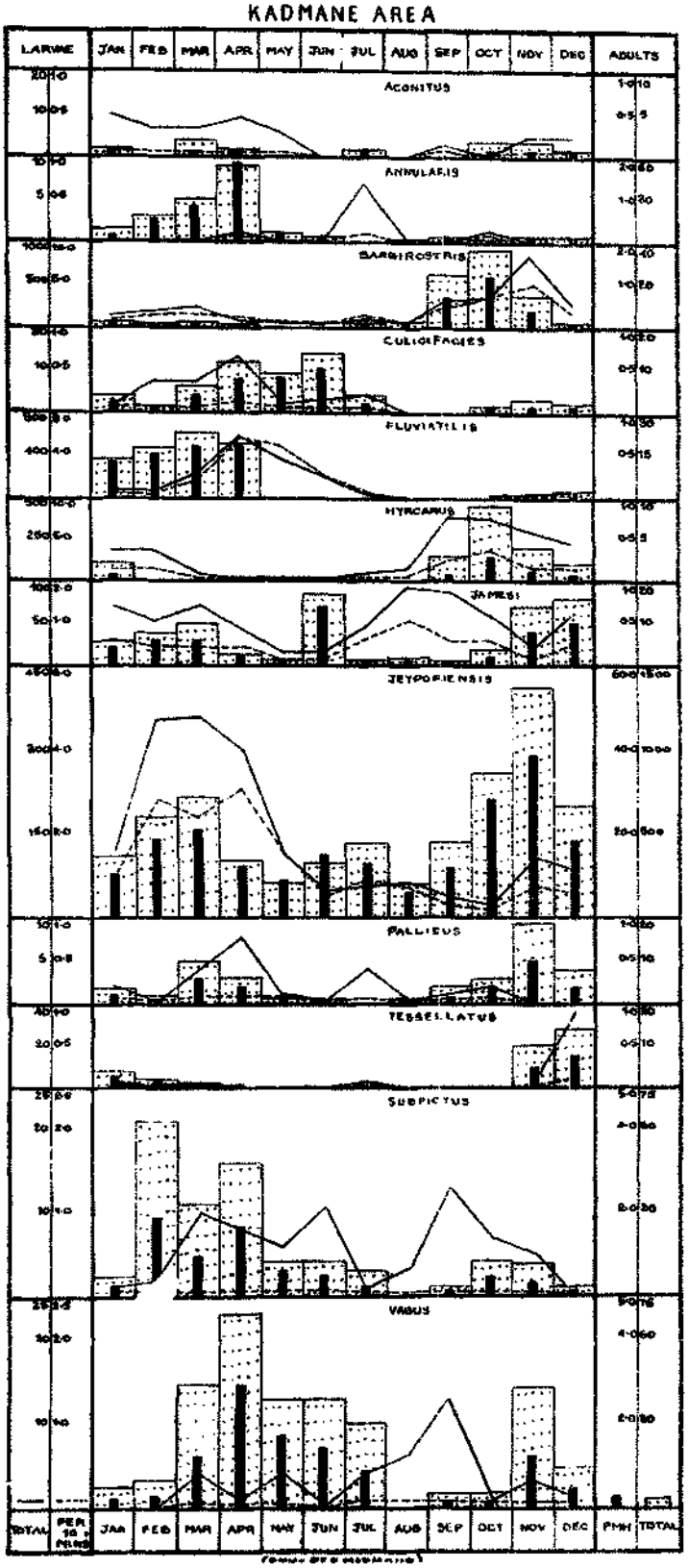


Chart 3.

SEASONAL PREVALENCE OF ANOPHELINES
KADMANE AREA



captures are consistently in excess of the larval collection, while at others they may be far below the latter.

The comparison of adult and larval densities on a collection-time basis is rather difficult.

While the charts referred to above, give information as to the distribution and abundance of the anopheline species recorded, each species may now be considered individually.

A. aitkeni.—The capture of very few adults of this species in man-made shelters is in agreement with the remarks of Christophers (1933) that it " is a wild and shy species not frequenting houses or cattlesheds ".

The bulk of the larvæ was collected from the area of high rainfall, after the summer and winter rains.

A. insulæflorum.—No adults of this species were collected.

Larvæ, although collected in all the three areas, were most abundant in the high rainfall area and were usually taken along the edges of streams, often not far from larval habitats of *A. fluviatilis* and *A. varuna.* March, April and May appear to be the peak months with the maximum in April.

A. annandalei.—Only a single adult specimen was taken in the intermediate rainfall area.

A. barbirostris.—This appears to a large extent to be a wild species, not frequenting man-made shelters during the day. The high larva-adult ratio also seems to indicate a low rate to daytime resting indoors. Night and window-trap collections, which will be referred to later, seem to indicate that it leaves the shelters soon after feeding.

The adult and larval collections indicate that the highest seasonal prevalence is between September and November.

A. hyrcanus.—This species is similar to *A. barbirostris* in its larva-adult ratio, owing to the small numbers collected in dwellings. Adult and larval peaks in its seasonal prevalence appear to occur in general from September through December, with their maximum in October-November. Undoubtedly the great abundance of this species during these months is mainly due to the presence of extensive paddy fields.

A. leucosphyrus.—According to Christophers (1933) this is a wild species found breeding in deep jungle, and does not rest in man-made shelters during the day. Larvæ were collected in all the three areas, the maximum number taken being in the high rainfall area. The two adults were collected in the high rainfall area at a time (July, October) when the larval figures were also high.

A. tessellatus.—Christophers (1933) has described this species as more or less domestic.

Although recorded from all the three areas, it was comparatively more common in the low rainfall area than in the other two. The seasonal prevalence shows its main peak during the south-west monsoon (July, August), while a secondary peak in November, which may be associated with the north-east monsoon. The species seems to disappear during the hot months of April and May.

The same pattern prevails in the intermediate rainfall area also.

In the high rainfall area, this species is virtually absent during the summer, there being a peak only in the winter—December.

The highest prevalence of this species during the rainy season in the comparatively dry area is apparently due to the larvæ requiring small pools for breeding. These pools develop and are maintained better during the summer rains in rolling plains than in high rainfall areas.

A. aconitus.—Christophers (1933) has described this species as widespread in the Oriental Region, and according to Puri (1945) it is found in all areas of moderate to heavy rainfall.

This species was rare in the high rainfall area, but found in large numbers in the intermediate and low rainfall areas.

Both larvæ and adults are either absent or are found in very small numbers in all the three areas during the hot weather.

A. varuna.—Although larvæ of this species were collected in fairly large numbers in practically all the three areas, very few adults were found. According to Iyengar (1924) this species breeds in stagnant fresh water in ditches during and soon after the monsoon. Puri (1945) recorded it as breeding in clear water pools and in slow-running streams which may account for the almost complete absence of larvæ in the two high rainfall areas during the monsoon. In the low rainfall area, however, they persist throughout the year, the maximum abundance being in June and July when the monsoon has just commenced and there are plenty of clear water pools. In the intermediate and high rainfall areas also, they are found just before the monsoon and after the monsoon during the winter months.

A. fluviatilis.—As this species is the alleged chief malaria vector in Malnad, its distribution and abundance received particular attention.* Analysis of the data collected shows that this species displays irregular larva-adult ratios in all the three areas.

As adult collections were exclusively made from dwellings, the real adult densities may be considerably greater than those indicated by the data under review in this paper. Collections from out-door resting places were not made simultaneously but at a later period.

The seasonal prevalance of this species in the area as a whole is in the pre-monsoon period with the peak in April. The most striking feature of the prevalance of this species in all its stages, in the high and intermediate rainfall areas is its virtual disappearance following the onset of the south-west monsoon and its re-appearance in December. This phenomenon was also recorded by Sweet and Rao (1933) at Mudigere.

It may be attributed to the fact that, during the monsoon, the habitats of this species get flushed out. Where such flooding is less extensive, as in the low rainfall area, *A. fluviatilis* is present, during the monsoon, though in smaller number.

An interesting observation made during these studies was that the bulk of the collections in all the three areas came from cattlesheds.†

*Details will be shortly published in a separate communication.

†Further observations on this species will be discussed at length in another paper.

A. culicifacies.—Larvæ occur usually in slow-moving fresh water and pools in sandy river beds. This may explain its scarcity in the high rainfall area, where there are less opportunities for the collection of quiet shady pools or slow-moving streams.

Peak periods in the intermediate rainfall area occur from February to May and from April to July in the low rainfall area. The hot weather does not seem to affect this species as much as the south-west monsoon when the larval habitats are possibly flushed out. During the monsoon, adult populations become so depleted that they are not appreciably built up until early in the following year.

In none of the three areas can *A. culicifacies* prevalence be correlated with paddy cultivation, since the peak levels are attained during the months when fields are fallow. This phenomenon is possibly due to the fact that no irrigation system exists in the three areas. In south-east Madras, where extensive irrigated areas exist, Russell and Rao (1941) observed a close correlation between the irrigation season and abundance of *A. culicifacies.*

A. jeyporiensis.—This species was collected in significant numbers in all the three areas, though exhibiting a marked predilection for the high rainfall area. The adult prevalence ratios in the three areas (low, intermediate and high) are roughly 1 : 5 : 14, but larval collections do not exhibit such wide disparity, the approximate ratios being 3·5 : 1 : 2.

In the low rainfall area there are two peaks of adult abundance. The peak in February-March is accompanied by a corresponding larval peak, while the other in October is attained after the larval peak in September.

In the intermediate rainfall area larval densities are marked by three peaks, January, April and September. The peak in April coincides with a period of minimum adult population (April-May). The adult peak in October-November is accompanied by the lowest larval densities and is preceded by a larval peak in September.

In the high rainfall area, there are again two peaks of adult abundance ; the one in February-March is accompanied by a corresponding larval peak but that in October-November is at a time when the larval population is at its lowest.

The finding of *A. jeyporiensis* var. *candidiensis* in the low and high rainfall areas is of importance even though only seven specimens were taken. Where such a variety is out-numbered by the type form, it is possible that it may be a recessive genetic combination.

A. majidi.—The adults of this species were very scarce in the catching stations in all the three areas under study. A single adult was collected in the high rainfall area though the larvæ were collected in all the three areas during early part of the year—January to May, the maximum number of larvæ were from the area of high rainfall.

A. subpictus.—This " is a markedly domestic form, breeding especially in the neighbourhood of habitations and to be caught often in large numbers in occupied dwellings " (Christophers, 1933). Larva-adult ratios are almost similar in all the three areas, and the seasonal prevalence is almost uniform. Though it has

been recorded throughout the year in all the three areas, the data seem to indicate that there is a late winter and early spring rise with the peak in February, and adult population is rather low during the remainder of the year.

The presence of this species during April seems to indicate its ability to withstand low humidity and high temperatures.

A. vagus.—This species is similar to *A. subpictus* in that it is prevalent in all the three areas under study and displays a wide variety of larval habitats. Unlike *A. subpictus*, it appears to be more adapted to withstand higher rainfall as shown by the progressively increasing numbers collected in the intermediate and high rainfall areas. In these two areas, the adult prevalence is high during April, May and June, whereas in the low rainfall area the peak is attained only in June. In the former, there is also a secondary peak in November which is not evident in the low rainfall areas.

Larvæ were collected in maximum numbers in the low rainfall area.

A. turkhudi.—Only five adults of this species were taken during the entire period of study and they were confined to the low rainfall area. Since no larvæ of this species were encountered, it has to be classified as a rare species. All the adults collected were taken at the beginning of the hot weather.

A. jamesi.—This species exhibits a predilection for the low rainfall area though it is common in all the three areas. Larvæ were collected from tanks and seepage pools with grass growing along the edges. The preponderance of larval over adult catches suggested that *A. jamesi* does not utilize dwellings as daytime shelters to a large extent. This is corroborated by the night and window-trap collections.

There appear to be two seasonal peaks for larval and adult populations. During April-May there is a decline, while during the south-west monsoon there is an increase in the prevalence of the larvæ, although a corresponding rise in adult population seems to develop only in the low rainfall area.

October and November are the periods of a second general decline. The seasonal prevalence is almost similar in the three areas.

A. annularis.—This species has been found in all the three areas, but exhibits a marked predilection for the low rainfall area. It shows a marked seasonal prevalence, the peak being in the late winter months.

The peak does not coincide with the period of paddy cultivation in any of the three areas.

A. pallidus.—This species was encountered in great abundace in the low rainfall area, but was less common in the intermediate area. Its density decreased appreciably in the area of high rainfall. It may be interpreted from this that this species is adapted to survive hot dry periods with considerable efficiency.

An extreme disparity between the larval and adult collections was at times noticed in the low rainfall area. During the first five months, and again in November and December, however, there appears to be a rough correlation between the two populations. There is a minor peak in the adult population during the hot weather—April and May—corresponding to high larval catches. The

highest seasonal prevalence of adults, however, occurs during the period after the south-west monsoon (September to November).

In the intermediate rainfall area, the densities of adults and larvæ build up and then decline from September to January, coincident with the single paddy crop dependant on the seepage and runoff of the south-west monsoon.

In the high rinfall area, the runoff is so rapid that paddy is cultivated during the monsoon itself, when all mosquito breeding is drastically curtailed due to the fast currents.

The larva-adult ratios also seem to indicate that the high rainfall area is less favourable for adults as there is a progressive increase in the ratio according to intensity of rainfall.

A. karwari.—According to Christophers (1933), this species has been recorded from the eastern, southern and Malabar areas of the Indian region, occurring in houses and cattlesheds and readily feeding on man. Larvæ are found in seepage pools and small fresh water springs with marginal vegetation (Puri, 1945). Such habitats are widely distributed in the three areas.

The fact that both larvæ and adults were taken in all the three areas seems to indicate the adaptability of this species to the three types of local climatic conditions. In all the three areas, adults appeared in July and August, dwindling off in September in the intermediate rainfall area, and in October in the low and high rainfall areas. Larvæ, on the other hand, were found right through the year in the low and high rainfall areas, while in the intermediate area they were restricted to January and February.

A. maculatus.—This species has been recorded from various parts of peninsular India, notably in the Nilgiris and many localities along foot of Himalayas (Christophers, 1933). Only five adults in all were recorded from the three areas during the winter months. The species apparently is very rare in the whole area under study.

A. philippinensis.—In spite of the fact that larvæ of this species were found in sufficiently large numbers in the low and intermediate rainfall areas, the comparative scarcity of adults in the catching station seems to indicate that, often, it is not safe to draw conclusions as to the seasonal prevalence, on collection of adults from such places only. Larvæ were usually collected in tanks covered with water-weeds.

The complete absence of larvæ of this species from the high rainfall area may be possibly due to the lack of suitable collections of water for larval habitat. In the low rainfall area, larvæ were collected in too small numbers to draw any conclusions, although they were taken at the same period as in the intermediate rainfall area.

A. splendidus.—Christophers (1933) states that this species is found in small numbers in houses and cattlesheds and the low numbers collected in the three areas would seem to confirm this statement. This is also in agreement with results of night-time and window-trap collections. The concentration of this species in the low rainfall area, and particularly during the hot months, seems to indicate its adaptability for dry conditions.

NIGHT-TIME HAND AND WINDOW-TRAP COLLECTIONS.

As a part of the programme in investigating the bionomics of the vector species, night hand-collections were carried out routinely every week from February, 1952, to January, 1953, in the three areas from human dwellings and cattlesheds which had been selected as fixed catching stations for daytime collections. Collections were made for thirty minutes at intervals of four hours, commencing at 6·00 p.m., and terminating at 6·00 a.m.

Window-traps were set up only in the low rainfall area and were operated in cattlesheds from July, 1952, to January, 1953.

Night catches duplicate the daytime adults densities to a remarkable degree not only in pattern but also in the absolute numerical sense for many species. It would appear, therefore, that, as far as these studies were concerned, the daytime catch from man-made structures was a satisfactory method for estimating adult densities. The species which exhibited marked deviations from day-time catches, were *A. barbirostris*, *A. hyrcanus*, *A. jamesi*, *A. splendidus* and *A. tessellatus*. This can be attributed to their outdoor resting habits, as commented upon earlier when describing these species individually.

DISCUSSION.

A discussion of the above data can be prefaced by a significant quotation from Boyd (1949). " For each species of Anopheles there is (in theory at least) a set of general environmental conditions most favourable for development, and variations from these optimum environmental conditions are reflected in variations in the population density of the species. The general environment varies, of course, from region to region and thus controls the distribution of a species in an area where there are no absolute barriers to dispersal. Within a given region, the local distribution of a species is controlled by reactions to the environmental differences among the available range of habitats. The general environment varies in time as well as in space ; and this variation is reflected in the characteristic seasonal distribution of the different anophelines in a given area ".

Adult occurrences in human dwellings, mixed dwellings and cattlesheds presuppose that a mosquito has entered on the previous night to feed and rest or has entered at dawn merely to rest. Negative collections or insignificant numbers of adults collected within these shelters do not automatically prove that a species does not frequent such shelters or is not commonly present in the local environment. This is especially true for those species where larvæ were collected but adults were not found.

Larvæ are static and are not likely to shift from one habitat to another unless mechanically propelled by a flood. Since the larvæ cannot select their habitat, there must be a close relation between the oviposition behaviour of the adult mosquito and the physical requirements of the larvæ. In the absence of preferred habitats, however, gravid mosquitoes may oviposit in unusual situations. Thus the routine dipping for larvæ in selected areas may at times give reliable information as to species prevalence, but at other times only a confusing or erroneous

indication. How true this is, is borne out clearly in the intermediate and high rainfall areas where some species virtually disappear during the southwest monsoon and reappear suddenly after the rains.

The seasonal abundance of a species depends to a large extent upon the presence of preferred habitats. If these are absent either during the larval or adult stages, a species cannot become abundant however favourable other conditions may be. Therefore any consideration of either larval or adult habitats necessitates taking into account the requirements of the species as a whole. In many cases the limiting factors of species distribution may apply to the adults. A diurnal mosquito ill-adapted to low humidities is bound to be prevalent in areas of high humidity, and the larval habitats will be conditioned by this factor. In the foregoing studies, *A. jeyporiensis* may be cited as an example of this type in the three areas. It is abundant in the high rainfall area and shows a progressive diminution with decreasing rainfall and consequently humidity. In the reverse case, a species which is able to survive only in dry hot areas shows poor adaptation to areas of high rainfall and humidity, where the required larval habitats will be scarce. In these studies, *A. pallidus* furnishes an admirable example of such a species.

Climate exercises an important influence on the longevity of the mosquitoes and this is of great importance from the point of view of malaria epidemiology, since the efficiency of a given species as a vector would depend on the ability of individual mosquitoes to live long enough for the incubation period of the plasmodia to be completed. Obviously a long-lived species would make a better vector than a short-lived one. But it is almost impossible to disentagle the effect of temperature from that of humidity on mosquito longevity. Presumably each such species has its own optimum temperature and humidity.

While the rainfall gradient may exert an influence on the abundance of anopheline larvæ or adults, numerous instances can be quoted from the foregoing data in which there seems to be some other basis for the abundance of some species. There are certain local factors which profoundly influence the incidence of these species. A high larva-adult ratio may sometimes be an indication of shortened adult longevity. This would be of significance in the case of subceptible species as it may imply that the mosquito, though it took a blood meal, was unable to survive the extrinsic incubation period of the plasmodia. A low larva-adult ratio would, on the other hand, indicate an enhanced longevity of the mosquitoes. This would account for a large number of adults being found when a corresponding low larval population is met with.

SUMMARY.

1. The anopheline fauna in the Western Hill Tracts of Mysore State was surveyed for two years in three areas near Saklaspur Town (Hassan District) selected on the intensity of rainfall.

2. Standardized methods of population sampling, namely, collection of larvæ from breeding places and of imagines from indoor resting places, were employed. These were supplemented later by night catches and window-trap collections.

3. Twenty-three anopheline species were recorded from the area and their seasonal prevalence were analysed from various standpoints, namely larval prevalence and habitats, adult prevalence and diurnal resting habits, larva to adult ratio during the different seasons and relation of the species to intensity of rainfall.

ACKNOWLEDGEMENTS.

Grateful thanks are due to the Director of Public Health in Mysore and to the authorities of The Rockefeller Foundation for facilities extended ; and to Dr. B. Ananthaswamy Rao, who was Superintendent, Bureau of Malariology, at the time of these studies, for constant guidance and encouragement. The faithful co-operation of the entomological staff of the Malaria Investigation Centre, Saklaspur, has rendered it possible to accumulate these data. The co-operation of the Director, Regional Mateorological Centre, Madras, in providing the mateorological data is also gratefully acknowledged.

This paper is published with the permission of the Director of Public Health in Mysore, Bangalore.

REFERENCES.

Boyd, M. F. (1949) ... ... *Malariology.* Vol. I, pp. 308-309. W. B. Saunders Company, Philadelphia and London.

Christophers, S. R. (1933) ... ... *Fauna of British India. Vol. IV. Diptera. Family culicidae. Tribe Anophelini.* Taylor and Francis Co., London.

Iyengar, M. O. T. (1924) ... ... *Ind. J. Med. Res.*, **12**, pp. 23-29.

Puri, I. M. (1945) ... ... *Synoptic tables for the identification of the full-grown larvæ of the Indian anopheline mosquitors.* Health Bulletin No. 16. Manager of Publications, Delhi.

Russell, P. F. and Ramachandra Rao, T. (1941) ... ... *J. Mal. Inst. Ind.*, **4**, pp. 263-295.

Sweet, W. C. and Rao, B. A. (1933) ... *Rec. Mal. Surv. Ind.*, **3**, pp. 663-674.

Indian Journal of Malariology, **8**, 1, *March* 1954.

EFFECT OF LIME ON RESIDUAL ACTIVITY OF D.D.T.*

BY

S. R. BHOMBORE,

N. L. SITARAMAN,

AND

K. S. NANJUNDAIAH.

(*Malaria Investigation Centre, Saklespur, Mysore State.*)

(March 30, 1954.)

INTRODUCTION.

In the course of routine application of D.D.T. as an indoor residual insecticide in malaria control in an irrigated tract of Mysore State (Channarayapatna area, Hassan District), it was frequently observed that the villagers were in the habit of white washing their houses either immediately before or after spraying, in connection with some religious functions. Observations made in different countries on the effectiveness of D.D.T. residual deposits in anopheline control reveal that far from uniform results are obtained by different workers. Causes for this lack of uniformity may be due to several factors. The mosquito species being studied, is undoubtedly an important factor, since species differ markedly in habits and also in response to minimal exposures to D.D.T. Another factor of undoubted importance is the surface on which D.D.T. is being sprayed. The influence exerted by various types of surfaces on the duration of the residual toxicity of D.D.T. has been reported by a number of workers (Clapp *et al.*, 1947 ; Sundararaman and Peffly, 1949 ; Downs *et al.*, 1951) ; but references to the effect of lime as such on the residual activity of D.D.T. are comparatively scanty. Clapp *et al.* (1947) showed that adding common salt (N_aCl) to the whitewash mixture, lengthened the duration of action of D.D.T. But Hocking (1947) reported that

*The studies and observations upon which this paper is based were conducted by the Bureau of Malariology, Department of Public Health, Government of Mysore, with the support and under the auspices of the Division of Medicine and Public Health of The Rockefeller Foundation.

limewash neutralized the residual action of D.D.T. against *A. gambiæ* and *A. funestus* in East Africa. Hadaway and Barlow (1947) reported rapid loss of D.D.T. toxicity when applied on whitewash in the form of kerosene solutions and emulsions, though water-suspensions gave better results. The whitewash they used contained iron oxide (content 2·6 per cent). Maier *et al.* (1948) recommended that walls should be whitewashed before being sprayed.

As the duration of the residual effect of D.D.T. is of paramount importance in malaria control programme, these studies were undertaken to elucidate whether lime had any effect on the residual toxicity of D.D.T.

AREA OF STUDY.

Village Anegola selected for these studies lies on the Mysore-Channarayapatna Road in Kikkeri Hobli, Krishnarajpet Taluk of Mandya District, Mysore State. It is one of the series of irrigated villages fed by the North Ramadevara Channel of the Hemavathi River. The irrigation season extends from June to January of the subsequent year, the main cultivated crops being paddy and sugarcane. The average annual rainfall for ten years (1941-50), as recorded at the nearest raingauge station, four miles away, is 28.45 inches. The inhabitants are typical agriculturists and their economic condition is poor. The houses are all mixed dwellings, with mudwalls (plastered or unplastered), country tile roofs and are poorly lighted and without any ventilation, except for a central opening in the inner courtyard. A preliminary malariometric survey revealed that the village is endemic for malaria (spleen rate 30·3 per cent., average enlarged spleen ·5). The transmission season in the entire irrigated tract is from September to January as judged from dispensary figures at Channarayapatna.

PLAN OF EXPERIMENT.

The houses in Anegola were grouped into two divisions 'A' and 'C'. In 'A' division, all the houses were sprayed on September 19, 1951 with D.D.T.* at the rate of 200 mg. per square foot and limewashed the following day. The limewash was applied by the villagers themselves under the supervision of a Health Inspector. In 'C' division, the houses received limewash mixed with an aliquot portion of D.D.T. to leave a residue of 200 mg. per square foot. The mixture was supplied to the housecowners with instructions for the mode of application and the work was supervised by a Health Inspector.

For assessing the duration of residual toxicity of D.D.T. in the different areas, weekly anopheline collections, infant parasite rates and spleen rates in children between the ages of two and 12 years, were used. A group of villages which had been routinely sprayed with 200 mg. of D.D.T. since 1950 and another group which was left unsprayed, were used as comparison areas.

*The D.D.T. used was Du Pont's Detane 50 per cent wettable powder and was applied with Meyer's petrol-driven power sprayers.

ENTOMOLOGICAL OBSERVATIONS.

Routine weekly collections of anopheline were started in Anegola and the comparison villages from the third week of July, 1951, six weeks after the commencement of the irrigation season and continued till September, 1952. These collections were made from selected catching stations during 08·00 -12·00 hours. Sixteen species of anopheline were recorded, viz., *aconitus*, *annularis*, *barbirostris*, *culicifacies*, *fluviatilis*, *hyrcanus*, *jamesi*, *jeyporiensis*, *karwari*, *pallidus*, *splendidus*, *subpictus*, *tesselatus*, *turkhudi*, *vagus* and *varuna*. Dissections could not be carried out to incriminate the vector but from the data collected from other places in the district, *culicifacies* and *fluviatilis* appear to be the vector species, the former probably more important than the latter because of its greater abundance. For the purpose of comparison, the anophelines collected were classified as *culicifacies* and *fluviatilis* (presumed vectors) and *other anophelines*, and the data collected from the various areas has been reduced to per man-hour collections and presented in the form of graphs for easy reference (Chart 1).

Observations in the various areas from July to September, 1951 (the pre-treatment period) show that from the third week of July, there was a gradual and steady increase in the densities of all anophelines including *A. culicifacies* and *A. fluviatilis* till the last week of August, after which there was a decline. The peak was reached in the third week of August except in Division 'C' in which the peak was recorded in September. *A culicifacies*, however, reached its highest density in September in both the divisions. In the comparison area in which D.D.T. alone was applied in September, 1950, density of all anophelines before treatment was 19·3 per man hour. After spraying, the mosquito density dropped to zero and remained continuously so for a period of thirty-seven weeks when a second treatment of 200 mg. D.D.T. was given during May, 1951. From then onwards, collections were negative till the end of December, 1951. During January, 1952, anophelines reappeared but the density attained was only 0·3 per man-hour. In February, it dropped down to zero again and remained at that level till August when anophelines appeared again. At no stage during the observations, the mosquito densities in these villages attained the level found in unsprayed villages. Infant parasite rates too remained negative throughout in these groups of villages.

Natural fluctuations in mosquito density observed in the unsprayed villages, are shown in Chart I. The density began to decline towards the end of August, and reached the lowest level during October. In October, the marked fall in the density can be attributed to the high rainfall in the area, which obviously was responsible for flushing out the larvæ from the breeding places. From November onwards till the end of the irrigation season (subsequent January), there was a steady and gradual increase in the density. With the onset of dry season from February onwards, there was again a steady decline. At the commencement of the irrigation season in the middle of June, the density was at its lowest, the per man-hour figures for *A. culicifacies*, *A. fluviatilis* and other anophelines being 1·4, 0·07 and 3·4 respectively. With the return of favourable conditions from July onwards, there was again a steady increase in the density and the trend was similar to that observed during the previous year. At the end of September, 1952, when

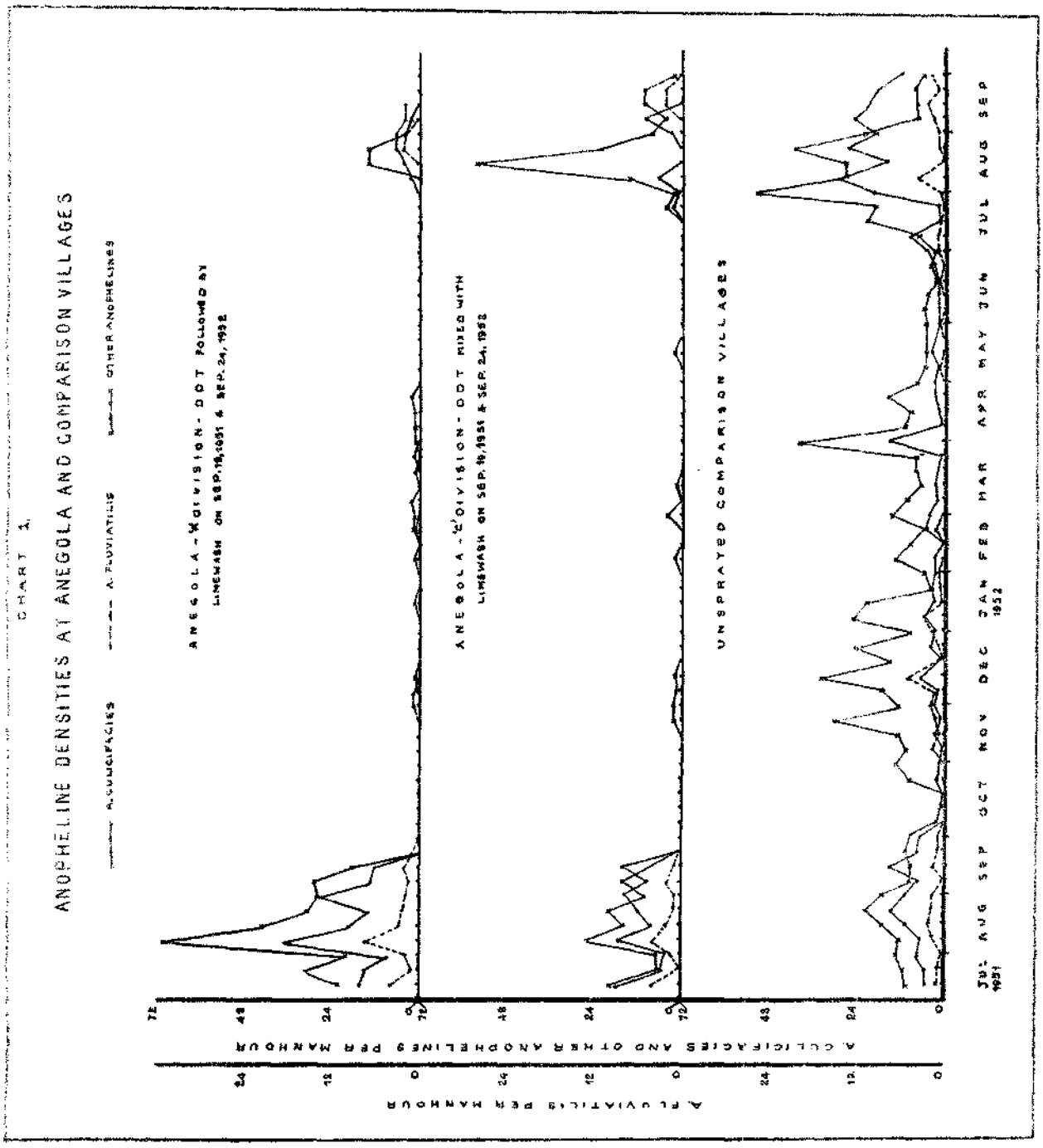


CHART 1.
ANOPHELINE DENSITIES AT ANEGOLA AND COMPARISON VILLAGES
A. CULICIFACIES
A. FLUVIATILIS
OTHER ANOPHELINES
ANEGOLA - 'A' DIVISION - DDT FOLLOWED BY LIMEWASH ON SEP. 15, 1951 & SEP. 24, 1952
ANEGOLA - 'B' DIVISION - DDT MIXED WITH LIMEWASH ON SEP. 15, 1951 & SEP. 24, 1952
UNSPRAYED COMPARISON VILLAGES
A. FLUVIATILIS PER MANHOUR
A. CULICIFACIES AND OTHER ANOPHELINES PER MANHOUR
72
48
24
0
12
JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP
1951
1952

one year's observations were completed, the densities of *A. culicifacies*, *A. fluviatilis* and other anophelines were 15·9, 1·3 and 6·1 per-man hour, respectively.

In 'A' division of Anegola Village where all the houses were sprayed with 200 mg. D.D.T. followed by limewash the day following, the density dropped to zero after D.D.T.-spraying. No *A. culicifacies* or other anophelines were encountered and except for a single specimen of *A. fluviatilis* caught flying in the catching station in the week following the treatment, the density of all anophelines remained zero for nine weeks after treatment. Solitary specimens of *A. fluviatilis* were collected during November and December (per man-hour figure was, however, too low for any serious consideration), and a single specimen in February. Except for a single specimen collected during March, five months after treatment, *A. culicifacies* was completely absent for 11 months. A few other anophelines were occasionally collected during December to April, after which they too were completely absent till about the middle of August, 1952.

With regard to 'C' division where the houses were treated with 200 mg. D.D.T. mixed with limewash, density showed characteristic drop to zero and remained so for a period of seven weeks after treatment. During the tenth, eleventh and thirteenth week after spraying, other anophelines appeared in small numbers, the per man-hour figure varying from 1·0 to 2·0. During January-July, 1952, only two *A. culicifacies* and seven other anophelines, but no *fluviatilis*, were collected. From the last week of July onwards, there was a gradual rise in the number of mosquitoes and at the end of one year's observations, i.e., the third week of September, the maximum per man-hour figure recorded for all anophelines was 56·0. The peak figures were attained in the month of August as in the comparison unsprayed villages.

INFANT PARASITE RATES.

In the comparison village treated with 200 mg. dosage, the infant parasite rate was 26·06 but dropped to zero two months after D.D.T. treatment, and remained so throughout the period of observation. In the unsprayed group of villages, infant parasite rate was positive from October, 1951, to April, 1952, and in July, 1952, the highest (8·33) being in October declining to 1·9 in December and January and rising to 7.69 in February, 1952, going down to 1·9 and 2·85 in March and April respectively. It was lowest (1·56) in July, 1952. Analysis of the monthly infant parasite indices indicates that even in the unsprayed villages, there was a natural decline, though it was not as marked as in the villages sprayed with 200 mg. D.D.T. only.

At Anegola, no blood smears from infants were taken during the pre-spray period, and all the smears collected after spraying, have been continuously negative for malaria parasites. The number of infants in Anegola is no doubt very small but it is clear that during the period of observation, there was no transmission going on in the sprayed villages.

SPLEEN RATES.

Spleen rates and average enlarged spleen studied at different intervals among the children between two and 12 years of age in the various villages, have been

tabulated (Table I). In the groups of comparison villages, the spleen surveys were first made in June, 1950 and then in October the same year, after which every six months till April, 1952. In the unsprayed villages, however, the survey corresponding to October, 1950 was not carried out. In Anegola only two surveys in October, 1951, and April, 1952, were carried out. The two surveys—one in June and the other in October, 1950 (only about a fortnight after D.D.T. application), carried out in the group of villages which were treated with 200 mg. D.D.T. per sq. ft. of wall surface, show a decline due to some natural causes. This natural but gradual decline is also revealed by the spleen rates taken in the group of unsprayed village. This decline, however, is in contrast to the abrupt fall observed in the spleen rates recorded in all the treated villages—comparison groups as well as at Anegola.

TABLE I.

Spleen survey findings at Anegola and comparison villages

Months of survey.	ANEGOLA.		COMPARISON VILLAGES.			
	200 mg. D.D.T. with lime.		200 mg. D.D.T. only.		Unsprayed villages.	
	Spleen rate (per cent.)	Average enlarged spleen	Spleen rate (per cent.)	Average enlarged spleen	Spleen rate (per cent.)	Average enlarged spleen
June, 1950 ...	...	...	91·4	3·6	46·9	2·7
October, 1950 ...	...	...	58·1	3·7	...	...
April, 1951 ...	...	...	49·4	2·7	41·6	2·4
October, 1951 ...	30·3	1·5	41·9	1·9	47·0	1·7
April, 1952 ...	7·5	1·4	20·2	1·8	39·4	1

Date of spray at Anegola=September 19, 1951.
Date of spray in 200 mg. D.D.T. area=September 11, 1950 : May 30, 1951.

DISCUSSION.

The object of this experiment was to study the comparative effect of lime on the residual toxicity of D.D.T. when the two are applied mixed with each other, as against the former being applied soon after the walls have been treated with D.D.T. A reference to the graphs (Chart I) shows that during September, 1951, to September, 1952, the prevalence of the vector species as well as of the other anophelines has been so low and variable in both the divisions of Anegola that no definite conclusion can be drawn as to the relative merits of the two modes of treatment.

A study of the post-treatment entomological data from Anegola as well as from the comparison villages indicates that the residual toxicity of D.D.T. sprayed either mixed with lime or lime washed after spraying, was effective for a period of ten to 11 months, the densities being only slightly higher in houses treated with D.D.T. and lime than in those in which only D.D.T. was applied. These observations are quite contrary to those of Hocking (1947) who reported that limewash

neutralized the residual action of D.D.T. against *A. gambiæ* and *A. funestus* in East Africa. That the insecticide was persistant in its action for a long period, is borne out by the fact that the day-time collections exhibited oscillations, with the crest of the waves too low to be considered serious. But at the end of these periods, when there was waning of the insecticidal action, succeeding waves increased in their amplitude and a sufficiently high density was soon reached.

SUMMARY.

(1) Field trials to determine the effect of lime on residual activity of D.D.T. were undertaken at Anegola Village where the level of anophelism was high.

(2) In villages where 200 mg. D.D.T. per sq. ft. was sprayed, repeated by another treatment of 200 mg. seven months later where no lime wash was done in any form, the level of anophelism was low or negligible for a period of nearly fifteen months.

(3) At Anegola where 200 mg. D.D.T. was sprayed either mixed with lime or the latter subsequently applied, a low mosquito density was maintained for a period of nearly ten months.

(4) In the untreated comparison area, both during the pre and post-spraying periods, the level of anophelism was high.

(5) From the infant parasite rates, there is evidence that transmission of malaria was going on in the unsprayed area during the months of October, November, December and January, whereas in the sprayed area there were no positive infant blood smears.

ACKNOWLEDGEMENTS.

The authors wish to express their grateful thanks to the authorities of the Rockefeller Foundation—Dr. R. B. Watson, Mr. J. C. Carter and Dr. C. Brooke Worth,— to Dr. T. Chandrasekharaiya, Director of Public Health with the Government of Mysore, and to Dr. B. Ananthaswamy Rao, Superintendent, Bureau of Malariology, Department of Public Health, Bangalore, for their constant encouragement and guidance.

This paper is published with the kind permission of the Director of Public Health in Mysore, Bangalore.

REFERENCES.

CLAPP, J. M., FAY, R. W. and SIMMONS, S. W. (1947) ... *U.S.A. Pub. Hlth. Rpts.*, **62**, p. 158.

DOWNS, W. G., BORDAS, E. and NAVARRO, L. (1951) ... ... *Science*, **114**, p. 250.

HADAWAY, A. B. and BARLOW, F. (1947) ... *Bull. Ent. Res.*, **38**, p. 489.

HOCKING, K. S. (1947) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, p. 589.

MAIER, J., RUNDTORFF, R. C. and SUAREZ, M. (1948) ... ... *Amer. J. Trop. Med.*, **28**, p. 889.

SUNDARARAMAN, S. and PEFFLY, R. L. (1949) *J. Nat. Mal. Soc.*, **8**, p. 267.

VISWANATHAN, D. K., RAMACHANDRA RAO, T., HALGERI, A. V. and KARANDKAR, V. S. (1950) ... ... *Ind. J. Mal.*, **4**, p. 533.

Indian Journal of Malariology, **8**, 1, *March* 1954.

TREATMENT OF MALARIA WITH A SINGLE DOSE OF AMODIAQUIN (CAMOQUIN).

BY

J. C. PATEL

AND

S. D. DALAL.

(*King Edward Memorial Hospital, Bombay*-12.)

[March 19, 1954.]

AMODIAQUIN (camoquin)—a new 4-aminoquinoline compound—has lately created considerable interest in the treatment of malaria. A number of authors (Halawani *et al.*, 1947 ; Simeons and Chattre, 1947 ; Chaudhuri and Chakravarty, 1948 ; Chaudhuri *et al.*, 1950 ; Ejercito and Duque, 1948 ; Patel and Mehta, 1948 ; De Lucene, 1950 ; Hamilton, 1950 ; Hockenga, 1951 ; Mien and Rosado, 1951 ; Villarejos, 1951 and Ansari, 1952) have found it useful and a safe addition to the antimalarial drugs. Most of the above-cited authors have used it in intermittent therapy and some have reported results with a single large dose in the treatment of clinical attacks of malaria.

METHOD.

Since the authors' last publication (Patel and Mehta, 1948), they have carried out further clinical trials with a larger single dose on patients admitted in the wards of the King Edward VII Memorial Hospital, Bombay. The drug was administered to those patients who had a typical clinical picture of malaria and whose blood showed malarial parasites. The thick drop of blood, using Field's method of staining, was examined for the detection of parasites four times a day, beginning twenty-four hours after the administration of the drug, until they were found to be negative for asexual parasites on two consecutive occasions. Records of temperature and of the occurrence of paroxysms were also kept in every case. Thirty-three cases were treated with the drug : seven showed *P. falciparum*, 20 *P. vivax*, and six mixed infections. The distribution of patients according to sex and age is given in Table I.

TABLE I.

Distribution of malaria patients according to sex and age.

Infection.	Sex.		Age in years.			
	Males.	Females.	0–9	10–19	20–29	30 and over
P. falciparum ...	6	1	0	0	5	2
P. vivax ...	18	2	0	0	14	6
Mixed ...	5	1	0	0	4	2

DOSAGE.

The drug was administered by mouth in tablet form and without any special time relationship to the malaria cycle. Each tablet of amodiaquin contained 0·05 gm. of the base in the form of the hydrochloride. On diagnosis, ten tablets (0·5 gm.) were given orally in a single dose, usually in the forenoon under personal supervision. This dose is about 10 mg. or more per kg. body weight for an average Indian.

RESULTS OF TREATMENT.

All the 33 patients were treated in accordance with the above schedule. There was one case which differed from the rest which has been described below in detail. The other 32 cases quickly became free from fever, and asexual parasites disappeared promptly. The immediate effect on temperature and parasites is shown in Table II. The average duration of temperature in *P. vivax* infection was 18·0 hours and *P. falciparum* infection 14 hours after the administration of the drug. In *P. vivax* infection, the average time required for clearance of the peripheral blood of asexual parasites was 42·2 hours and *P. falciparum* infection 48 hours. Fever subsided from 12 hours to 36 hours after the administration of the drug. Parasites disappeared at the earliest in 30 hours and the maximum time required was 54 hours.

Effect on gametocytes.—Gametocytes were present in the blood of three patients before institution of the treatment. Amodiaquin had no effect on them. Other workers also did not report any effect on the gametocytes. As the period of observation in these cases was very short, the authors cannot opine on the prevention of the development of gametocytes in the peripheral blood as observed by Simeon and Chattre (1947).

Average time of disappearance of fever has varied with different series (Halawani *et al.*, 1947 ; Simeons and Chattre, 1947 ; Chaudhuri and Chakravarty, 1948 ; Ejercito and Duque, 1948 ; Patel and Mehta, 1948 and Ansari, 1952) and in the majority of them it is less than 24 hours with a single dose. There has been no appreciable difference between the dosage of 0·5 gm. to 1·0 gm. of the drug and with the type of plasmodia.

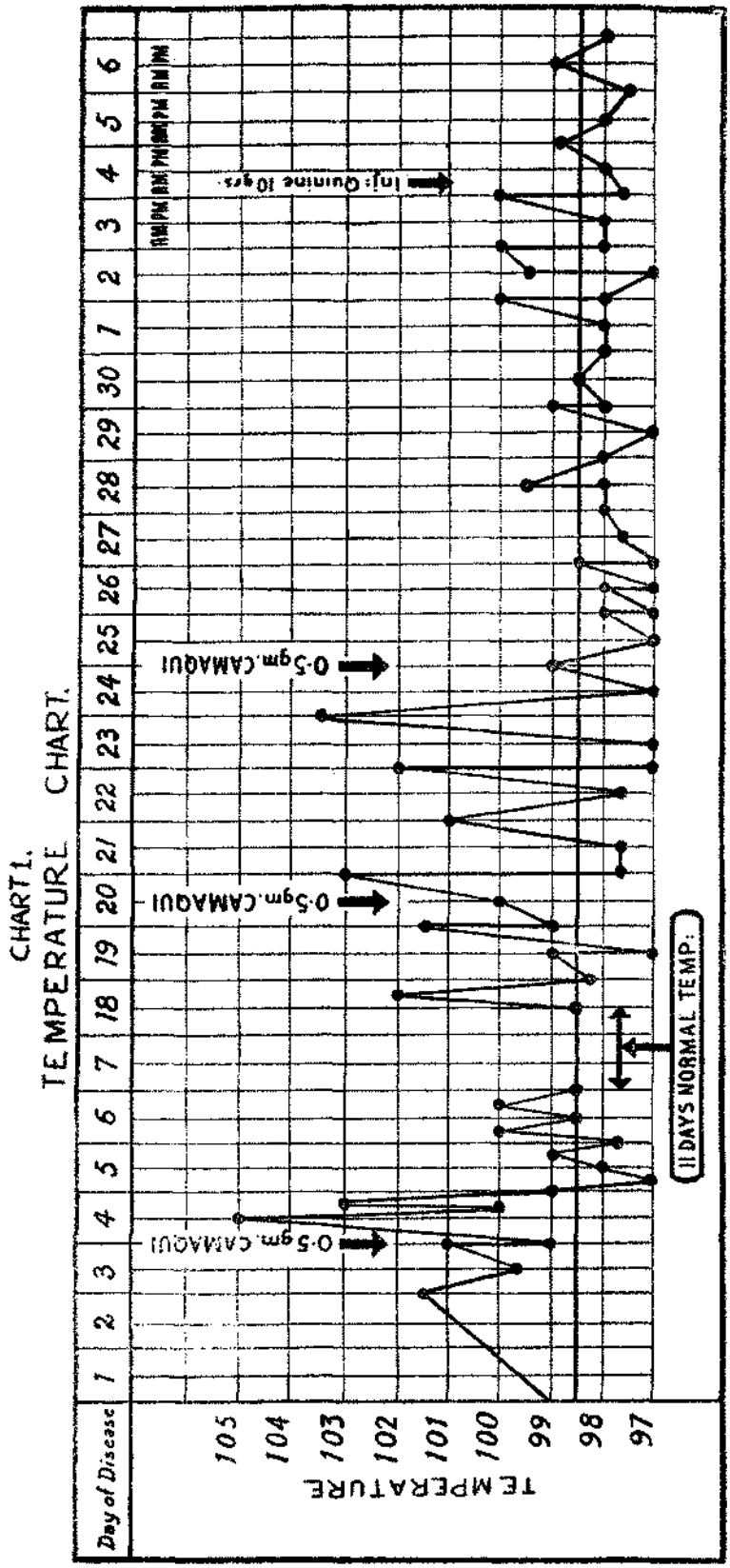


CHART 1.
TEMPERATURE CHART.
Day of Disease
TEMPERATURE
105
104
103
102
101
100
99
98
97
0·5gm. CAMAQUI
0·5gm. CAMAQUI
0·5gm. CAMAQUI
Inj: Quinine 10 grs.
11 DAYS NORMAL TEMP.

TABLE II.

Duration of fever and asexual parasites.

Duration of fever in hours.		Duration of asexual parasites in blood in hours.	
M.T.	B.T.	M.T.	B.T.
14	18·0	48	42·2

RELAPSES.

The study of relapses was handicapped because of shortage of beds which prevented the keeping of the patients in the hospital for as long as required for this purpose. Most of the patients were discharged two days after clearance of fever and the asexual parasites from the peripheral blood. It is known that relapses do occur in *P. vivax* infection when treated with amodiaquin (Halawani *et al.*, 1947 ; Chaudhuri and Chakravarty, 1948 ; Ejercito and Duque, 1948 ; Chaudhuri *et al.*, 1950 ; De Lucene, 1950 ; Hockenga, 1951 ; Mien and Rosado, 1951 ; Villarejos, 1951 and Ansari, 1952). Three patients were observed in the present series who had relapses with parasitæmia and fever. They again responded to amodiaquin. One such patient was admitted to the hospital for at least three relapses, which each time responded to amodiaquin. Later, he was given proguanil 0·1 gm. once a week, and he has not reported since. Hockenga (1951) has observed two such cases. Ansari (1952) finds relapses are frequent with a dosage lower than 10 mg./kg.

TOXIC ACTION.

No untoward effects were observed. De Lucene (1950) has noticed mild gastrointestinal disorders.

AN UNUSUAL CASE OF MALARIA RESISTANT TO AMODIAQUIN.

D. K., male, aged 30, was admitted on November 2, 1948, with a history of fever with rigors on alternate days which subsided after four hours with sweating. The patient also complained of severe headache. A history of similar attacks previously was not available. On examination, the patient was found to be fairly well-built and nourished. Temperature 102° F., pulse 100/min., respiration 26/min., tongue—moist and not coated. The respiratory, cardiovascular and nervous systems were normal. The liver was not enlarged and the spleen not palpable. Blood examination showed R.B.C. 4·1 million/c.mm., Hb 80 per cent, W.B.C. 8200/c.mm. with a differential leucocytic count of polymorphonuclear 60 per cent, lymphocytes 30 per cent and monocytes 10 per cent. A thick blood film stained by Field's method showed heavy infection with the ring forms of *P. falciparum*. Amodiaquin (0·5 gm.) ten tablets were given on November 4, 1948. The temperature came down to normal after 56 hours but the parasites persisted for 92 hours after which the patient went away.

The patient was readmitted on November 18, 1948, with a history of fever with rigors for two days. Ring forms of *P. falciparum* were found in the peripheral blood smear ; ten tablets (0·5 gm.) of amodiaquin were given on November 20, 1948. The temperature, however, continued as before and the parasites persisted in the peripheral blood for 96 hours. A further ten tablets (0·5 gm.) of amodiaquin were given on November 25, 1948. The temperature seemed to be settling down, but on November 28, the patient had another bout of fever with persistent parasitæmia, leading to the administration of quinine 5 grs. t.d.s. on December 4, 1948. The next day, the patient became drowsy with higher temperature and was given injectible quinine hydrochloride 10 grs. intramuscularly and proguanil tablets (0·1 gm. t.d.s.). The temperature subsided and the patient was discharged on December 6, 1948. The temperature is shown in chart 1. The patient had no symptoms of gastrointestinal irritation.

DISCUSSION.

It is obvious that the results are better than those obtained by Patel and Mehta (1948) with a single dose of five tablets (0·25 gm.). The average duration of temperature for *P. vivax* infection, in that series, was 26·5 hours and the average time required for the clearance of the peripheral blood of asexual parasites was 60 hours.

It is evident from the present series and from all the reports published, including that of Patel and Mehta (1948), that amodiaquin has negligible or no toxic effects when given in one single dose of 0·5 gm. (about 10 mg. per kg. body weight). It has a powerful action on the parasites and a more powerful action in bringing the temperature down to normal than most other antimalarial drugs administered orally. All workers agree that the parasitæmia persists longer than the fever and no difference of action has been noted on the three different types of parasites. It does not prevent relapses and some cases have been reported that are resistant to its action. In the present series, only one case was observed where the parasites persisted in the peripheral blood in the same numbers, although amodiaquin was used in a dosage of 0·5 gm. The drug was personally administered to the patient and was repeated four days later. The patient did not have nausea, vomiting or diarrhoea, so it may be presumed that it was absorbed from the gastrointestinal tract. In spite of repeated large doses, the patient had both fever and parasitæmia. In the first attack the fever had responded to amodiaquin, but the parasites persisted. Hence, it may be considered that the parasites had actually acquired a resistance to amodiaquin. This patient subsequently responded to quinine. Mica and Rosado (1951) found that five per cent of the cases were resistant to the drug. Ansari (1952) also suggests that it is ineffective in certain cases. Thompson (1948) did not observe any signs of acquired resistance to camoquin and chloroquine in the three strains of *Plasmodium lophurae* in chicks, but he did notice it in the case of chloroguanide (proguanil).

SUMMARY.

1. The results of clinical trials of 33 cases of malaria with a single dosage of 0·5 gm. of camoquin orally administered, are presented.

2. There were no toxic effects and the drug showed no action on gametocytes.

3. Four cases of relapse were observed and one of them (*P. falciparum*) developed resistance to even 1·0 gm. of the drug, showing that malarial parasites may develop resistance to camoquin.

4. It is suggested that a single large dose of amodiaquin is an effective, safe and quick remedy for malaria in practice.

ACKNOWLEDGEMENT.

The authors thank their colleagues and the Dean of the King Edward Memorial Hospital, Bombay-12, for facilities ; and to Messrs. Parke, Davis & Co. for a free supply of the drug. They are also grateful to the numerous house physicians of the hospital who have helped in blood examinations and a follow-up of the patients.

REFERENCES.

Ansari, M. Y. (1952) ... ... Single dose of camoquin in the treatment of malaria. *Ind. J. Med. Sci.*, **6**, p. 306.

Chaudhuri, R. N. and Chakravarty, N. K. (1948) ... ... Clinical trials of cam-aqi in malaria. *Ind. J. Mal.*, **2**, p. 115.

Chaudhuri, R. N., Rai Chaudhuri, M. N., Ghosh, S. and Datta, B. N. (1950) ... Comparison of action of paludrine (proguanil), chloroquine and camoquin in single doses. *Ind. J. Mal.*, **4**, p. 135.

De Lucena, D. T. (1950) ... ... Treatment of malarial infection with cam-aqi (Camoquin). *Rev. Brasileria Med.*, **5**, p. 588. Abstracted in *Trop. Dis. Bull.*, **48**, p. 863.

Ejercito, A. and Deque, M. (1948) ... Cam-aqi in the treatment of human malaria. *J. Phillipine Med. Assoc.*, **14**, p. 633.

Hamilton, C. S. P. (1950) ... ... Camoquin and chloroquine in the treatment of malaria. *Ind. J. Mal.*, **4**, p. 200.

Halawani, A., Baz, I. and Markos, F. (1947) Preliminary report on the antimalarial cam-aqi. *J. Roy. Egyptian Med. Assoc.*, **30**, p. 99.

Hockenga, M. T. (1951) ... ... The treatment of malaria with a single dose of camoquin. *Amer. J. Trop. Med.*, **31**, p. 139.

Mirn, R. M. and Rosado, P. N. S. (1951) Experimental use of new antimalaria drugs in Amazonia. *Trop. Dis. Bull.*, **48**, p. 235.

Patel, J. C. and Mehta, J. M. (1948) ... Cam-aqi in the treatment of malaria. *Ind. J. Med. Sci.*, **2**, p. 675.

Simeons, A. T. W. and Chatterje, K. D. (1947) Preliminary report on a new synthetic antimalarial. *Ind. Med. Gaz.*, **82**, p. 255.

Thompson, P. E. (1948) ... ... On the ability of *Plasmodium lophurae* to acquire resistance to chloroguanide, camoquin, and chloroquin. *J. Inf. Dis.*, **83**, p. 250.

Villarejos, V. M. (1951) ... ... Experiments with amodiaquin (camoquin), a new synthetic antimalarial. *Amer. J. Trop. Med.*, **31**, p. 703.

Indian Journal of Malariology, **8**, 2, *June* 1954.

PROBLEMS IN TROPICAL PUBLIC HEALTH AMONG WORKERS AT A JUTE MILL NEAR CALCUTTA.

VI. The prevalence of filariasis in the labour force with a note on its transmission.

BY

ELI CHERNIN.

(*Department of Tropical Public Health, Harvard School of Public Health, Boston.*)

[March 8, 1954.]

THE mill compound of the Ludlow Jute Company, Chengail, Howrah District, West Bengal, is 17 miles south of Calcutta on the banks of the Hooghly River in an environment which is essentially rural in nature. However, the compound proper is urban in character because of the large number of mill workers and their dependants that are housed within it. Since it is generally accepted that bancroftian filariasis in India occurs predominantly in urban rather than rural areas (Iyengar, 1939), an investigation of the prevalence of filariasis in the compound-resident labour force was undertaken during the period April-June, 1952. This investigation also served as a corollary of studies then in progress on malaria and intestinal parasitic infections among these workers and their supervisory personnel (Chernin, 1954*a* : 1954*b* : 1954*c* : 1954*d* : 1954*e*).

Reference may be made to the earlier papers in this series for a description of the compound and the surrounding area as well as for details of entomological studies that formed a portion of the malaria project.

MATERIALS AND METHODS.

Some 6,000 to 7,000 workers are regularly employed in the Ludlow Mill at Chengail. Approximately one-half of this force is composed of people indigenous to the vicinity of the mill and who mostly reside in the nearby villages. The rest of the workers come from provinces other than West Bengal and, with rare exception, live in rented company-owned quarters within the compound. These "labour lines" cover an area 350 yards long and 125 yards wide in which

reside an estimated 4,500 persons, some 1,500 of whom are dependents. Within the lines there are but few Bengalis, so that Oriyas and Madrassis are the largest groups represented. The quarters in the labour lines have been sprayed with D.D.T. three times yearly since 1948 and community sanitation is of a much higher order in the lines than in the surrounding villages.

Thick blood smears (an estimated 12-15 cm. blood per slide) were prepared between 9·30 and 11·30 p.m. during the course of 12 night-time visits to the labour lines. Workers submitted to the preparation of blood smears on a purely voluntary basis, but circumstances precluded on-the-spot physical examination of these persons. However, details of age, sex, province of origin, length of employment at Ludlow, and number of yearly visits home were recorded for each person. All blood films were air-dried, stained with Giemsa's, and examined microscopically under low magnification for the detection of infections and under oil immersion for specific identification of microfilariæ.

BLOOD SMEAR EXAMINATIONS.

The results of the 658 thick-smear examinations are shown by sex and by province of origin in Table I. Forty-four of 571 males were microfilaria-positive (7·7 per cent), while only one positive was found among the 87 females examined (1·1 per cent). Although the highest proportion of microfilaria positives occurred among workers from Uttar Pradesh, this provincial group was one of the smallest in the line population and is therefore of minor consequence. Attention is thus focussed on the workers from Orissa since this was the largest provincial group in the compound population and since 10·4 per cent of this segment had detectable microfilaremia.

TABLE I.

Distribution by sex and by provincial groups of microfilaria-positives among compound-resident workers at the Ludlow Mill, Chengail.

Provincial groups	Males		Females		Totals		
	Number examined	Number positive	Number examined	Number positive	Number examined	Number postive	Per cent positive
Orissa ... ... (1300)	277	29	2	0	279	29	10·4
Madras ... ... (1100)	112	3	81	0	193	3	1·5
Bihar ... ... (500)	100	5	3	1	103	6	5·8
West Bengal ... (600)	57	4	1	0	58	4	6·9
Uttar Pradesh ... (250)	20	3	0	0	20	3	15·0
Madhya Pradesh ... (125)	5	0	0	0	5	0	0·0
TOTALS ... ... (3875)*	571	44	87	1	658	45	6·8†

*Plus other groups to an estimated total of 4,500 persons in the lines.
†The adjusted rate for the entire compound population is 5·5 per cent.

Table II presents, in summary form, the age distribution of all the microfilaria-positive cases. Infections were rare or absent in the age groups under 20 or over 50 ; 95 per cent of all the microfilaria-positives occurred between these extremes with the highest rate of infection in the age group 40-49. The intensity of microfilaremia was estimated by counting all embryos in each thick film. A summary of these findings is presented in Table III, and indicates that the

TABLE II.

Distribution by age of microfilaria-positives among compound-resident workers at the Ludlow Mill, Chengail.

Age groups.	Number.		Per cent positive.
	Examined.	Positive.	
<9	14	0	0·0
10–19	62	1	1·6
20–29	229	17	7·4
30–39	186	13	6·9
40–49	109	13	11·9
50–59	53	1	1·9
60 +	5	0	0·0
TOTALS ...	658	45	6·8

TABLE III.

Distribution of microfilaria-positives by intensity of microfilaremia. All microfilariæ were counted in an estimated 12 to 15 cm. of blood in thick film.

Microfilaremia groups (Count per 12-15 cm.).	Microfilaremia positives.	
	Number.	Per cent.
1–9	28	62·2
10–49	11*	24·5
50–99	4	8·9
100–149	1	2·2
150–199	0	...
200–249	1	2·2
TOTALS	45	100·0

*One person with *W. malayi*. All others in tabulation are *W. bancrofti*.

largest proportion of workers had very light filaremia : thus, 62·2 per cent of the positive thick films contained nine or less microfilariæ while only 4·4 per cent had counts of 100 or more microfilariæ. All of the microfilariæ were identified as those of *Wuchereria bancrofti* with the exception of specimens on a single slide which proved to be those of *W. malayi*. This infection occurred in a 28 year old male Oriya who, upon careful questioning, insisted he had never been out of India nor had he been in any other areas of India apart from his native province and West Bengal. Assuming this information to be correct, this isolated case is of interest since the geographical distribution of *W. malayi* infection in India is still not well defined (Iyengar, 1937).

WORKERS WITH MICROFILAREMIA.

Of the 45 workers who were microfilaria-positive, 37 were available for further questioning and examination after the conclusion of the survey. The mean number of years of employment at Ludlow for this group was 10·9 ; nearly 45 per cent had worked at Ludlow for one to five years, 27 per cent for 6 to 15 years, and the balance for periods of up to 30 years. Almost 50 per cent of the group visited their native province at least once each year, a further 25 per cent made these visits once in two to three years, while the remainder rarely or never went home. Physical examinations of these 37 workers were conducted by the medical staff and revealed hydroceles in five, epididymitis in six, and chyluria in one. Enlarged inguinal glands were found in nearly half of the 37 workers ; however, this condition is so common in the labour population that it is of questionable significance in connection with filariasis.

NATURAL INFECTIONS WITH *CULEX FATIGANS*.

While no intensive attempt was made to collect large numbers of mosquitoes in a search for filaria-infected specimens, some specimens of *C. fatigans* were taken in the Ludlow labour lines and in the lines of the adjacent Gagalbhai Mill during the course of routine catches directed at anophelines (Chernin, 1954*a*). A total of 97 *C. fatigans*, 70 from Ludlow and 27 from Gagalbhai, were dissected during the period April to July, 1952, of which two proved to be infected (Table IV). One specimen taken from Gagalbhai contained a single sausage-stage larva dissected from the thoracic muscles, while three mature larvæ were found in the proboscis of the second infected specimen which had been taken in the Ludlow lines. These specimens all corresponded morphologically to the descriptions of the mosquito stages of *W. bancrofti*.

Mention may also be made of the fact that *Mansonioides uniformis* and *M. annulifera* are both present in the area surrounding Ludlow, although only in very small number (Chernin, 1954*a*). That these species are only infrequently encountered is probably due to the fact that relatively few ponds and tanks in the nearby area contained *Pistia* and that these waters tended to dry out during the rainless periods of the year.

TABLE IV.

The results of dissections of Culex fatigans *taken from the workers' quarters at Ludlow and at an adjacent mill during April to July,* 1952.

Months (1951)	Number.		Per cent positive.
	Dissected.	Positive.	
April ...	18	0	0·0
May ...	21	1*	4·8
June ...	18	1†	5·5
July ...	40	0	0·0
TOTALS ...	97	2	2·1

*One sausage-stage larva in thoracic muscles.
†Three mature larvæ in proboscis.

COMMENT.

According to dispensary statistics, overt elephantiasis of the extremities is exceedingly rare among the Ludlow workers. However, other manifestations of filarial infection, particularly lymphangitis and genital involvements, are more frequently seen, and these are more commonly among workers from Orissa than among those from any other provincial group. It is significant in this connection that every Oriya who was found to be microfilaria-positive during the present survey, came from the heavily endemic districts of Puri or Cuttack. The Oriyas constitute the largest single goup among the compound residents and one can estimate on the basis of the data in Table I that some 135 are probably infected in all. The other provincial groups together might be expected to contribute an additional 125 cases, bringing the total expected number of filaria-infected persons in the labour lines to 260, or about 5·5 per cent of the total population. This prevalence of microfilaria-positives among compound-resident workers at Ludlow is almost half the 9·5 per cent microfilaria rate reported for the general population of Calcutta (Knowles and Basu, 1934), and both figures fall below the 10 per cent level which these authors define as the upper limit for areas of "slight" filarial endemicity.

These comparative figures are of interest because a somewhat higher rate of infection might have been anticipated among the line-resident workers in view of the crowded living conditions that were complicated further by the presence of large numbers of workers from areas of greater endemicity. The rate of filarial infection in the workers compares even more favourably with the data reported by Iyengar (1941) for two rural villages in Birbhum District northwest of Calcutta. Microfilariæ of *W. bancrofti* were found in 17 per cent of the villagers examined there and frank cases of elephantiasis were in evidence. *Anopheles philippinensis* was incriminated as the vector in this rural area in the absence of *C. fatigans*, the occurrence of which had until then been thought to be the factor that strictly limited *W. bancrofti* infections to the urban areas of India. However, *Anopheles*

philippinensis is a very rare species in the Ludlow area (Chernin, 1954*a*) and therefore cannot be of any significance in filarial transmission there. Furthermore, the finding of naturally infected *C. fatigans* is evidence that transmission of the infection can be effected in the lines, despite the community sanitary measures and the possible militating effects of the D.D.T. spray.

Considering all of the data presented, it is plain that the problem of filariasis among the Ludlow workers is not nearly of the same proportions it assumes in more heavily endemic regions nor is it nearly as significant to the local disease complex as is malaria. However, it can be assumed that even a five per cent prevalence of filarial infection among the workers results in some mental distress and illness and that these are in turn reflected in time lost from work, in costs of medical and surgical treatment, and in hampered efficiency.

SUMMARY.

A survey of the prevalence of filariasis was conducted among compound-resident workers at the Ludlow Jute Company's mill at Chengail, Howrah District, West Bengal, from April through June, 1952. Of 658 thick blood smears taken at night, 45 (6·8 per cent) were positive for microfilariæ, 44 with *Wuchereria bancrofti* and one with *W. malayi*. Workers from Orissa constituted the largest single provincial group in the population and of these 10·4 per cent had detectable parasitæmia. Details of the age distribution and the intensity of filaremia are presented together with the results of physical examinations of positive cases. A total of 97 *Culex fatigans* taken from workers' quarters were dissected and two contained larval stages corresponding to those of *W. bancrofti*. The prevalence of filariasis in the worker population is compared with data from other sources and the significance of the infection at Ludlow is discussed.

ACKNOWLEDGEMENTS.

This is the sixth of a series of reports originating from a project sponsored by the Ludlow Manufacturing and Sales Company (Boston) in conjunction with the Harvard School of Public Health. The author is indebted to the officers of the company, to its staff members in Calcutta and at the Chengail Mill, and to Dr. D. L. Augustine, Harvard School of Public Health, for their unstinting co-operation and interest. The author also wishes to express his thanks to Dr. K. L. Basu Mallick, lately Chief Medical Officer at the Chengail Mill, for performing the physical examinations referred to in the text, to Mr. Ravi Iyengar, Entomological Assistant, and Mr. N. N. Chatterjee, Laboratory Technician, for their able efforts during the course of this study.

REFERENCES.

CHERNIN, ELI (1954*a*) ... ... Problems in tropical public health among workers at a jute mill near Calcutta. I. Malaria in the labour population. *Amer. J. Trop. Med. Hyg.*, **3**, pp. 74-93.

Idem (1954*b*) ... ... Problems in tropical public health among workers at a jute mill near Calcutta. II. A study of intestinal parasites in the labour force. *Ibid.*, **3**, pp. 94-106.

Idem (1954*c*) ... ... Problems in tropical public health among workers at a jute mill near Calcutta. III. Intestinal parasites in the European supervisory staff and their food-handler servants. *Ibid.*, **3**, pp. 107-111.

Idem (1954*d*) ... ... Problems in tropical public health among workers at a jute mill near Calcutta. IV. Hæmoglobin values and their relation to the intensity of hookworm infection in the labour force. *Ibid.*, **3**, pp. 338-347.

Idem (1954*e*) ... ... Problems in tropical public health among workers at a jute mill near Calcutta. V. Eosinophile levels and their relation to intestinal helminthiasis in the labour force. *Ibid.*, **3**, pp. 348-355.

Iyengar, M. O. T. (1937) ... ... Public health aspects of filariasis in India. *Ind. Med. Gaz.*, **72**, pp. 300-307.

Idem (1939) ... ... Filariasis in India. *Proc. 3rd Intl. Congr. Trop. Med.*, Amsterdam, **1**, pp. 249-257.

Idem (1944) ... ... Occurrence of *Wuchereria bancrofti* infections in a rural area. *Ind. J. Med. Res.*, **29**, pp. 677-679.

Knowles, R. and Basu, B. C. (1934). ... Mosquito prevalence and mosquito-borne diseases in Calcutta City. *Rec. Mal. Surv. Ind.*, **4**, pp. 291-320.

Indian Journal of Malariology, 8, 2, June 1954.

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

*XVI. Effect of ketogenic diet on the course of blood-induced infection in rats.

BY

S. P. RAMAKRISHNAN.

(*Malaria Institute of India, Delhi.*)

(May 18, 1954.)

THE effects of starvation on the course of infection in rats were described by the author (Ramakrishnan, 1953). It was considered that inhospitable conditions for parasite multiplication in starved hosts were possibly due to two of several factors, namely, that in host starvation some essential nutrient(s) required by the parasite was absent or that ketonæmia or ketosis and allied phenomena in the starved host were inimical to parasite multiplication. The former possibility was experimentally tested by the author and colleagues (Ramakrishnan *et al.*, 1953) and it was found that parasitæmia was considerably higher in starved animals to which methionine was administered. In this paper, the results of experimental verification of the second possibility, are recorded.

METHODS.

Ten rats, each eight weeks old, were used in the experiment. Five of them were on a ketogenic diet, and the rest on standard diet, served as controls. Both groups were preconditioned on respective diets for one week prior to inoculation. The animals were separately housed in special cages where they had no access to their excreta.

The composition of experimental and standard diets is given in Table I. The proportions of proximate principles and calorific values of the experimental diet are given in Table II. Diet was given to each animal once a day on an *ad lib.* basis.

*The investigation was financed from a grant by the Indian Council of Medical Research. This paper forms part of a thesis submitted for Doctorate degree in Public Health.

TABLE I.

Composition of the diets.

Diet group.	Parts per 100 gm. of diet.								
	Whole wheat flour.	Whole milk powder.	Butter.	Calcium carbonate.	Dried brewer's yeast.	Table salt.	Steenbock's salt mixture.	Glucose.	Vitamin supplement.
Standard	72	23	...	1	3	1	...	...	...
High fat (ketogenic)	...	...	93	...	...	...	1	5	1 Vitamin 'B' complex.

TABLE II.

Calorific value, protein, fat and carbohydrate content per 100 *gm. of diets.*

Diet groups.	Calorific value of 100 gm. of diet.	Content per 100 gm. of diet.		
		Protein.	Fat.	Carbohydrate.
Standard ...	378·8	16	7·4	61·8
High fat (ketogenic) ...	700·0	0·56	75·3	5·4

The actual food consumed by rats on the two kinds of diet, was determined by weighing every day, the quantity of food left over in the cages. The results are given in Table III. Animals were weighed twice weekly and their weight progress is summarized in Table IV. The different indices of the course of infection in the experimental and control animals are shown in Table V. The average enlarged spleen volume was determined by the water displacement method described by the author (Ramakrishnan, 1952).

TABLE III.

Relative consumption of the diets.

Diet groups.	Average daily consumption of food in gm. per rat.		
	February.	March.	April.
Standard ...	9·0	4·5	4·9
High fat (ketogenic) ...	5·0	3·0	2·8

TABLE IV.

Weight progress of animals on the two diets.

Diet groups.	Average weight in gm. of animals at different periods.		
	Commencement.	Inoculation.	Death or end of observation.
Standard	93·6	134·0	137·0
High fat (ketogenic) ...	90·0	80·0	54·0

TABLE V.

The course of infection in rats on the two diets.

Age of animals in weeks.	Number of animals.	Diet groups.	Average parasitæmia per 10,000 erythrocytes.		Average enlarged spleen volume in c.c.
			Daily.	Peak.	
	5	Standard.	784	2,690	1·8
8	5	High fat (ketogenic).	139	666	0·52

RESULTS AND DISCUSSION.

It was apparent that the food consumption of rats on the ketogenic diet was lower than that of controls (Table III). This was perhaps due to the food being less palatable than the standard diet. The difference in food consumption was reflected in the weight of the animals during the period of observation (Table IV). While the animals on standard diet progressively increased in weight, the experimental animals lost weight. To that extent, the animals were not of the same nutritional status at the time of inoculation.

The course of parasitæmia in the experimental animals was considerably milder than in controls (Table V). The average daily parasitæmia was 139 as against 784 in the control group. The peak density was 666 as against 2,690. These differences are statistically significant.

The development of ketosis during complete absence of food, even for as short a period as two or three days in man, has been recognized for a long time (Keys *et al.*, 1950). Although none of the animals on ketogenic diet died during the 16 days of patent parasitæmia, every one of them died within 20 days of commencement of latency. As uninfected controls were not maintained, it is not possible to definitely ascribe the cause of death to infection.

The average enlarged spleen volume of the experimental animals was smaller than that of the controls (Table V). One possible reason for this may have been due to the fact that the parasitæmia in the experimental animals was low and consequently the antigenic stimulus was also low. A second reason was also possible, namely, the experimental diet due to its deficiency in protein and carbo-hydrate, impaired the defensive mechanism of the host.

SUMMARY.

A ketogenic diet of a high fat content fed to infected rats, does not favour parasitic growth to the same extent as in controls fed on a balanced diet.

Ketosis of starvation in addition to lack of essential nutrilites like methionine and probably PABA, appears to be responsible for poor parasitic growth in starved rats.

ACKNOWLEDGEMENT.

The author is grateful to Mr. Chanan Singh, M.Sc., Assistant Research Officer, Indian Council of Medical Research, for his help in the feeding and care of the animals.

REFERENCES.

KEYS, A., BROZECK, J., KENSCHELL, A., MICKELSON, O. and TAYLOR, L. H. (1950) ... ... *The biology of human starvation*. First edition. The University of Minnesota Press, Minneapolis.

RAMAKRISHNAN, S. P. (1952) ... ... *Ind. J. Mal.*, **6**, p. 189.

Idem (1953) ... ... *Ibid.*, **7**, p. 53.

RAMAKRISHNAN, S. P., KRISHNASWAMI, A. K., SATYA PRAKASH and CHANAN SINGH (1953) *Ibid.*, **7**, p. 225.

Indian Journal of Malariology, **8**, 2, *June* 1954.

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

*XVII. Effect of different quantities of the same diet on the course of blood-induced infection in rats.

BY

S. P. RAMAKRISHNAN.

(*Malaria Institute of India, Delhi.*)

(May 22, 1954.)

THE experimental study previously reported by the author (Ramakrishnan, 1953) dealt with extreme conditions of starvation and their effect on the course of infection. Further experiments were undertaken to determine the course of infection in partially starved subjects (rats) as such extreme conditions of starvation are rare with particular reference to man.

METHODS.

Six replicate experiments were carried out using a total of 92 rats. In each experiment, 3 to 12 rats were fed on half diet, and three to six others were fed on a standard† full diet. Both the experimental and the control animals were then inoculated with a million parasites each. Data were recorded separately regarding the percentage of animals which died of acute infection, the average daily parasitæmia per 10,000 erythrocytes, and the peak parasitæmia reached in each animal. Thus, in all 41 animals were fed on half and 27 animals on full diet and infected. As a further control, 24 rats were maintained on half diet but without any experimental infection.

*The investigation was financed by the Indian Council of Medical Research. This paper forms part of a thesis submitted for Doctorate degree in Public Health.

†The standard diet contained the following proportions :-

Pea-nuts	8	parts
Whole wheat flour...	70	,,
Skimmed milk powder	16	,,
Dry brewers' yeast	2	,,
Table salt	1	,,
Calcium carbonate	1	,,
Shark liver oil	2	,,
	300	,,

In addition to above, each rat received 2 gm. germinated Bengal gram a day.

Each animal had been pre-conditioned on diet intended for it for a period of two weeks prior to experimental inoculation except in the case of the first two experiments in which this pre-conditioning was prolonged (29 days).

All animals were weighed twice a week regularly. In the first experiment, the amount of food was arbitrarily fixed to be equal in weight to ten and five per cent of the weight of each animal in the control and experimental groups, respectively. The rationing was seen to be inadequate for growth of the young animals. The control animals were consuming all the food offered to them and were recording poor growth, indicating that they themselves were in a state of under nutrition. And the experimental animals were in a much worse state and six out of sixteen died within a week of commencement of conditioning. The scale of rationing was changed to 15 and 7·5 per cent. For sometime, this seemed to be adequate, but a few weeks later, with the onset of summer, the food requirements of the control group were found to be less. A change was made again in the rationing which was reduced to 12 and 6 per cent. At this stage, it was noticed that animals of experimental group were increasing in weight instead of losing it. Arbitrary rationing proved erroneous for the purposes of investigation, but it was continued up to the termination of this experiment.

From the second experiment onwards, a new procedure for feeding was adopted. Control animals were given food of known weight in excess of their daily consumption. The quantity of food left over every morning in each individual cage was weighed and the actual daily consumption per 100 gm. body weight of each control animal was determined. Each experimental animal was provided with 50 per cent of this amount of food by weight per 100 gm. body weight. The change in rationing was made twice a week as before. This method appeared to be rational and ensured that control animals were well and fully nourished while the experimental animals were half nourished or under-nourished.

The average spleen volume of the animals were determined by the water displacement method described by the author (Ramakrishnan, 1952).

RESULTS.

In each experiment, the animals were of the same age group, and all conditions like care, exercise and composition of food, were identical. The only variable was the quantity of food. But the increase or decrease in weight was not consistent with the quantity of food consumed as seen in Table I. It appeared that the quantity of the diet consumed was not the only factor in increasing or reducing the weight of an animal. This emphasized individual variations in the response of animals to any particular diet.

Table II shows that, excluding the three animals in the first experiment which lived longer presumably on account of factors of under-nourishment not being precisely secured, the remaining 21 animals lived for a varying period of time ranging from five to 46 days with an average longevity of 19·4 days. The 41 infected animals, fed on half diet, had a longevity varying from five to 25 days. As the variation in longevity of the half-fed rats even in the absence of experimental infection is pretty wide, no statistical evidence for the differences being significant, could be furnished.

TABLE I.

Nutritional status of control and experimental animals at the time of inoculation.

Experiment number.		1		2		3		4		5		6	
Average age, in weeks, at commencement		7–9		7–9		24		7–9		24		27	
Groups.		Control.	Experimental.	Control.	Experimental.	Control.	Experimental.	Control.	Experimental.	Control.	Experimental.	Control.	Experimental.
Number of animals ...		6	8	3	6	5	12	5	12	4	4	6	11
Average weight in grammes	Commencement	71·5	74	76	99	180	157·5	129	127	179	145	147	147
	Inoculation ...	125	64	131	98	195	142	151	99·5	227·5	144	182	138
	Increase or decrease	+53·5	−10	+55	−1	15	−15·5	+22	−27·5	+48·5	−1	+35	−9

TABLE II.

Effect of undernourishment on the longevity of uninfected controls.

Experiment number	Age in weeks.	Number of half-fed uninfected controls.	Day of death from the end of pre-conditioning.
I.	7–9	6	8, 5, 13, 143, 132, 171
II.	7–9	2	10, 19
III.	24	8	11, 17, 15, 19, 18, 15, 16, 15
IV.	7–9	3	30, 17, 30
V.	24	2	13, 11
VI.	27	3	33, 46*, 46*, 33, 46, 46

*Returned to colony from 46th day.

Table III shows that, out of 41 experimentally infected half-starved animals, 30 (73·2 per cent) died during the stage of acute parasitæmia. In 27 control infected animals fed on standard diet, 16 (59·3 per cent) died of acute infection. This difference, however, is not statistically significant. Table III also shows the average daily parasitæmia per 10,000 erythrocytes in the experimental and control animals in each experiment as well as the peak parasite densities reached. These two indices are generally much lower in the under-nourished animals than in the control animals on full diet. In the sixth experiment, however, the peak parasitæmia in the experimental animals was slightly higher than in the control animals.

A statistical analysis of the differences observed between peak parasitæmia and average daily parasitæmia in the experimental and control animals in each of the six experiments show that in 11 out of 12 instances, the experimental group had a lower index, and in six the differences were statistically significant, but not in the remaining five. The consistency of the lower indices in each case and the significance of the differences in six out of 12 trials, lends support to the experimental data, yielding a logical conclusion.

TABLE III.

Course of infection in half and full fed rats.

Experiment number.	Age of animals in weeks.	Group.	Number of animals.	Number of animals which died of acute infection.	Average parasitæmia per 10,000 erythrocytes.	
					Daily.	Peak.
I.	7-9	Experimental	7*	4	276	708
		Control	6	1	296	739
II.	7-9	Experimental	5*	4	145	365
		Control	3	2	378	856
III.	24	Experimental	8	8	67	222
		Control	3	1	399	1068
IV.	7-9	Experimental	10	10	170	785
		Control	5	5	457	1182
V.	24	Experimental	1	1	164	620
		Control	4	4	777	2462
VI.	27	Experimental	10	3	204	737
		Control	6	3	240	593

*Survivors challenged with two million homologous parasites in the fifth month of infection with negative results.

Three parasitic relapses were encountered among the nine survivors of acute infection among full-fed control animals in experiments I, II and VI. Scanty parasites were patent only for a day or two during the relapses. In all, only eleven half-fed animals survived the acute infection and of these only one (the sole survivor out of five in experiment II) developed a relapse which lasted for thirteen days and ended fatally. In this case, the parasites during the relapse remained patent for an unusually long period, namely, 13 days ; and the parasitæmia was considerably high for a relapse with a peak of 108 parasites per 10,000 erythrocytes. This density is considerably more than in the relapses not only of the control animals of the present investigation, but of a large number of animals observed in

the course of other studies by the author and his colleagues (Ramakrishnan *et al.*, 1951). It will be observed that it is extremely difficult in laboratory experiments to secure an adequate number of survivors amongst half-fed infected animals and still more difficult to get an adequate number with relapses.

The consistently smaller average spleen volume of the experimental group as compared to the controls indicated that the immunity acquired by the former was less than the latter (Table IV). But the degree of immunity acquired by the experimental animals was found to be adequate enough to nullify a challenge inoculation in the fifth month of the original infection.

TABLE IV.

Effect of diet on the size of spleen of infected rats.

Experiment number.	1		2		3		4		5		6	
Group of animals on	Number of animals.	Average enlarged spleen volume in c.c.	Number of animals.	Average enlarged spleen volume in c.c.	Number of animals.	Average enlarged spleen volume in c.c.	Number of animals.	Average enlarged spleen volume in c.c.	Number of animals.	Average enlarged spleen volume in c.c.	Number of animals.	Average enlarged spleen volume in c.c.
Half diet, uninfected	5	0·32	1	0·4	8	0·34	2	0·25	1	0·4	...	...
Half diet, infected	6	0·4	5	0·6	8	0·4	10	0·54	1	0·8	10	0·9
Standard diet, infected	6	1·6	1	0·2	5	1·8	4	0·9	4	2·9	6	1·8

DISCUSSION.

On a combined analysis of the effects of starvation previously described by the author (Ramakrishnan, 1953) and the present results, the effect of nutrition on the host parasite relationship would appear to be somewhat as follows. Under-nourishment of the host affects the nutritional requirements of the parasite, and therefore its rate of multiplication, and still further its capacity for antigenic stimulation. As regards the acquisition of specific immunity on the part of the host against the particular pathogen, it is not possible to obtain clear-cut evidence, because of the fact that in under-nourished host, the antigenic stimulus itself is quantitatively lower. Possibly on account of the lowered virulence of the parasite under conditions of partial starvation of the host, its capacity to relapse also seems to be greatly diminished. But when in certain under-nourished hosts, the parasite is somehow able to maintain itself and relapse, on account of the lack of sufficiency of stimulation of specific immunity on the part of the host, it reaches concentration higher than in fully fed animals in which the stimulus to the specific immunity potential is of a higher order. But the parasitic density reached during relapse in an under-nourished host is not so high as during primary parasitæmia of a fully fed animal, and yet with such density, the host appears to react very unfavourably with a fatal end. This would perhaps only be due to the

fact that, on account of under-nourishment during the relapse, the host is unable to withstand even a milder degree of parasitæmia than it was able to during the course of primary parasitæmia. In normal times, a good majority of the communities may be said to be under conditions of poor nourishment. Quite possibly, while under-nourishment of the host in such cases is not favourable to as rapid and as high a degree of multiplication of the parasites as in well nourished individuals, when relapses occur amongst a certain number of these infections, the hosts react unfavourably to the effect of parasitic multiplication. In normal times, the degree of undernourishment may not be so acute as very largely to interfere with the rate of multiplication of the parasites. In famine, however, conditions are different. During the acute period of famine, the degree of starvation reached in a good proportion of the community, is very high. This apparently greatly reduces the capacity of the parasites to multiply, and hence during the period of semi acute starvation in famine, examinations of the peripheral blood may fail to reveal patent parasitæmia as shown experimentally by the author (Ramakrishnan, 1953). When, however, either the famine conditions disappear or special efforts are undertaken to establish feeding centres and the hosts are provided with increased nourishment, dormant infections may manifest themselves patently. When a good many amongst them relapse, mortality rate is considerably enhanced. Thus, during the acute famine phase, direct mortality due to malaria is by no means high, though the mortality due to starvation completely masks deaths due to other causes. In the Bengal famine of 1943, conditions similar to what are described above, actually seem to have occurred. The Famine Enquiry Commission's Report on page 118 states, " From about December, 1943, onwards, there was a change in the clinical picture seen in hospitals. Most of the beds were filled with cases of malaria. The number of cases of famine œdema gradually diminished during the early months of 1944. Cases of acute starvation and emaciation became relatively rare. Patients in general were thin and weak, and obviously required plenty of nourishing food to restore them to health. The majority were anæmic. There was, however, a genuine improvement in the state of nutrition ".

The malaria transmission in Bengal is generally from July to December. Famine conditions reached their peak at the commencement of 1943. Relief measures with all their limitations were instituted by about September of the same year. In December, 1943, about two to three months after the relief measures were commenced, the reported deaths from malaria were 202·6 per cent in excess of the quinquennial average. From July to December, 1943, malarial deaths were 125·1 per cent in excess of the quinquennial average. In the first six months of 1944 when transmission was low or absent, the malarial deaths were 126·1 per cent above the quinquennial average. It would thus seem clear that until relief measures were established by about September, 1943, the bulk of the deaths was directly due to starvation, and malaria played very little part as a force or mortality. Later on when facilities for feeding the communities were organized and starvation *per se* became a factor of lesser importance as a cause of death, the infections acquired amongst starved men from July, 1943, onwards, became much more manifest after feeding was begun and malaria began to exert itself as a very distinct force of mortality. On account of the fact that the infections acquired in the earlier part of the malaria season could not establish themselves

but remained latent and became manifest towards the last quarter of the year at a time when feeding arrangements were made available, relapses continued well into the first quarter of the following year amongst people who had been exposed to the rigour of starvation and perhaps even later could only get partial nourishment. These were the people who succumbed more readily to the effect of relapses.

CONCLUSIONS.

Under-nourishment of the host affects the parasite much more than the host during the stage of primary parasitæmia. Both the average daily parasitæmia and the peak density reached, are lower in the under-nourished host. Perhaps, on this account the capacity of the parasite to relapse, is also low. But when it does relapse, the densities reached are much higher than in the case of fully fed hosts, presumably because of the absence of development of an adequate degree of acquired immunity, either on account of the effect of under-nourishment on the defence mechanism of the host or on account of a lower antigenic stimulation during the period of primary parasitæmia. Though the density reached during the relapse in an under-fed host is not as high as in the case of a fully fed host in primary parasitæmia, the host reacts very adversely to such relapses.

Applying the above findings to the epidemiology of malaria in man, under-nourishment met with in normal times presumably has a tendency to reduce the average daily parasitæmia and the peak density reached in the primary attack. But when relapses occur, the host reacts adversely, and as a result of it, malaria mortality during relapses is considerably enhanced in under-nourished communities.

In famine conditions, during the period of active malaria transmission there is practically a very low grade parasitæmia on account of the absence of nutrition for the parasite. The direct malaria mortality cannot be assessed under such conditions as extreme starvation by itself accounts for a great deal of mortality. When famine conditions lessen in their intensity and simultaneously facilities for feeding are established, the latent parasitæmia becomes patent, and on account of the previous gross under-nourishment of the host, considerably increases malaria mortality. Mortality continues to remain high on account of the long continued relapses which occur in a certain proportion of cases even when transmission has ceased and the hosts often react very adversely during such relapses.

SUMMARY.

The primary parasitæmia has been found to be less intense in the under-nourished animals than in the well-nourished controls. A fatal parasitological relapse occurred in only one animal that survived out of a group of five on half diet. Both the duration and the parasite density of the relapse, were considerably longer and higher, respectively, than in relapses in several animals on normal diet.

The effect of semi-starvation on host-parasite relationship in rodent malaria is discussed and inferences in relation to human malarial have been made.

ACKNOWLEDGEMENT.

The author is grateful to Mr. Chanan Singh, M.Sc., for his help in the care and feeding of animals.

REFERENCES.

FAMINE ENQUIRY COMMISSION REPORT ON BENGAL (1945) ... ... *Govt. of India Press*, New Delhi, p. 118.

RAMAKRISHNAN, S. P. (1952) ... ... *Ind. J. Mal.*, **6**, 2, pp. 189-198.

Idem (1953) ... ... *Ibid.*, **7**, pp. 53-60.

RAMAKRISHNAN, S. P., SATYA PRAKASH and KRISHNASWAMI, A. K. (1951) ... *Ibid.*, **5**, pp. 447-454.

Indian Journal of Malariology, **8**, 2, June 1954.

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

*XVIII. Effect of diet different in quality but adequate in quantity on the course of blood-induced infection in rats.

BY

S. P. RAMAKRISHNAN.

Malaria Institute of India, Delhi.

(May 10, 1954.)

THE growth of Plasmodium in a susceptible host is most intimately related to the environment in which it finds itself, and environment in its turn is controlled by at least three known factors, namely (1) adequate availability of nutrients required by the parasites, (2) capacity of the host to exert its innate immunity to any infection, and (3) degree of specific immunity acquired by the host. All these factors are influenced to some extent by the food available to the host. Broadly speaking, the food can be adequate or otherwise in quantity or quality. Quantitative deficiency gives rise to under-nutrition and qualitative deficiency to malnutrition. The effects of starvation as well as under-nutrition have already been reported by the author (Ramakrishnan, 1953 : 1954).

In the present report, the effect on the course of infection in rats, of different types of diet in general consumption by human beings, is considered. The diets were adequate in quantity in every case.

METHODS.

One hundred and thirty-two rats were used for the study which consisted of six experiments some of which were replicates. The animals in each group, experimental as well as control, were nearly of the same age and sex distribution.

Different diets used in the experiments were generally of the types representing vegetarian, lacto-vegetarian and mixed (containing meat). A few were specially designed for high calorific values to determine their effect, irrespective

*The investigation was financed from a grant by the Indian Council of Medical Research. This paper forms part of a thesis submitted for Doctorate degree in Public Health.

of the proportion of proximate principles. Composition and calorific values of the different diets, and the relative proportion of proximate principles in each, are shown in Tables I and II, respectively. As far as possible it was ensured that each diet was not deficient in any of the vitamins or minerals.

TABLE I.

Composition of diets.

Diet.	Diet number.	Parts of ingredients per 100 gm. of diet.									Remarks.
		Whole wheat flour.	Whole milk powder.	Pea-nut kernel.	Rice.	Lean meat.	Calcium carbonate.	Dried brewer's yeast.	Table salt.	Steenbock's salt mixture.	
Standard	1	72	23	...	...	...	1	3	1	...	...
Rice and milk	2	...	23	...	72	...	1	3	1	...	...
Rice	3	...	...	...	95	...	1	3	1	...	2 drops of adexolin and wheat germ oil per rat per week.
Wheat	4	95	...	...	...	...	1	3	1	...	2 drops of adexolin per rat per week.
Meat (high)	5	...	...	...	...	95 (dry)	1	3	1	...	2 drops of adexolin per rat per week.
Meat (low)	6	...	...	...	79	16 (dry)	1	3	1	...	2 drops of adexolin per rat per week.
Rice and pea-nut	7	...	...	18	77	...	1	3	1	...	2 drops of adexolin and wheat germ oil per rat per week.

NOTE.—Vegetables at the rate of 5 mg. per rat per day were given to animals on rice, rice and milk and their controls on standard diet.

TABLE II.

Calorific values, protein, fat and carbohydrate content per 100 gm. of diets.

Diet number.	Diet.	Calorific value per 100 gm. of diet.	Content per 100 gm. of diet of :		
			Protein.	Fat.	Carbohydrate.
1.	Standard	378·8	16·0	7·4	67·8
2.	Rice and milk	375·8	12·4	6·5	66·7
3.	Rice	342·0	8·0	0·38	76·1
4.	Wheat	345·8	12·9	1·7	69·7
5.	Meat (high)	563·4	54·1	1·1	37·9
6.	Meat (low)	379·2	15·7	6·7	63·5
7.	Rice and pea-nut	378·2	11·6	7·5	65·6

The ingredients of each diet, except meat, were mixed together dry in required proportions and the quantity prepared at a time was sufficient to last for two to three weeks and was stored in a refrigerator. Meat diets were prepared daily by boiling the calculated amount of meat in water for 10 minutes and mixing it with the rest of the ingredients already in cold storage. The amount of diet placed in the cage for each animal once a day, was in excess of its consumption in 24 hours.

The period of pre-conditioning of experimental animals on vegetarian and lacto-vegetarian diets varied from three to four weeks. Those on meat diets were pre-conditioned for two weeks, and the group on different isocalorific diets for one week.

The average enlarged spleen volume was determined by the water displacement method described previously by the author (Ramakrishnan, 1952). The dose, method of inoculation and the criteria for assessment of results, were the same as described previously by the author (Ramakrishnan, 1954).

RESULTS.

Eighteen uninfected controls on different diets survived the period of observation (four to five months) indicating that the various diets included in the experiments were not deficient to the extent of causing death.

Table III records the nutritional status of animals on different diets at the time of inoculation. All groups, except those on rice and pea-nut, showed an increase in weight at the end of the pre-conditioning period.

TABLE III.

Nutritional status of animals on different diets at the time of inoculation.

Group	Vegetarian				Lacto-vegetarian				Mixed			
Experiment number	I, II, V and VI.				I and II.				III to VI.			
Age of animals at commencement, in weeks	42	10	8	28	42	10	42	10	9	24	28	8
Diets	Rice	Rice	Wheat	Rice and pea-nut	Rice and milk	Rice and milk	Wheat and milk	Wheat and milk	Meat high	Meat low	Meat high	Meat high
Average weight, in grammes, at commencement	129	89	95	148	133	90	160	92	68	146	147	86
Average weight, in grammes, at inoculation	182	172	102	148	193	188	194	170	132	155	158	106
Average weight, increase or decrease	+53	+83	+7	0	+60	+98	+34	+78	+64	+9	+11	+20

It is of interest that in accordance with popular impressions, diets with rice contributed to the greatest increase in weight, while wheat alone or with milk gave rise to less increase (Table III). Except in younger age groups, meat diet resulted only in a moderate weight increase.

VEGETARIAN DIET AND INFECTION.

In the first experiment (Table IV), the animals used were forty-two weeks old. The average daily parasitæmia in the experimental animals on rice diet was 125 as against 72 in those on the standard diet. The difference, however, is not statistically significant within the limits of the experiment, being restricted to two animals in each group. The peak parasitæmia reached is lower in the experimental animals than in the control. Taken together, in older animals no difference is established as regards the parasitic behaviour with rice and standard diets, respectively. In the second experiment in which younger and relatively larger number of animals were used, the average daily parasitæmia is higher in the experimental animals on rice diet as also the peak parasitæmia. The higher daily parasitæmia is statistically significant though not the higher peak parasitæmia. Taken together, it would seem that in younger animals, rice diet tends to favour the parasitic multiplication during the stage of primary parasitæmia. In the fifth experiment in which vegetable protein in the shape of pea-nut was added to the rice diet, there is practically no difference either in the average daily parasitæmia or in the peak parasitæmia reached, as compared with the control animals on a standard lacto-vegetarian diet. In the last experiment, comparing the effect of wheat diet and standard diet, there is no difference as regards the peak parasitæmia reached. But the average daily parasitæmia is slightly higher than in the experimental group, but the difference is not statistically significant.

TABLE IV.

Course of acute infection in rats on vegetarian diets.

Experiment number	Age of animals, in weeks	Number of animals	Diet	AVERAGE PARASITÆMIA PER 10,000 ERYTHROCYTES.	
				Daily	Peak
I.	42	2	Rice	125	195
		2	Standard	72	261
II.	10	7	Rice	381	1335
		7	Standard	182	691
V.	28	11	Rice and pea-nut	311	650
		12	Standard	308	637
VI.	8	5	Wheat	996	2400
		5	Standard	829	2436

In general, the average enlarged spleen volume of animals on vegetarian diet was smaller than that of animals on standard diet (Table VII) which is consistent with the hypothesis earlier assumed that vegetarian diets in general, and rice diet in particular, were associated with a lesser degree of operation of the forces of immunity.

TABLE V.

Course of acute infection in rats on mixed diets.

Experiment number	Age of animals, in weeks	Number of animals	Diets	Average parasitæmia per 100,000 erythrocytes	
				Daily	Peak
III	9*	15	Meat (high)	413	988
		4	Standard	397	936
IV	24	11	Meat (low)	594	1238
		3	Standard	631	1623
V	28	11	Meat (high)	431	996
		8	Meat (low)	198	467
		12	Standard	308	637
VI	8	5	Meat (high)	1046	2352
		5	Standard	829	2436

*Vegetables 5 gm. a day per rat were given.

MIXED DIET AND INFECTION.

The course of acute infection in animals on a diet with a high proportion of meat was generally more severe than in animals on standard diet (Table V). In the case of animals fed on a low meat diet but isocalorific with standard diet, the average daily parasitæmia was lower as also the peak parasitæmia. The difference between a low meat diet isocalorific with standard diet and standard diet, was alone statistically significant. This shows that a mixed diet favours the host better than a diet of equal calorific value but without any meat. Mixed diets of higher calorific values secured by larger proportions of meat, seem to have either an adverse effect on the host, or a favourable effect on the parasites, presumably the latter. Though the differences are not statistically significant, the fact that the differences are consistent in each of the three experiments, and the differences are in the same direction with respect to both the indices, namely, average daily parasitæmia and peak parasitæmia reached, would add considerable validity to the data recorded and the conclusion reached.

As regards survivals from acute infections, by far the largest number of deaths occurred in the group fed on a mixed diet (Table VI). Likewise, the survival rate was least in the group on rice and pea-nut, the highest in animals fed on rice diet, and middling on those fed on wheat.

Relapses occurred in three out of nine survivors on rice diet, neither of the two animals on wheat diet, five out of nine survivors on a standard lacto-vegetarian diet, and none out of the survivors on meat diet (the period of survival being however not more than two to three weeks after the subsidence of acute infection

and the establishment of latency) showed relapses. Excluding the animals on meat diet, which for some reason did not survive for long after the subsidence of primary parasitæmia, rice diet seems to favour relapses as opposed to wheat diet or rice fortified by pea-nut. But the proportion of relapses on rice diet is certainly not higher than in the case of a standard-lacto-vegetarian diet. These results, however, do not seem to be of any special significance.

The average enlarged spleen volume of animals on meat diet was equal to that of animals on a standard lacto-vegetarian diet and larger than that of animals on vegetarian diet.

TABLE VI.

Course of chronic infection in rats on different diets.

Groups	Vegetarian				Lacto-vegetarian				Mixed			
Experiment numbers	I to VI				I, II, V and VI.				III to VI.			
Age of animals, in weeks	42	10	28	8	42	10	42	10	9	24	28	8
Diets	Rice	Rice	Rice and pea-nut	Wheat	Rice and milk	Rice and milk	Wheat and milk	Wheat and milk	Meat (high)	Meat (low)	Meat (high)	Meat (high)
Number of survivors of acute infection	2*	7*	2	3	2*	7*	2*	7*	15	1	0	4
Mortality of survivors within three months of inoculation	0	0	2	0	0	4	0	4	15	0	0	0

*Challenged in the fourth month by two million homologous parasites with negative results.

TABLE VII.

The effect of different diets on the average enlarged spleen volumes of infected rats.

Diet groups	Vegetarian						Lacto-vegetarian						Mixed				
Experiment numbers ...	I, II, III, V and VI						I, II, V and VI						III, IV, V and VI				
Age of animals in weeks	42	10	28	8	42	10	8	9	10	24	28	42	8	9	24	28	28
Numbers of animals whose spleens were measured	2	6	7	2	2	6	2	4	7	3	12	2	1	14	10	11	8
Diet	Rice	Rice	Rice and pea-nut	Wheat	Rice and milk	Rice and milk	Control animals on standard diet consisting of wheat and milk.						Meat (high)	Meat (high)	Meat (low)	Meat (high)	Meat (low)
Average enlarged spleen volume in c.c.	0·8	1·1	1·2	1·0	1·2	1·0	1·8	1·7	1·2	2·0	1·1	1·0	3·0	0·8	1·4	1·8	1·9

DISCUSSION.

The same age group of animals could not be used in all the experiments owing to their non-availability. As already stated, it was ensured that the experimental and control animals of any particular experiment were of the same age.

It was not possible to ensure the same calorific value, nor identical proportions of proximate principles in each diet. But such variations are "normal" in the different diets of human beings of different countries, climates and races. Indeed, it has not been possible to arrive at precise standard requirements of protein to man (Jolliffe, Tisdall and Cannon, 1950). Within certain limits, the proximate principles are capable of being interconvertible in the living animal (Hawk, Oser and Summerson, 1949). In addition, fats and carbohydrates have a certain sparing action on proteins. In view of the above, minor differences in the proportion of proximate principles in a diet, seem to be of little importance.

The results of the present experiments confirm the findings by Passmore and Sommerville (1940) that the severity of first attacks of malaria in individuals, is not mitigated by a good state of nutrition. Presuming that diets inclusive of meat promote better nutrition, it was found that when meat in a diet was excessive, the course of acute infection was more severe. When meat was "adequate" in quantity in a diet which was isocalorific to standard diet, the acute infection tended to be mild. In the final analysis of host parasite relationship, vegetarian and lacto-vegetarian diets seem to affect the host more favourably than meat diet, but a mixed diet with low proportion of meat, affords the host the best assistance.

The rôle of protein in the diet in relation to course of infection appears to be different in the present experiment from that in studies made by Seeler and Ott (1945) on avian malaria. They found that protein deficiency in chicks caused a more severe acute infection than in controls, and that the deficient birds were unable to clear parasites from the peripheral blood as rapidly as well nourished ones. The diet composition in their experiments was of pure chemical foods, while in the present series it was of natural food stuffs. Animals fed on diet with high proportion of protein of animal origin (meat) as compared to those with less proportion of meat, suffered from a more severe acute as well as chronic infection. Between two isocalorific diets one with meat and the other with milk, the former was responsible for a milder acute infection than the latter. Animals fed on vegetarian diets with low protein and high carbohydrate content, suffered from a more severe acute infection than their controls on lacto-vegetarian diet. In no instance, however, was the infection so severe as in animals on a diet with high proportion of meat protein.

As already stated, animals fed on a rice diet, enriched by protein of peanut, suffered from a milder acute infection than those fed on a diet of rice alone. This could probably have been due to the higher plant protein content of the diet increasing the capacity of the host to acquire immunity.

Brooke (1945) concluded from experiments on avain malaria that birds on experimental diets which contained high proportion of carbohydrates and poor in practically all other nutrient values, suffered from a more severe acute infection, a greater tendency to relapse, and less resistance to superinfection, than control animals fed on balanced diet. In the author's experiments also, rats on vegetarian

diets rich in carbohydrates but adequate in accessory factors, suffered from a more severe acute infection than their controls on lacto-vegetarian diet. But the infection was considerably milder in the former in comparison with that in animals fed on a high protein (meat) diet.

The relapse rates in animals on vegetarian and lacto-vegetarian diets were about equal while relapses were absent in survivors on meat diet. But as against the absence of relapse, a larger number of animals on meat diets apparently succumbed to chronic infection, as compared with those on vegetarian and lacto-vegetarian diets.

The infection in animals fed on diets which included milk, was consistently milder than those fed on pure vegetarian or mixed diets. It almost seemed that milk contained a protective factor which was absent in diets without it, whether of low protein content as in the case of vegetarian diets or of excessive protein content as in the case of diet with high proportion of meat. In two experiments, however, where the experimental diet contained the same proportion of meat, and was isocalorific with standard diet, the course of infection was mild, but in view of the high mortality in animals on such a diet, despite the mild course of infection, it seemed that protein of milk was superior to that of meat in promoting the defensive mechanism of the host.

SUMMARY AND CONCLUSIONS.

Vegetarian diets of a high carbohydrate content, containing rice or wheat, gave rise to a more severe acute infection than balanced diet. Very few of the animals on such a diet died of chronic infection and the degree of acquired immunity was sufficient to nullify a challenge inoculation.

Animals on a diet with a high proportion of meat, suffered from a much more severe acute as well as chronic infection than those on any other diet. This seemed logical in view of the fact that such a diet probably provided in abundance all the nutrients required by the parasites, far in excess of its capacity to enhance the defensive mechanism of the host.

Among the different proteins constituting different diets, milk protein seemed superior to others in its beneficial influence on the host.

ACKNOWLEDGEMENT.

The author is grateful to Mr. Chanan Singh, M.Sc., for his help in the care and feeding of animals.

REFERENCES.

BROOKE, M. M. (1945) ... ... *Amer. J. Hyg.*, **41**, pp. 81-108.

HAWK, P. B., OSER, B. L. and SUMMERSON, W. H. (1940) ... ... *Practical physiological chemistry*. 12th edition. The Blackiston Company, Philadelphia and Toronto.

JOLIFFE, N., TISDALL, F. F. and CANNON, P. R. (1950) ... ... *Clinical nutrition*. B. Hoeber Ind. Medical Book Depot, Harper Brothers, New York.

PASSMORE, R., and SOMMERVILLE, T. (1949) ... ... *J. Mal. Inst. Ind.*, **3**, pp. 447-455.
RAMAKRISHNAN, S. P. (1952) ... ... *Ind. J. Mal.*, **6**, pp. 189-198.
Idem (1953) ... ... *Ibid.*, **7**, pp. 53-60.
Idem (1954) ... ... *Ibid.*, **8**, pp. 87-94.
SEELER, A. O. and OTT, W. H. (1945) ... *J. Inf. Dis.*, **77**, pp. 181-184.

Indian Journal of Malariology, **8**, 2, *June* 1954.

STUDIES ON *PLASMODIUM BERGHEI* VINCKE AND LIPS. 1948.

*XIX. The course of blood-induced infection in pyridoxine or Vitamin B_6 deficient rats.

BY

S. P. RAMAKRISHNAN.

(*Malaria Institute of India, Delhi.*)

(May 22, 1954.)

It has been well established that vitamins are required by malaria parasites, and that the requirements probably differ from species to species (Editorial, *Lancet*, 1947). Trager (1949) reviewed the literature on vitamin requirements of *Plasmodia*, and the available information on the subject is summarized in Table I.

Seeler and Ott (1945) deduced that pyridoxine was essential to *Plasmodium lophurae* for its growth in ducklings from their experiments on biochemical antagonism between the vitamin and quinine as well as atebrin in a manner similar to that between PABA and sulphonamides. Storeck, Eisen and John (1947) showed striking impairment of antibody response to sheep erythrocytes as well as loss of lymphoid tissue in pyridoxine deficient rats.

As far as is known, the experiment described here is the first to establish any direct relationship between pyridoxine deficiency of the host and malaria.

METHODS.

The experiment was carried out on 12 albino rats, ten of these were six to seven weeks old and the remaining two were eighteen weeks old. The sex composition was equal. The animals were kept in specially designed cages in order to prevent coprophagy.

A diet deficient in pyridoxine (Appendix 1) modified from that used by Gubler, Cartwright and Wintrobe (1949) in their experiments, was served daily *ad lib.* in aluminium cups to each of the six animals under experiment. Of the two control groups of three animals each, one received the same diet as the

*The investigation was financed by a grant from the Indian Council of Medical Research. This paper forms part of a thesis submitted for a Doctorate degree in Public Health.

experimental group and was supplemented by an aqueous solution containing 75γ of pyridoxine hydrochloride per rat per day, administered orally. Animals of second control group were fed on standard diet (Appendix I) also on an *ad lib.* basis.

TABLE I.

Effects of vitamin deficiency in different hosts on their respective malaria parasites.

Host	Parasite	Deficiency	Parasitæmia in deficient hosts as compared to controls	Probable effect on immunity mechanism of deficient hosts as compared to that of controls	Observer (s)
Duck	*P. lophuræ*	Biotin	Higher	Depressed	Trager (1949).
Chicken	*P. lophuræ* and *P. cathemerium*	Biotin	Higher	Depressed	Seeler, Ott and Gundel (1944).
Chicken	*P. lophuræ*	Thiamine	Higher	Depressed	Seeler and Ott (1946)
Pigeon	*P. relictum*	Thiamine	Higher	Not known	Brooke (1945).
Chicken	*P. gallinaceum*	Pantothenic acid	Lower	Not known	Brackett, Waletzky and Baker (1946).
Chicken	*P. lophuræ*	Pantothenic acid	Lower	Not known	Seeler and Ott (1944).
Chicken	*P. lophuræ*	Folic acid	Higher	Not known	Seeler and Ott (1946).
Chicken	*P. lophuræ*	Vitamin A or Niacin	Lower	Not known	Roos, Hegsted and Stare (1946).
Albino mouse	*P. berghei*	Vitamin A or Niacin	Lower	Depressed	Fabiani and Grellet (1952).
Chicken	*P. lophuræ*	Riboflavin	Lower	Depressed	Seeler and Ott(1944).
Monkey	*P. knowlesi*	Vitamin C	Lower	Not known	Mackee and Geiman (1946)
Albino rat	*P. berghei*	PABA	Lower	Not known	Hawking (1953)

The period of pre-conditioning of the animals prior to inoculation was prolonged and lasted 121 days in the case of experimental group as well as the controls. The average daily consumption of food per rat, of all the animals, was determined. The animals were weighed twice a week throughout the observation period which lasted till the death of the animals. The maximum period inclusive of the pre-conditioning period was 231 days.

The average enlarged spleen volume was determined by water displacement method described by the author (Ramakrishnan, 1952). The method and dose of inoculation, and determination of indices to assess the course of infection, were the same as described by the author (Ramakrishnan, 1953).

RESULTS.

The daily average food consumption of rats did not remain constant for animals in any of the three groups (Table II). Peak food consumption for all the animals was in the second month of observation. From the third month onwards, it fell for all the groups, the highest reduction was in the experimental group on pyridoxine deficient diet, the least in the group on standard diet, and intermediate in the group on the fortified experimental diet. These differences were probably due to impairment of appetite of the first and the last groups of animals.

TABLE II.

Average daily food consumption per rat.

Diet group.	AVERAGE FOOD CONSUMPTION IN GRAMMES PER RAT PER DAY.						
	January.	February.	March.	April.	May.	June.	July.
Pyridoxine deficient ...	13·2	15·4	8·0	5·8	5·4	5·3	9·1
Pyridoxine deficient *plus* pyridoxine hydrochloride	13·3	15·6	11·2	8·3	4·7	...	...
Standard ...	8·0	12·6	12·4	10·3	9·1	...	...

The difference in the food consumption was reflected in the weights of the animals (Table III). At the end of the pre-conditioning period, the group on the deficient diet showed a smaller increase in weight per cent than the group on the non-deficient diets.

TABLE III.

Weight of animals in the course of the experiment.

Diet group.	Number of animals.	AVERAGE WEIGHT IN GRAMMES.		
		At commencement.	On inoculation.	At the end of observation or death.
Pyridoxine deficient ...	6	94	171	172
Pyridoxine deficient diet *plus* pyridoxine hydrochloride ...	3	112	240	277
Standard (18 weeks) ...	2	286	266	270
R. 1164 (6 weeks. ...	1	60	192	184

The signs of pyridoxine deficiency in rats were reviewed by Sinclair (1952) quoted by Bacon (1953). The signs included changes in the skin, neurological manifestations such as a characteristic gait and occasional epileptiform fits, and hæmatological changes such as microcytic anæmia.

Every one of the experimental rats displayed dermal changes, namely patchy hyperæmia giving rise to a generalized brown colouration of the fur. No neurological manifestations were seen in any of the rats. Hæmatological investigation of the animals (Table IV) showed that they were anæmic. Apparently the vitamin deficiency was not absolute, but only relative. The group on the fortified experimental diet also suffered from anæmia, indicating that pyridoxine deficiency was not the only factor responsible for the anæmia.

TABLE IV.

Haematology of pyridoxine deficient and control rats.

Diet groups	Number of observations	Average total erythrocytes per c.mm	Average number of polychromatophilic cells per 10,000 erythrocytes
Pyridoxine deficient	6	4,670,000	134
Pyridoxine deficient *plus* pyridoxine hydrochloride ...	2	4,510,000	188
Standard diet ...	2	6,170,000	57

The acute infection of pyridoxine deficient rats was mild as compared with that of the controls* (Table V). The daily average parasitæmia in the three groups was in the ratio 1 : 5 : 5. Similarly peak parasitæmia in the three groups was in the ratio 1 : 2 : 2·5 respectively. No mortality occurred in the deficient group from acute infection, while it was 100 per cent in the two control groups.

TABLE V.

Effect of pyridoxine deficient and control diets on P. berghei *infection in albino rats.*

Diet group	Number of animals	Age in weeks	Average parasitæmia per 10,000 erythrocytes		Percent animals died of acute infection	Relapse rate per cent.	†Average enlarged spleen in c.c.
			Daily	Peak			
Pyridoxine deficient ...	6	6	112	346	0	16·6	1·2
Pyridoxine deficient diet *plus* pyridoxine hydrochloride ...	3	7	461	1040	100	...	3·0
Standard ...	3	18‡	556	1293	100	...	2·8

*The difference in average daily and peak parasitæmia between experimental and standard diet control animals, is found to be statistically significant.

†The animals on pyridoxine deficient diet lived with latent infection for 34 to 140 days and their spleen volumes were measured at death, while in remaining two groups the spleen volumes were of acute infection.

‡The course of parasitæmia in a six weeks old animal was essentially the same as in the other two animals 18 weeks old.

The only animal in the whole series which suffered from a relapse was among the deficient group. It occurred after a latency of 49 days and lasted for one day, the parasitæmia was 3 per 10,000 erythrocytes.

The average enlarged spleen volume was lowest in the experimental group, highest in the group on fortified experimental diet and intermediate in that fed on standard diet (Table V).

DISCUSSION.

The results of the experiments showed that pyridoxine is a vitamin essential for the growth of *Plasmodium berghei* as its multiplication was markedly lower in deficient animals than in the controls. Consequent to the low parasitæmia, apparently the antigenic stimulus was slight and gave rise to a smaller degree of splenic enlargement as already mentioned.

It was seen that the group on fortified experimental diet showed a more intense parasitæmia than the one on deficient diet, resulting in greater degree of cellular response as shown by nearly three times the enlargement of spleen. It is not, however, possible to conclude whether this greater degree of cellular activity was due solely to a greater antigenic stimulus or to the hosts not being subject to deficiency in pyridoxine being able to exhibit a better degree of defence.

The anæmia *per se* of the deficient group could not account for the low parasitæmia, as the group on fortified experimental diet also showed an identical anæmia, but a considerably more enhanced parasitæmia.

Certain limitations are inherent in *vivo* studies as the present and numerous others listed in Table I. Vitamin deficiency affects the parasite, firstly by its effects on its growth and secondly by its effects on host defence mechanism. A relative biotin deficiency in rats, as described later, gives rise to a higher parasitæmia than in non-deficient animals. In the case of certain other vitamin deficiencies like that of vitamin A and riboflavin (Table I), it appeared that the deficiency exercised both its direct as well as indirect effects on parasitic growth, but the former was so marked that the latter effect was not patent. In the case of pyridoxine deficiency it appeared that it affected the parasite more by its direct effects than indirectly through the host defence mechanism.

SUMMARY.

Pyridoxine or Vitamin B_6 was found to be an essential requirement for *Plasmodium berghei*.

The splenic enlargement of the deficient animals was less than that of non-deficient animals. This was probably due to the low antigenic stimulus in the former.

ACKNOWLEDGEMENT.

The author is grateful to Mr. Chanan Singh, M.Sc., for his help in the care and feeding of animals.

REFERENCES.

Bacon, J. S. D. (1953)	*Nature*, **171**, p. 456.
Brackett, S., Waletzky, E. and Baker, M. (1948)	*J. Parasitol.*, **32**, p. 453.
Brooke, M. M. (1945)	*Amer. J. Hyg.*, **41**, p. 81.
Editorial (1947)	*Lancet*, **17**, p. 642.
Fabiani, G. and Grellet, P. (1953)	*C. R. Soc. Biol.*, **146**, pp. 441-444. Abstract in *Trop. Dis. Bull.*, **50**, p. 12.
Gubler, C. J., Cartwright, G. E. and Wintrobe, M. M. (1949)	*J. Biol. Chem.*, **178**, p. 989.
Hawking, F. (1953)	*Brit. Med. J.*, May 13, p. 1201.
Mackee, R. W. and Geiman, Q. M. (1946)	*Proc. Soc. Expl. Biol. Med.*, **63**, pp. 313-315.
Ramakrishnan, S. P. (1952)	*Ind. J. Mal.*, **6**, pp. 189-198.
Idem (1953)	*Ibid.*, **7**, pp. 53-60.
Roos, A., Hegsted, D. M. and Stare, P. J. (1946)	*J. Nutrition*, **32**, pp. 473-484.
Seeler, A. O. and Ott, W. H. (1944) ...	*J. Inf. Diseases*, **75**, pp. 175-178.
Idem (1946)	*Ibid.*, **77**, pp. 82-84.
Seeler, A. P., Ott, W.H. and Gundel, M.E. (1944)	*Proc. Soc. Exptl. Biol. Med.*, **55**, pp. 107-109.
Sinclair, H. M. (1952)	Quoted by Bacon, J. S. D.
Storeck, H. C., Eisen, N.H. and John, H.M. (1947)	*J. Exptl. Med.*, **85**, pp. 365-371.
Trager, W. (1949)	*Bact. Rev.*, **13**, pp. 105-110.

Appendix I.

The composition of the deficient diet was as follows :-

BASAL DIET.

Casein (E Merck Darmstadt)	27·0 parts
Cane sugar	64·0 ,,
Coconut oil	4·0 ,,
Shark liver oil	1·0 ,,
Steenbock's salt mixture No. 40	4·0 ,,
	100 parts.

The above was supplemented by two drops each of adexolin and wheat germ oil per rat per week administered orally. The basal diet was prepared every two to three weeks and stored in the refrigerator. The following proportion of vitamins was added daily to every kg. of basal diet and served to the animals.

Thiamine hydrochloride	10·0 mg.
Riboflavin	10·0 mg.
Panthenol	40·0 mg.
Choline chloride	500·0 mg.
Niacin	100·0 mg.
PABA	600·0 mg.
Inositol	1000·0 mg.

The experimental non-deficient diet consisted of the above basal diet and supplements, further supplemented by 75γ of pyridoxine hydrochloride per rat per day administered orally.

The composition of the standard diet was as follows :-

Whole wheat flour	72·0 parts
Whole milk powder (Nespray)	23·0 ,,
Dried Brewers' yeast	3·0 ,,
Calcium carbonate	1·0 ,,
Table salt	1·0 ,,
	100 parts.

Indian Journal of Malariology, **8**, 2, *June* 1954.

NOTE ON NATURAL PARASITIC INFECTIONS FOUND IN *RATTUS RATTUS* OF DELHI MUNICIPAL AREA.

BY

SATYA PRAKASH.

(*Malaria Institute of India, Delhi.*)

(June 10, 1954.)

A TOTAL of 616 specimens of *Rattus rattus*, obtained from the municipal health organization of Delhi, have been used since 1950 at the Malaria Institute of India in routine studies on *Plasmodium berghei* infections in albino mice and rats. The former were used as recipients of sub-inoculation of blood from the latter animals which had been treated by various drugs in order to substantiate radical cure.

The routine practice was to examine blood smears stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944) from every animal on receipt from the municipality. Almost every animal was autopsied on death. The note records the different natural infections encountered in the rats and an analysis of their seasonal prevalence.

Three protozoon parasites were encountered in the blood and one helminth in its encysted form in the liver. The protozoon parasites were *Trypanosoma lewisi* (Lewis, 1879), *Babesia decumani* (Macfie, 1915) and *Hepatozoon muris* (Balfour, 1905). The helminthic infection detected was that of *Hymenolepis diminuta* (Rudalphi, 1819). Table I indicates the number of rats examined every month and the natural infections observed in them.

The lowest infection rate for *trypanosomiasis* was 15 per cent in February, and the highest 70 per cent in April. In the month of September, the infection rate was again 60 per cent. *Babesia* infections were conspicuously absent during the first four months of the year. The lowest rate of 2·5 per cent was recorded in the month of May, which rose to 16 and 18 per cent in June and September, respectively, and was the highest in December (20 per cent). *Hepatozoon* infections were also absent in the first three months of the year ; during April, May and June the infection rate was 16 per cent and reached a peak of 28 per cent in July. The lowest rate (six per cent) was found in October. Like *trypanosomiasis*, *tænia* infections were found throughout the year. In August, the rate was three per cent (lowest), and 81 per cent (highest) in December.

TABLE I.

Average monthly prevalence of endo-parasites in Rattus rattus *during the years* 1950, 1951 *and* 1952.

Month.	Number of rats examined	Number showing trypanosome	Per cent.	Number showing babesia	Per cent.	Number showing hepatozoon	Per cent.	Number showing tænia	Per cent.
Jan.	22	4	18	...	...	...	...	4	18
Feb.	26	4	15	...	...	...	...	9	34
Mar.	27	14	51	...	...	...	...	9	33
Apr.	24	17	70	...	...	4	16	5	20
May	44	20	46	1	2·5	7	16	17	38
Jun	89	25	29	14	16	14	16	32	37
Jul.	84	28	34	3	3·6	24	28	25	29
Aug.	34	14	41	1	3	5	15	1	3
Sep.	45	27	60	8	18	6	13	18	40
Oct.	68	14	20	5	7	4	6	36	53
Nov.	88	26	29	4	5	8	9	39	44
Dec.	65	12	19	14	20	7	10	53	81
TOTAL	616	205	33	60	9·8	79	13	248	60

Parasites in the blood were observed to be patent for varying number of days. Trypanosomes were found to be continuously present for a period of 35 days in Rat 280, *Babesia* remained patent for 37 days in Rat 14, and *Hepatozoon* for 15 days in Rat 452.

Tænia cysts were generally seen in the liver and occassionally in the spleen, lung and kidney. The highest number of cysts encountered in a single animal was eighteen in the liver of Rat 477. The size of the cysts varied from that of a small millet to the size of a pea.

ACKNOWLEDGEMENT.

Thanks are due to the Health Department of Delhi Municipality for their co-operation in the constant supply of rats.

REFERENCES.

BALFOUR, A. (1905) ... ... *J. Trop. Med. Hyg.*, **8**, p. 240.
JASWANT SINGH and BHATTACHARJI, L. M. (1944) ... ... *Ind. Med. Gaz.*, **79**, p. 102.
LEWIS, T. R. (1879) ... ... *Quart. J. Micro. Sci.*, **19**, p. 109.
MACFIE, J. S. W. (1915) ... ... *Ann. Trop. Med. Parasit.*, **9**, p. 457.
RUDOLPHI, C. A. (1819) ... ... *Clinical parasitology* edited by Graug and Faust, p. 442. Lea and Febiger, Philadelphia.

Indian Journal of Malariology, **8**, 2, *June* 1954.

NATURAL IMMUNITY OF DOMESTIC PIGEONS (*COLUMBA LIVIA* GMELIN) TO EXPERIMENTAL INFECTIONS WITH *P. GALLINACEUM*.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., Ch.B.(Edin.), D.P.H.(Eng.), D.T.M. & H. (London),

C. P. NAIR,

A. DAVID

AND

K. S. KRISHNAN.

(*Malaria Institute of India, Delhi.*)

(May 18, 1954.)

JASWANT Singh, Nair and David (1951) examined blood smears of 294 domestic pigeons (*Columba livia Gmelin*) obtained in Delhi and its environs, for plasmodial infection but none was found positive. Further evidence of such absence of natural plasmodial infections in pigeons has been recorded by Jaswant Singh, Krishnan and David (1952). David and Krishnan (1953) found that pigeons in Delhi area are naturally immune to both blood and sporozoite-induced experimental inoculations of *P. relictum*. The susceptibility of pigeons to blood and sporozoite-induced infection of *P. gallinaceum* was examined and the results of that work are presented in this paper.

METHODS AND MATERIAL.

Altogether 41 pigeons and 31 fowls were used for the experiment. All the pigeons, except one, were obtained from Delhi bird market and their individual weights varied between 0·25 and 0·37 kg. One pigeon was a very young one (0·15 kg. body weight) picked up from the nest when it was about two weeks

old, and reared in the laboratory for a period of one week in a screened wire cage. The fowls used were all obtained from the local poultry farm.

P. gallinaceum was obtained from the strain maintained in fowls at the Institute. Sporozoites were obtained by feeding laboratory bred *A. aegypti* mosquitoes on infected fowls showing sexual forms of the parasite. Infected mosquitoes were dissected eight to ten days after, either in normal saline or in normal fowl serum. The inoculum in blood-induced infection was determined in terms of the number of parasitized erythrocytes per kg. body weight of the bird, and in the case of the sporozoite-induced infection, in terms of pairs of infected glands expressed as mosquito equivalents. The route of inoculation in all the experiments was intravenous (wing vein).

In order to determine the exact period up to which sporozoites and trophozoites circulate in the peripheral blood of pigeons after injecting them, as well as to detect subsequent sub-patent infection in any of them, sub-inoculations were made from these birds to normal fowls immediately to within a period of 30 days after inoculation. The subinoculations were either in the form of whole blood or emulsion of the organs in 3 to 5 c.c. normal saline.

Attempts were made to find out the respective influence of fowl and pigeon blood on the susceptibility and/or refractoriness of *P. gallinaceum* infection. For this, whole blood in 5 to 15 c.c. volume was given intravenously either from normal fowls to pigeons or from normal pigeons to fowls, five minutes to 24 hours before the recipient birds got inoculation of *P. gallinaceum* trophozoites. In one case, blood containing *P. gallinaceum* was mixed up with pigeon's blood and injected intravenously to a normal fowl.

In all the cases, blood smears from normal birds were examined on three consecutive days prior to their inoculation and every alternate day thereafter during the entire period of observation which generally lasted from 15 to 64 days. Sufficient controls were kept appropriate to the investigation by inoculating normal fowls with trophozoites and sporozoites of *P. gallinaceum* simultaneously with the corresponding inoculations to pigeons.

EXPERIMENTAL.

1. *Susceptibility to sporozoite and blood-induced infections.*—Thirteen pigeons (12 adult birds and one young bird) were inoculated intravenously with blood forms of *P. gallinaceum*. The inoculation varied from 50 to 4,000 million parasitized erythrocytes per kg. body weight. Another six pigeons were inoculated by the same route with crushed glands of 10 to 22 *A. aegypti* mosquitoes showing heavy *P. gallinaceum* sporozoite infection. Side by side, three mosquitoes (*A. aegypti*) infected with the same strain of the parasite were fed on another normal pigeon.

Blood smears of sixteen (out of the twenty birds) were examined for a period of 54 to 64 days and the remaining four (sporozoite inoculated =1 ; and blood inoculated =3) for 11 to 37 days. No parasites could be detected in the peripheral blood of any of these birds during the entire period. After the due observation period, these birds were sacrificed and smears of brain, lungs, heart, spleen, liver and kidneys were examined for any evidence of exo-erythrocytic stages of the

parasite but with negative result. Histological study was made of the tissues collected from Bird 12 (Table I) but no tissue forms of the parasite could be seen in these either.

TABLE I.

Susceptibility of pigeons to blood and sporozoite-induced P. gallinaceum *infection.*

Pigeon number	Inoculation.		Observation period (days)	Result (Blood and organ smears)
	Blood	Sporozoites		
	Number of parasites/kg. body weight of bird (in millions)	Mosquito equivalent (numbers)		
1 to 5	50	...	64	Negative
6	100	...	64	Negative
7 and 8	200	...	64	Negative
9	300	...	54	Negative
10	400	...	64	Negative
11	600	...	37	Negative
12†	800	...	15	Negative‡
13	4000	...	15	Negative
14	...	10	60	Negative
15 and 16	...	14	11, 60	Negative
17	...	16	60	Negative
18	...	20	60	Negative
19	...	22	60	Negative
20	...	3*	30	Negative

†About two weeks old bird picked up from the nest and reared in the laboratory for a period of one week before using for the experiment.
‡Including tissue sections.
*Mosquito bite.

II. SUBINOCULATION RESULTS.

(a) *Blood passage from pigeons to fowls 15 days after infecting them with blood forms of parasite and subsequent inoculation from fowl to fowl.*—Two pigeons (Numbers 21 and 22) were inoculated with 100 million parasitized cells per kg. body weight from a donor fowl showing heavy *P. gallinaceum* infection. When there was no evidence of parasitæmia on blood examination during the first fifteen days, two fowls (Numbers 1 and 2) were inoculated with 5 c.c. blood each from Pigeon 21 and 22, respectively. These fowls, in turn, were kept under observation

for similar period of 15 days after which subinoculations were made from them to other normal fowls (Number 3 from Number 1 and Number 4 from Number 2). On similar lines, serial subinoculations were made up to fourth passage as listed below :—

Initial inoculation from pigeons to fowls and subsequent serial sub-passages from fowl to fowl.

Experiment I		*Experiment II*	
Pigeon	21	Pigeon	22
Fowl	1	Fowl	2
Fowl	3	Fowl	4
Fowl	5	Fowl	6
Fowl	7	Fowl	8

In all the cases, the volume of blood inoculated was 5 c.c. and route of inoculation intravenous. Donor birds (pigeons as well as fowls) after subinoculation from them were again kept under observation for a further period of 30 days after which they were sacrificed and smears from brain, lungs, heart, spleen, liver and kidneys were examined. In all cases, neither the tissue smears nor the blood smears showed any parasites.

(*b*) *Tissue passage to fowls from pigeons, 15 days after inoculating them with blood forms of the parasites.*—In another trial, four pigeons (Number 23 to 26) were given intravenous inoculation of 400 to 800 million parasitized erythrocytes per kg. body weight of the bird 15 days after these birds were killed, and tissue emulsion of each consisting of brain, lungs, heart, spleen, liver and kidneys, was injected intravenously to Fowls 9 to 12, respectively. After lapse of 30 days, these fowls were ultimately sacrificed and their organ smears examined. Neither these, nor the blood smears collected during the 30 days observation of the fowls, and 15 days observation of pigeons, revealed any type of parasite.

(*c*) *Tissue passage of fowls from pigeons inoculated with sporozoites and subsequent blood passage from fowl to fowl.*—Six pigeons (Numbers 27 to 32) were given intravenous inoculation of crushed glands of *Aedes ægypti* mosquitoes infected with *P. gallinaceum* in doses varying from 9 to 50 mosquito equivalents. These pigeons were sacrificed 48 to 240 hours after inoculation and tissue emulsion of each bird injected separately into normal fowls intravenously (Numbers 13 and 18). The details are shown in Table II. The smears of organs of these pigeons were not found positive at the time of inoculation. Similarly, no parasites could be detected by histological study of various organs of Pigeon 25 that was sacrificed 96 hours after receiving the heaviest dose of inoculum (50 pairs of infected glands). Blood examination of the subinoculated fowls also gave negative results throughout the period of 15 to 30 days over which they were observed. At this stage, 5 c.c. blood was subinoculated to one normal fowl each from three of the above birds and they in turn were observed for 18 to 33 days. Organ smears of all these fowls after subinoculation or completion of observation, as the case may be, proved negative.

TABLE II.

Details of inoculation of normal fowls with tissues emulsion of the pigeons, inoculated with P. gallinaceum *sporozoites and subsequent blood passage from some of these fowls to other normal fowls.*

INITIAL INOCULATION.		SERIAL PASSAGE I (EMULSION OF ORGANS).			SERIAL PASSAGE II (BLOOD INOCULATION).		
Number of the pigeon.	Inoculation, mosquito equivalent.	Interval between inoculation of the pigeons and the injection of the tissue emulsion to fowls (hours).	Number of the fowl injected.	Observation period (days).	Fowl number.	Interval between tissue injection of the fowl and blood passage from it (days).	Observation period (days).
27	9	48	13	30	...	...	...
28	20	48	14	30	...	...	...
29	50	96	15	15	15	18	18
30	13	144	16	20	...	...	...
31	26	192	17	20	16	30	33
32	14	240	18	22	17	22	23

(*d*) *Subinoculation of fowls from pigeons soon after their inoculation with trophozoites or sporozoites of* P. gallinaceum.—Effect of subinoculation of fowls from pigeons shortly after their inoculation, either with blood forms or sporozoites of *P. gallinaceum*, was observed in eight cases. Three pigeons, Numbers 33, 34 and 35, were inoculated intravenously into the right wing vein, blood forms of the parasite at the rate of 150 to 600 million per kg. body weight of the bird. Five c.c. blood was withdrawn from the left wing of the birds, five to seven minutes, 2·5 minutes and 1·5 minutes, respectively, after that and subinoculated to one healthy fowl each, Numbers 19 to 21. The fowl inoculated with the 1·5 minutes sample became positive within 24 hours and ran a normal course of infection. The other two birds remained negative during 30 to 32 days observation period. The blood samples used for inoculation, on examination, also showed that with the exception of 1·5 minutes specimen, all the others were negative. Smears were made from some pigeons 5, 10 and 15 minutes after trophozoite inoculation and they too proved negative for parasites.

Pigeons 36 to 40 were given intravenous injection of 19 to 45 pairs of positive crushed salivary glands of *A. ægypti* into the right wing vein. Similar to the above blood phase experiment, 3 to 5 c.c. blood was withdrawn from the left wing vein of these birds within one to eight minutes after the sporozoite inoculation, and injected intravenously to healthy Fowls, 22 to 26 respectively, as shown in Table III. In some cases, when it was difficult to get sufficient quantity of blood from wing veins of the pigeons, they were killed and blood drawn from heart to make up the required quantity. Of all the recipient

fowls, except Fowl 26 that received the sample of blood drawn from pigeon within four minutes of the inoculation, the rest remained negative during 42 to 50 days observation period. In Fowl 26, parasites could be detected on the eighth day after blood inoculation. All the pigeons in this trial, with the exception of three which had to be killed for collecting blood from the heart, were kept under observation for 45 to 60 days and their blood smears during the period were throughout negative.

TABLE III.

Results of subinoculation of fowls from pigeons after their inoculation with trophozoites or sporozoites of P. gallinaceum.

Pigeon.			Fowl.			
	Inoculation.					
Number	Blood forms of parasite (million/kg. body weight)	Sporozoites (mosquito equivalent)	Number	Time lapse between inoculation and subinoculation (minutes)	Observation period (days)	Results (Blood smear fowls)
33	400	...	19	5 to 7	32	Negative
34	150	...	20	2·5	17	Positive within 24 hours
35	600	...	21	1·5	30	Negative
36	...	19	22	2·5	30	Negative
37	...	19	23	2·45	30	Negative
38	...	45	24	1 to 8	50	Negative
39	...	30	25	1 to 7	42	Negative
40	...	31	26	1 to 4	30	Positive eighth day.

III. *Effect of normal fowl blood on the susceptibility of* P. gallinaceum *in pigeon; and of pigeon blood on the susceptibility of the parasite in fowls.*—Effect of normal fowl blood on the susceptibility of *P. gallinaceum* in pigeon was determined in one case. Pigeon 41 was given 5 c.c. blood and within 24 hours, 15 million parasitized (*P. gallinaceum*) erythrocytes were given intravenously. The bird remained negative during the following 30 days.

As a parallel, effect of pigeon blood on the susceptibility of *P. gallinaceum* in fowls was investigated in 4 fowls. Two fowls received 8 to 15 c.c. pigeon blood as a single injection. Five minutes thereafter in one case, and 24 hours after in the other, 60 million parasites were inoculated intravenously. In another case, 5 c.c. pigeon blood was given once a day for six days and after the last injection, the bird was infected with 50 million parasites. In the fourth, 8 c.c. blood was mixed up with 13 million parasites and given as a single injection (Table IV). All the four fowls became positive within 24 hours after the infective blood inoculation

just like the control Fowl 27 that had received the trophozoite inoculation without the prior administration of pigeon blood.

TABLE IV.

Effect of pigeon blood on the susceptibility of P. gallinaceum *in fowls, and fowls blood on the susceptibility of this species in pigeons.*

Bird		Pigeons		Fowls			
Number		41	27	28	29	30	31
Normal blood injected (c.c.)	Fowl	5	...	...	...	...	...
	Pigeon	...	...	15	10	8	5
Frequency ...		Thrice, alternate day	...	Once	Once	Once	Daily for 6 days
Inoculum. Million *P. gallinaceum* parasites/kg. body weight		15	60	60	60	15	50
Interval between normal and infective blood inoculation ...		24 hours*	...	24 hours	5 minutes	Mixed blood	24 hours
Results (First positive)		Negative†	24 hours	24 hours	24 hours	24 hours	24 hours

*After the last injection of fowl blood.
†Observation period—30 days.

DISCUSSION.

No patent parasitæmia occurred in pigeons after inoculation with heavy doses of *P. gallinaceum* sporozoites or the parasitized erythrocytes (Table I).

The negative results obtained with (i) tissue subinoculations from pigeons inoculated with blood forms of the parasite, to fowls (Fowls 9 to 12) and (ii) several serial sub-passages of blood from pigeons to fowls first, and subsequently from fowl to fowl (Fowls 1 to 8) indicate that infective blood inoculation to pigeons does not give rise to either phanerozoic type of infection or subpatent infection of the blood. The former assumption is substantiated further by the fact that microscopic examination of the tissues of the young pigeon (Number 12, Table I) 15 days after the inoculation of infective blood, showed no parasites.

The findings in the experiment in which tissue subinoculation was made to fowls from pigeons (inoculated previously with sporozoites) and subsequently subpassaged from fowl to fowl, indicate that the parasites do not develop even to the infective pre-erythrocytic stages.

Perhaps it may even be concluded that no pre-erythrocytic forms are formed in these birds because the organs such as brain, lungs, heart, spleen, liver and

kidney of Pigeon 25 which was sacrificed 96 hours after inoculation of the salivary glands of 50 infected mosquitoes (Table II), showed no parasites on histological studies.

The test to determine the fate of the inoculated trophozoites and sporozoites has shown that they do not remain in the peripheral blood for more than four minutes (Table III).

Huff (1950) has shown that *P. gallinaceum* infection occurs in various combinations depending upon the type of the avian host. Pheasants inoculated with the sporozoites of *P. gallinaceum* give rise to both pre-erythrocytic and erythrocytic stages. In turkeys and canaries, the infection stopped with the pre-erythrocytic stages, whereas in guinea fowl, California Valley quail, and ring neck doves, neither pre-erythrocytic nor erythrocytic stages were found.

With regard to domestic pigeons, Huff (*loc. cit.*) found no pre-ertythrocytic stages in skins biopsied after 42 and 70 hours intracutaneous inoculation of sporozoites of *P. gallinaceum* from 30 infected mosquitoes. In the present experiments, though the sporozoites were inoculated intravenously, the results obtained are in conformity with those of Huff (*loc. cit.*) with regard to the high degree of natural immunity of pigeons to the pre-erythrocytic stage of *P. gallinaceum*. Another observation recorded by Huff (*loc. cit.*) was that, though no pre-erythrocytic stages of *P. gallinaceum* were found in the local site of intracutaneous inoculation of the pigeon, two erythrocytic parasites were found in the bird on the eighteenth day and none on subsequent days. Moreover, parasitæmia of a very low grade resulted in one of four subinoculated chicks. The low grade parasitæmia was not, however, demonstrable in the present experiments. As the birds belong to the same species, this relative difference in the susceptibility of *P. gallinaceum* infection is not clear. Perhaps it may be due to some variation in the physiological state of the host than to any other immunological factor.

Immunity of pigeons to *P. gallinaceum* was not altered by its prior inoculation of whole blood from a normal fowl. Likewise, the susceptibility of the fowls to *P. gallinaceum* infection could not be changed even to a slight degree by the inoculation of pigeon blood into the fowl (Table IV). Though much significance cannot be attached to the failure of fowl blood to influence the non-susceptibility of pigeons to *P. gallinaceum* infection due mainly to the limited nature of the trials, the unchanged course of infection in fowls inoculated previously with pigeon blood in different quantities and at different intervals, tend to indicate that no passive immunity existed in pigeons as a result of previous natural infections.

Both chicks and pigeons are said to possess Forssman antigen with this difference, that in the pigeon it is present only in organs whereas in the chicks it is present both in the organs and in erythrocytes (Boyd, 1939). Jacob (1945) discussing about the great parallelism between the absence of Forssman antigen and susceptibility to malaria pointed out that with the exception of chicks, all the others in which this antigen was present, represented the group of vertebrates that are not hosts to malaria. This hypothesis he could not substantiate, however, because injections of material known to contain Forssman hapten (*e.g.*, from horse kidney, or guinnea pig kidney or sheep red cell) at spaced intervals into pekin white ducklings to increase their resistance to *P. lophuræ* infection, yielded largely

negative results (Jacob, *loc. cit.*). Similarly, Becker and Schwink (1953*a*) reported that when horse kidney extract (" source of material " of Forssman hapten) was injected intravenously into chicks beginning an hour before their inoculation with duck erythrocytes containing *P. lophuræ*, the course of infection in chicks was not significantly influenced. This they thought was possibly due to the fact that organ tissues had a stronger affinity for horse kidney extract than plasma so that it was removed from the blood before its effect could be noted. Experiments on similar lines (Becker, Schwink and Prather, 1951 and Becker and Schwink 1953*b*) in which duck plasma or duck serum, or sero-mucoid separated from duck plasma or serum, or duck liver extract, was injected for sparing properties, resulted in an overall increase in parasite count in test chicks as compared with the control birds.

The above sparing phenomenon if it could be made applicable to the present preliminary experiments, one should expect a greater degree of infection in the fowls that received pigeon's blood than the controls that received no such injection, but the same was not evident. No conclusion is attempted to the effect that no sparing phenomenon was present, as it is obvious that measurement of that kind is not easy in a host which is even otherwise highly susceptible to the infection. Moreover, the injection of whole blood probably is not as close an approach as the serum or plasma or seromucoid to the actual sparing substance.

SUMMARY.

Experiments were carried out using 41 pigeons and 31 fowls to find out the susceptibility of pigeons to *P. gallinaceum* infection.

Total and solid natural immunity of the host was evident both against sporozoite and infective blood inoculations. Injection of large numbers of these sporozoites and parasitized erythrocytes resulted in rapid clearance of these forms from circulation and absence of developed parasitæmia.

Intravenous administration of normal fowl blood to pigeon and pigeon blood to fowls, prior to inoculation of infective blood containing *P. gallinaceum*, did not bring about any change either in the non-susceptibility of pigeons or in the susceptibility of fowls to this infection.

REFERENCES.

BECKER, E. R. and SCHWINK, T. M. (1953*a*) ... The nature of sparing phenomenon. II. Activity of *vivo* and nature in the sparing substance. *J. Infec. Dis.*, **92**, pp. 74-76.

Idem (1953*b*) ... ... The nature of the sparing phenomenon. III. Agglutination of duck erythrocytes by chicken plasma potentiated with alcoholic extract of horse kidney in *vivo* tests in State College. *J. Sci.*, **27**, p. 437.

BECKER, E. R., SCHWINK, T. M. and PRATHER, R. M. (1951) ... ... Hemagglutinin-inhibiting property of duck plasma exhibited in agglutination reactions involving duck erythrocytes and plasma of chick recovered from lophuræ malaria. *J. Infec. Dis.*, **89**, pp. 95-102.

BOYD, W. C. (1939) ... ... Quoted by Jacobs, H. R. (1945). Tabulæ Biologicæ, **17**, p. 113. (1939).

David, A. and Krishnan, K. S. (1953) ... Some observations on experimental infections in domestic pigeons (*Columba livia* Gmelin) with *P. relictum*. *Ind. J. Mal.*, **7**, pp. 113-115

Huff, C. G. (1951) ... ... Observations on the pre-erythrocytic stages of *Plasmodium relictum*, *Plasmodium cathemerium* and *Plasmodium gallinaceum* in various birds. *J. Infec. Dis.*, **88**, pp. 17-26.

Jacobs, H. R. (1945) ... ... Unsuccessful attempts to increase resistance of ducklings to *Plasmodium lophura* infections by previous injections of materials containing the Forssman antigen. *Amer. J. Trop. Med.*, **25**, pp. 151-153.

Jaswant Singh, Krishnan, K. S. and David, A. (1952) ... ... Natural infection of *P. relictum* in weaver bird, the baya (*Ploceus philipinus*). *Ind. J. Mal.*, **6**, pp. 471-473.

Jaswant Singh, Nair, C. P. and David, A. (1951) ... ... Five years' observation on the incidence of blood protozoa in house sparrows (*Passer domesticus* Linnæus) and in pigeons (*Columba livia* Gmelin) in Delhi. *Ibid.*, **5**, pp. 220-223.

Indian Journal of Malariology, **8**, 2, *June* 1954.

ANTIRELAPSE TREATMENT WITH PRIMAQUINE AND PYRIMETHAMINE.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B.(Edin.), D.P.H.(Eng.), D.T.M. & H.(London),

A. P. RAY, M.B.B.S.,

B. G. MISRA, L.M.F., L.T.M.(Cal.),

AND

C. P. NAIR, M.P.H.

(In collaboration with RAJINDAR PAL, M. I. D. SHARMA, B. S. KRISHNAMURTHY, M. K. MENON and DALIP SINGH.)

(*Malaria Institute of India, Delhi.*)

(May 25, 1954)

IN the past, many workers have attempted to solve the problem of radical cure in relapsing malaria which is primarily due to *P. vivax* infection. The success aimed at could not be achieved firstly because the chances of re-infection after cure could not be reduced in the absence of suitable malaria control measures in most parts of the country, and secondly, because pamaquin which is known to have antirelapse properties (Sinton *et al.*, 1930) is unfortunately saddled with two major handicaps of being a poor schizonticide and its margin of safety between therapeutic and toxic dose being considerably narrow.

On account of malaria control programmes in many states, the position of late has been changing. Thus *pari passu* with effective control measures aiming at interception of transmission and reduction in the reservoir of infection, chances

of reinfection would be considerably minimised. Now with the National Malaria Control Programme (Jaswant Singh, 1953) which was launched in April, 1953, the needs of a radical cure become all the more obvious, and in this connection the rôle of the newer 8-amino-quinolines like pentaquine (Drake *et al.*, 1946), isopentaquine and primaquine (Elderfield *et al.*, 1947), is gaining importance. They are comparatively less toxic than pamaquin. Primaquine is considered to be the least toxic of the series and has been shown to possess effective schizonticidal properties (Chakrabarty and Chaudhuri, 1953 ; Jaswant Singh, Ray, Basu *et al.*, 1953).

During his studies on pyrimethamine (daraprim), Coatney (1952) had observed that if the drug was continued in weekly doses after the treatment of acute attacks, up to a period of eight weeks, the rate of relapses was reduced. In the recommended dose of 25 mg. for an adult, the drug showed no harmful effect (Coatney *et. al.*, 1953 ; Myatt *et al.*, 1953).

So far, investigations on primaquine and pyrimethamine had been undertaken under hospital conditions in this country, and results of hospital investigations to determine the relapse rates after treatment with primaquine have already been reported earlier by Jaswant Singh, Ray, Basu *et al.* (1953 *loc. cit.*). It is essential that such a scheme should first be undertaken in the field.

Recently, a field station was established at Shivpuri, Madhya Bharat, in the autumn of 1953 where primaquine or pyrimethamine was used as an antirelapse measure. Side by side, hospital studies on the effect of weekly treatment with pyrimethamine were taken up at the Jail and the Police hospitals in Delhi. These have been reported in a previous communication (Ray *et al.*, 1953).

Six villages near Shivpuri (72 miles from Gwalior, on the main road Delhi to Bombay) were selected after a preliminary survey in August, 1953. While the areas to the west and the south of Shivpuri are flat, in the north and the east they are hilly and traversed by several streams. The annual rainfall varies between 28 and 40 inches. In 1953, it was 30·77 inches and the average for the previous four years was 36·2 inches. The spleen rates of the two villages, Singhanbas and Manihar, situated to the west and the south-west of Shivpuri, respectively, were 24 and 17 per cent. Two other villages, Bachhora and Thakurpura, situated towards the north of the town showed spleen rates of 33 and 34 per cent, respectively. Those of Kota and Bhagora, 9 miles to the east of Shivpuri, had spleen rates of 64 and 74 per cent.

Except in a few cases, spleen in the children belonging to the villages of Singhanbas and Manihar, was found to be soft and 'just palpable'. The picture at Bachhora and Thakurpura was more or less similar. On the contrary, majority of the children from Kota and Bhagora were found to have hard and fairly enlarged spleen, often three to four fingers below the costal margin. In 20 per cent of these cases, there was concomitant enlargement of the liver. A spleen survey was carried out also at the conclusion of the studies.

In September, 1953, a survey was carried out to determine the density of prepondering anopheline mosquitoes. Blood smears from all fever cases were taken and examined. Each village was visited daily and, wherever necessary,

house to house visits were made. This was necessary before spraying all villages with insecticides in order to effectively intercept transmission. Entomological data revealed that density of *A. culicifacies* was uniformly highest in all the villages. *Pari passu* with the commencement of treatment, the villages were sprayed with insecticides to eliminate further transmission as reported earlier (Ray *et al.*, 1953 *loc. cit.*). A further spraying programme was arranged in early March to intercept spring transmission, if any. Examination of blood smears revealed that up to early October, the prepondering species of malaria parasite was *P. falciparum*. Subsequently, as the number of *P. falciparum* cases dwindled, that of *P. vivax* increased progressively till early November.

All the 102 cases with *P. vivax*, 2 cases with *vivax* and *malariæ*, and 3 cases with *P. malariæ* infections, were placed under the one or the other antirelapse regime. Regime I consisted of 7·5 mg. of primaquine* administered twice daily for a period of 5 days, i.e., a total dose of 75 mg. at the rate of 15 mg. a day for an adult. Proportionate dose was given to the children. These cases were observed closely twice a day for any signs of toxic manifestations. Further, they were advised to restrict their activities as far as possible. But in actual practice, when they were afebrile, neither the children could be confined to the house nor was it possible to dissuade the villagers from going to the field.

Regime II consisted of two quinine-daraprim tablets† administered every 12 hours up to three such doses similar to that reported in an earlier paper by Jaswant Singh, Ray, Misra and Nair (1953). The total dose of quinine hydrochloride was 27 grain (equivalent to 37 grain of quinine sulphate) and 30 mg. of pyrimethamine (daraprim). This initial treatment was followed by a weekly dose of 25 mg. of pyrimethamine for eight weeks. Proportionate doses were given to children.

Subsequently, all these cases were followed-up for a period of 180 to 224 days (6 to $7\frac{1}{2}$ months). Periodically and at the conclusion of the investigation, blood smears were examined for evidence of any parasitic relapse.

Many of the cases which showed *P. falciparum* infection, were treated with different doses of quinine and pyrimethamine as reported earlier (Jaswant Singh, Ray, Misra and Nair, 1953 *loc. cit.*).

The cases treated in Delhi were mostly from the Police Force and only a few from the inmates of the Delhi Jail. A careful and systematic check was made in each case to determine the source of infection and the locality from where the case was admitted. Majority of the cases were found to have acquired the infection while away from Delhi either on leave or on training elsewhere. On account of intensive malaria control measures undertaken in Delhi, the actual number of cases, who appeared to have contracted malaria locally, was very few. However, during the investigations, heavy spraying of all police barracks and the hospital was carried out repeatedly. Both anti-adult and anti-larval measures were undertaken in the jail premises.

*Received through the courtesy of Messrs. Imperial Chemical Industries.

†Received through the courtesy of the Wellcome Laboratories. Each tablet contains 5 mg. of pyrimethamine and 0·3 gm. (4·5 grain) of quinine dihydrochloride.

The disposition of all cases under different regimes is shown in Table I below :—

TABLE I.

Distribution of malaria cases under the two regimes.

Experimental station.	Regime I (Primaquine).		Regime II (Quinine-daraprim) and 8 weeks follow-up with pyrimethamine.	
	Number of cases treated.	Number followed up throughout.	Number of cases treated.	Number followed up throughout.
Shivpuri ...	50	46	57	49*
Delhi ...	...	...	69	51
TOTAL ...	50	46	126	100

*Three cases left the area for short periods during December/January but returned again and were followed up. Careful history of their movements was taken. None of these three had any subsequent attack.

Thus it would be evident that although a total of 176 cases were placed under the two regimes, the actual number which could be followed-up throughout the period of observation was 146.

RESULTS.

Response to initial treatment.—During the treatment of acute infection, the rate of clearance of asexual parasites under the different regimes was recorded as shown in Table II. Records of temperature could be available only in those cases treated in the hospital at Delhi, but not at the field station due to difficulties associated under such conditions.

TABLE II.

Clearance of asexual parasites and relief of clinical symptoms (in hours).

Regime	Experimental station.	Total cases.	Parasite clearance (in hours).					Temperature clearance (in hours).			
			24	48	72	96	120 or over.	24	48	72	96
I	Shivpuri	50	11	15	21	2	1	Not recorded.			
			(22·0)	(52·0)	(94·0)	(98·0)	(100·0)				
II	(*a*) Shivpuri	57	27†	22	7	1	...	Not recorded.			
			(47·3)	(85·8)	(98·0)	(100·0)					
	(*b*) Delhi ...	69	43	23	3	...	...	42	26	1	...
			(62·3)	(95·6)	100·0)			(60·8)	(98·4)	(100·0)	

†Includes 3 *P. malariæ* cases. Figures in brackets represent percentage of cases.

From Table II it would be evident that within a period of 72 hours, clearance of asexual parasites from the peripheral blood was attained in 94 to 100 per cent in all cases ; 94 per cent under primaquine and 98 to 100 per cent under the combined regime with quinine and daraprim. Clinical response under the latter regime was observed to be similar to parasite clearance rate. However, during the first 48 hours, the rate of parasite clearance under primaquine was only 52 per cent as against 85 to 95 per cent under quinine-daraprim. All the three *P. malariæ* cases responded promptly to the regime.

Table III below shows the rate of gametocyte clearance.

TABLE III.

Clearance of gametocytes (in hours).

Regime.	Total cases showing gametocytes.	Clearance of gametocytes within hours.			
		24	48	72	96
I	16	7	9	...	...
		(43·7)	(100·0)		
II	13*	11	23	7	2
		(25·5)	(79·0)	(95·3)	(100·0)

*This includes cases from both stations. Figures in brackets represent percentage of cases.

About one third of the cases under each regime showed gametocytes at the time when treatment was begun. The rate of clearance within 48 hours was 100 per cent under primaquine as against 79·0 per cent under quinine-daraprim. Under the latter regime, 100 per cent clearance was attained much later (within 96 hours).

Under Regime I, there had not been a single case of relapse, clinical or parasitic.

Under Regime II, from the series treated in Delhi, there was one case of relapse 123 days after the initial treatment, *i.e.*, 67 days after completion of the follow-up course. In the series treated at Shivpuri, there was one case who had clinical manifestations and parasitæmia, 152 days after the initial treatment or 96 days after follow up treatment. In three other cases, evidence of parasitic relapse was observed when the blood smears were examined during the last survey. The relapses were observed 209-222 days after initial treatment or 153 to 166 days after completion of follow-up treatment. One more case under the same regime developed high temperature, in the night, 160 days after completion of the treatment (104 days after follow-up treatment). But the following morning he left the area to be treated in town. On interrogation after his return a few days later, it was ascertained that he had two bouts of fever and was treated with chloroquine by his physician, but blood smear was not examined. No parasite was detectable in his blood when he returned to the experimental area.

In none of the cases any toxic manifestations could be detected nor was there any kind of complaint from any source.

The final spleen survey showed considerable reduction in the spleen rate (Table IV).

TABLE IV.

Spleen survey.

Serial number of village.	Name of village.	Initial spleen survey		Final spleen survey.	
		Spleen rate (Per cent).	Remarks.	Spleen rate (Per cent).	Remarks.
1.	Singhanbas	24·0	Over 60 per cent. *Class 1 and 2.	9·3 (4 out of 43).	Class 2=1 Class 3=3
2.	Manihar ...	17·0	83 per cent. Class 1 and 2.	0·0	...
3.	Bachhora ..	33·3	Over 50 per cent. Class 1 and 2.	12·0 (3 out of 25)	Class 2=1 Class 3=2
4.	Thakarpura	34·0	58 per cent. Class 1 and 2.	9·0 (8 out of 83)	Class 1=1 Class 2=2 Class 3=5
5.	Kota ...	64·0	72 per cent. Class 2 to 4.	30·7 (4 out of 13)	Class 3=1 Class 4=2 Class 5=1
6.	Bhagora ..	74·0	68 per cent. Class 2 to 4.	36·0 (9 out of 25)	Class 2=1 Class 3=4 Class 4=2 Class 5=2

*Classification is after Hackett (Russell *et al.*, 1946).

The reduction in the spleen rates in villages 1 to 4 appears to be dramatic, whereas in villages 5 and 6, the rate was reduced to about 30 per cent only. The rapid reduction in villages 1 to 4 above, is not altogether unexpected, on account of the type of spleen encountered during the initial survey.

DISCUSSION.

Edgecomb *et al.* (1950) reported that with 22·5 mg. daily dosage for 6 days, primaquine had curative properties and that when the same was combined with 1·64 gm. of quinine (base), relapses were prevented "in practically 100 per cent of cases". By increasing the dosage to 60 mg. daily, no therapeutic advantage was observed but on the other hand toxic manifestations were evident. Later, this group of workers observed equally satisfactory result after a dose of 15 mg. daily for 14 days, subsequent to chloroquine treatment during acute attack.

Recent reports from Jaswant Singh, Ray *et al.* (1953) show that during an observation period of 1 to 2 years, there had been only 1 case of relapse in a

series of 20 cases of *P. vivax* treated with 10 mg. daily dose (5 mg. b.d.) of primaquine alone administered for 10 days.

In the present series, it is evident that during the period of observation, in none of the 46 cases treated with 15 mg. daily dose (7·5 mg. b.d.) of primaquine for 5 days, there was any relapse.

No toxic manifestation of any kind was encountered in the series treated earlier with 10 mg. daily under hospital conditions. Even when the dose was increased to 15 mg., and the patients were ambulatory throughout (some resuming to normal duties when afebrile) as in the present series, adverse side symptoms were conspicuously absent. Further, even without the aid of quinine, primaquine was effective in clearing the asexual parasites in 94 per cent of cases within 72 hours, and none of the cases proved refractory.

In view of these observations, the rôle of primaquine in the treatment of relapsing malaria, under hospital or rural condition, appears to be well established.

As regards the follow-up regime with pyrimethamine for 8 weeks, the results obtained in the present series appear to be satisfactory for all practical purposes, as only 6 out of 100 cases (6 per cent) which include the patient who gave history of clinical symptoms and responded to chloroquine, had relapses. In this respect, these observations show better results than reported by Coatney *et al.* (1953 *loc. cit.*) who had recorded 6 relapses out of 13 patients. But it may be pointed out that while their observation period varied from 419 to 557 days, in the present series it was for 180 to 224 days only. The alternative explanation may be that the dose of infection regulated the course of events. Coatney *et al.* (1953 *loc. cit.*) seem to believe that the dose of sporozoite may be a factor in the rate of cure. This is evident from the data reported by them that 4 out of 6 cases of relapses received the heaviest sporozoite dose. This may also be the explanation for the much higher rate of relapse in the Shivpuri series (an area without any previous control measures), as compared to Delhi series where on account of adequate control measures, repeated or heavy dose of infection would be unlikely.

In all cases under quinine-daraprim regime (initial treatment), parasite clearance was attained in 86 to 96 per cent of cases during the first 48 hours, and 98 to 100 per cent within 72 hours. During the same periods, clinical response in the series treated in Delhi was observed in 98 and 100 per cent of cases, respectively. Thus compared to the treatment regimes adopted earlier with a single or repeated doses of 25 mg. of pyrimethamine wherein the asexual parasite clearance was attained in 64 to 92 per cent cases within 48 hours and 88 to 100 per cent within 72 hours, and relief of clinical symptoms observed in 53 to 92 and 64 to 100 per cent of cases respectively during the same period (Jaswant Singh, Ray *et al.*, 1952), the present regime appears to be advantageous. This attributed largely to the inclusion of 27 grain of quinine dihydrochloride which in itself is an effective remedy.

A comparison on the rates of parasite clearance in Regimes I and II shows that within 48 hours complete clearance was attained in 52 per cent of cases under Regime I as against 86 to 96 per cent of cases under Regime II. The figures for 72 hours were 94 per cent under Regime I and 98 to 100 per cent under Regime II.

Thus, although primaquine appeared to be tardy in the beginning, in the final analysis it proved almost as effective as quinine-daraprim course.

Comparing the effects on gametocytes, Regime I proved to be definitely superior to Regime II.

Taking the overall picture, the following points emerge out of the present studies :—

(*a*) Both regimes are effective in preventing relapses but primaquine showed somewhat better results than pyrimethamine.

(*b*) For all practical purposes, both the regimes exert more or less the same effect on the asexual parasites. However, on critical analysis it may be said that Regime II is slightly better.

(*c*) The position is reversed in regard to their action on gametocytes, and Regime I is considered to be better of the two.

(*d*) None of the regimes gave rise to adverse side symptoms.

Thus though both regimes may appear to be effective, the final appraisal will depend on the price structure and more important is the ease with which these can be administered. While in Regime I the patient has to take the antimalarial on 5 consecutive days, in the other regime it is necessary that the patient takes the drug every week for 8 consecutive weeks.

From practical point of view it seems logical that implementation of Regime I would be easier as the drug administration begins when the patient is actually ill and as such he is likely to take the remedy even without medical supervision for at least 3 to 4 days. On the other hand, since the follow-up treatment with pyrimethamine begins after the initial treatment and is to be continued for 8 weeks, it would be difficult to ensure that the drug has been actually taken as, by nature, the people are reluctant to any kind of medication when they are free from ailments. Another apparent disadvantage is that two different types of tablets, quinine-daraprim, and pyrimethamine, are required under the second regime and this might be somewhat confusing to some.

For appraisal of the cost accounting, it would be necessary to compare the price structure of 75 mg. of primaquine as against 230 mg. of pyrimethamine and 27 grain of quinine dihydrochloride. However, the second regime can be modified by changing the initial treatment regime to any of the powerful schzonticidal drugs like quinine, mapacrine or the 4-aminoquinolines. Obviously the cost would vary according to the choice of such drugs.

The general picture after the final spleen survey appeared to be highly satisfactory particularly in villages 1 to 4. This is believed to be due, firstly, to the larger number of cases with Class 1 and 2 spleen, and concomitant treatment of all cases. Secondly, the application of insecticides helped considerably in cutting down subsequent re-infections. The comparatively lesser reduction, in Villages 4 and 5, is due to the number of cases showing Class 2 to 4 spleen, the evidence of which was still present during the final survey.

SUMMARY.

1. A total of 176 cases were placed under anti-relapse treatment ; 50 cases under primaquine (Regime I) out of which 46 could be followed-up, and of 126 cases under quinine-daraprim (Regime II), 100 could be kept under observation. The cases were observed for periods ranging from six months to seven-and-a-half months.

Sixty-nine of these cases were treated (Regime II) in hospitals in Delhi, and 107 cases were treated in the rural areas around Shivpuri in Madhya Bharat.

2. None of the cases under primaquine relapsed, whereas 6* out of 100 (6 per cent) had relapsed under quinine-daraprim.

3. Response during the initial treatment, as determined by parasite clearance rate, was slightly better under Regime II than I. Reverse was the case regarding the effect on gametocytes.

4. No toxic manifestations were observed under any of the regimes.

5. Taking all points into consideration, primaquine regime shows a few advantages over the other particularly because it is less complicated and has to be continued for short period.

6. In view of the results obtained, the authors are optimistic that now the problem of treatment of relapsing malaria is not as formidable as it had been before, and they, therefore, recommend to institute such regimes wherever possible in areas where control measures are operative as by this step not only much relief can be given to the people who have to suffer from frequent attacks, but the risk of transmission would be negligible due to the reduction in the carrier rates in an area where mosquito control measures are operative.

ACKNOWLEDGEMENT.

The authors wish to convey their deep sense of gratitude to the Director of Health Services, Madhya Bharat, and his staff, as well as to Drs. N. M. Sawant, District Medical Officer, Shivpuri, and K. K. Goel of the Civil Hospital for their willing co-operation and every possible help rendered at all phases of the investigation undertaken at Shivpuri. They also wish to thank Dr. K. C. Chopra of the Police Hospital, Delhi, and his staff, and Dr. Bachan Singh of the District Jail Hospital, Delhi, for extending necessary facilities in the conduct of the experimental studies.

REFERENCES.

CHAKRABARTY, N. K. and CHAUDHURI, R. N. (1953) ... ... *Ind. J. Med. Sci.*, **7**, p. 236.

COATNEY, G. R. (1952) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 490.

COATNEY, G. R., MYATT, A. V., HERNANDEZ, T., JEFFERY, G. M. and COOPER, W. C. (1953) ... ... *Amer. J. Trop. Med. Hyg.*, **2**, p. 777.

DRAKE, N. L., VAN HOOK, J., GARMAN, J. A., HAYES, R., JOHNSON, R., KELLY, G. D., MELAMED, S. and PECK, R. M. (1946) ... *J. Amer. Chem. Soc.*, **68**, p. 1529.

*Since writing this paper, there had been one other case which relapsed after nine months.–EDITOR.

Edgecomb, J. H., Arnold, J., Young, E. H. (Jr.), Alving, A. S., Eichelberger, L., Jeffery, G. M., Eyles, D. and Young, M. D. (1950)	*J. Nat. Mal. Soc.*, **9**, p. 285.
Elderfield, R. C., Kermer, C. B., Kupchan, S. M., Birstein, O. and Cortes, G. (1947)	*J. Amer. Chem Soc.*, **69**, p. 1258.
Jaswant Singh (1953)	*Bull. Nat. Soc. Ind. Mal. Mos. Dis.*, **1**, p. 1.
Jaswant Singh, Ray, A. P., Basu, P. C., Nair, C. P. and Misra, B. G. (1953) ...	*Ind. J. Mal.*, **7**, p. 289.
Jaswant Singh, Ray, A. P. Misra, B. G. and Basu P. C. (1952)	*Ibid.*, **6**, p. 441.
Jaswant Singh, Ray, A. P., Misra, B. C. and Nair, C. P. (1953)	*Ibid.*, **7**, p. 319.
Myatt, A. V., Harnandez, J. and Coatney, G. R. (1953)	*Amer. J. Trop. Med. Hyg.*, **2**, p. 788.
Ray, A. P., Rajindar Pal, Misra, B. G., Nair, C. P., Sharma, M. I. D. and Krishnamurthy, B. S. (1953)	*Ind. J. Mal.*, **7**, p. 309.
Sinton, J. A., Smith, S. and Pottinger, D. (1930)	*Ind. J. Med. Res.*, **17**, p. 793.
Russell, P. F., West, L. S. and Manwell, R. D. (1946)	*Practical Malariology*, p. 387. W. B. Saunders Co Philadelphia.

Indian Journal of Malariology, **8**, 2, *June* 1954.

ASSAY OF ANTIMALARIALS AGAINST THE SPOROGONY CYCLE OF *P. GALLINACEUM*.

Part II.

BY

M. G. NARAYANDAS, B.SC.(HONS.),

AND

A. P. RAY, M.B.B.S.

(Malaria Institute of India, Delhi.)

(June 1, 1954.)

In an earlier paper, Jaswant Singh *et al.* (1953) reported the effect of M3349, proguanil and pyrimethamine on the sporogony cycle of *P. gallinaceum* in *Ædes ægypti*. Using the same technique, further studies were carried out with some compounds of the 8-aminoquinoline series.

MATERIALS AND METHODS.

About 26,000 laboratory bred *Aedes aegypti* were used for these studies. The technique of mosquito feeding on infected fowls and drug administration was similar to that reported earlier (Jaswant Singh *et al.*, 1953).

Sample dissections were carried out from +5 day to +15 day, till either sporozoites appeared in the salivary glands or all mosquitoes had been dissected, whichever was earlier.

Oöcysts in all the mosquitoes were counted and measured. When sporozoites were detected in any batch, normal fowls were infected with two mosquito equivalent dose of sporozoites. Blood smears from these fowls were examined daily thereafter till patent parasitæmia was established.

Pamaquin base, pentaquin phosphate and primaquine phosphate were tested.

As pamaquin base is not soluble in water, it was first dissolved in alcohol, finely ground with gum acacia, then mixed with water (with 4 per cent glucose) and homogenized.

The other compounds, being soluble in water (with 4 per cent glucose), presented no difficulties. Mosquitoes were allowed to imbibe concentrations of drugs through pads of cotton which were changed twice daily.

DISSECTIONS AND RESULTS.

Pamaquin.—Gut infections were seen in all batches of mosquitoes which were allowed to imbibe concentrations of pamaquin varying from 0.001 to 0.1 per cent. Details have been given in Table I. In mosquitoes kept on glucose only (comparison group) and those on 0·001 per cent pamaquin-glucose solution, sporozoites appeared on the tenth day while those kept on 0·01 and 0·1 per cent solutions, showed sporozoites on the twelfth day. Normal fowls inoculated with the sporozoites from all batches developed patent parasitæmia between 10 and 13 days. Intensity and course of infection were similar in both experimental and the comparison group.

Table I.

Gut and gland infectivity rate in Ædes egypti after allowing them to imbibe different concentrations of pamaquin.

Number of days after infective blood feed.	Comparison group.			Experimental group.																
				0·0001 Per cent.			0·001 Per cent.			0·01 Per cent.			0·1 Per cent.			1·0* Per cent.				
	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.		
5	10	0	7	...	...	...	5	0	2	5	9	3	5	2	...	...	...	...		
8	15	0	6	...	...	...	5	0	1	5	0	1	5	0	3	2	0	24		
9	10	0	5	...	...	...	5	0	3	5	0	0	5	0	3	...	...	...		
9	15	0	8	...	...	...	5	0	2	5	0	2	5	0	3	1	0	1		
9	10	0	5	...	...	...	5	0	1	5	0	1	5	0	3	...	...	...		
10	15	2	7	...	...	...	5	0	2	5	0	1	5	0	2	1	0	1		
11	10	2	2	...	...	...	5	2	...	5	0	1	5	0	1	...	...	...		
12	5	4	...	...	...	...	5	1	...	5	0	1	5	4	...	...	...	...		
13	...	...	...	...	...	...	50	16	...	5	1	...	5	2	...	...	...	...		
14	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...		
15	...	...	...	...	...	...	...	...	...	34	10	...	5	3	...	...	...	...		

*Mosquitoes of this group were first fed on infective blood and then the drug 1·0 per cent pamaquin was given for 5 days.

†From 6 days onwards, the live mosquitoes were fed on standard glucose solution.

Note.—Oöcysts which were encountered in the gland-positive mosquitoes, have not been counted, measured and included in the gut-positive column.

The mortality amongst mosquitoes kept on 1·0 per cent pamaquin solution was extremely high, and as such few became available for dissection beyond the sixth day after the infective feed. But by +6 day, oöcysts were detectable in this batch. One of the two mosquitoes which survived up to +10 day, showed oöcysts though sporozoites were detectable in the control batch at the same period.

Pentaquin.—Infection of gut was observed from +5 day in all batches of mosquitoes fed on concentrations of 0·0001, 0·001, 0·01, 0·1 and 1·0 per cent of pentaquin. Details have been shown in Table II. The mortality rate in the mosquitoes increased as the concentration of the drug in the feeding medium increased. Sporozoites were first detectable on +9 day both in the comparison and experimental group.

TABLE II.

Gut and gland infectivity rate in Ædes ægypti *after allowing them to imbibe different concentrations of pentaquin.*

Number of days after infective blood feed.	Control.			0·0001 Per cent.			0·001 Per cent.			0·01 Per cent.			0·1 Per cent.			1·0 Per cent.		
	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.	Number dissected.	+gland.	+gut.
5	15	0	6	5	0	2	10	0	2	10	0	2	10	0	3	5	0	3
6	15	2	7	5	0	1	10	0	5	10	0	1	10	0	7	5	0	4
7	15	7	11	5	2	2	10	1	5	10	0	4	10	0	3	6	0	3
8	10	2	7	5	3	3	10	3	0	10	0	4	5	0	3	...	...	...
9	5	3	...	...	...	...	5	3	...	5	0	1	...	...	...	...	...	...
10	5	3	...	...	...	...	9	6	...	10	4	1	5	0	1	...	...	...
11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12	...	...	...	...	...	...	...	...	...	12	2	...	7	2	3	...	...	...
13	...	...	...	...	...	...	...	...	...	16	2	...	...	...	...	...	...	...

NOTE.—Oöcysts which were encountered in the gland-positive mosquitoes, have not been counted, measured and included in the gut-positive column.

Primaquine.—Details of observations have been shown in Table III. It clearly shows that infections of gut and gland were detectable in mosquitoes throughout the period of dissection from +5 to +15 day. In none of the concentrations, 0·0001, 0·001, 0·01, 0·1 and 1·0 (percentages), was there any variation in size or number of oöcysts as compared to the control series. Patent parasitæmia was established between 10 and 13 days in normal fowls which received inoculations of sporozoites from these mosquitoes.

TABLE III.

Gut and gland infectivity rate in Ædes ægypti *after allowing them to imbibe different concentrations of primaquine.*

Number of days after infective blood feed	Control.			0·0001 Per cent.			0·001 Per cent.			0·01 Per cent.			0·1 Per cent.			1·0 Per cent.		
	Number dissected.	+Gland.	+Gut.	Number dissected.	+Gland.	+Gut.	Number dissected.	+Gland.	+Gut.	Number dissected.	+Gland.	+Gut.	Number dissected.	+Gland.	+Gut.	Number dissected.	+Gland.	—Gut.
5	10	0	9	5	0	4	5	0	3	10	0	0	10	0	7	...	...	...
6	15	0	9	5	..	3	5	0	5	10	0	6	10	0	5	5	0	3
7	11	3	11	5	0	5	5	0	5	10	0	6	10	0	3	5	0	2
8	15	8	5	5	0	4	5	0	3	10	0	10	10	0	6	2	2	...
9	25	17	5	5	3	...	5	2	...	10	3	5	10	2	4	...	...	...
10	5	5	...	...	...	...	...	...	...	5	5	...	5	0	3	...	...	...
11	...	...	...	...	...	...	...	...	...	10	5	2	5	0	2	...	...	...
12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13	...	...	...	...	...	...	...	...	...	...	...	...	5	1	3	...	...	...
14	...	...	...	...	...	...	...	...	...	...	...	...	3	1	2	...	...	...
15	...	...	...	...	...	...	...	...	...	...	...	...	11	2	...	...	...	...

NOTE.—Oöcysts which were encountered in the gland-positive mosquitoes, have not been counted, measured and included in the gut-positive column.

DISCUSSION.

The inhibitory effect of proguanil on the sporogony cycle of *P. gallinaceum* in *Edes ægypti* was reported by Terzian (1947) and Terzian *et al.* (1949). Subsequently these observations were confirmed by Jaswant Singh *et al.* (1953) who reported that both proguanil and pyrimethamine exerted inhibitory influence in concentrations of 0·01 per cent and above. With regard to pamaquin, it was shown earlier that neither "plasmochin citrate" nor "pamaquin naphthoate" was able to prevent development of oöcysts or sporozoites (Terzian, 1947 ; Johnson and Akins, 1948 ; Terzian *et al.*, 1949). But subsequently, Terzian *et al.* (1951) reported that though development of oöcysts and sporozoites was unhampered, pamaquin has a marked toxic effect on the latter and these were completely destroyed depending upon the concentration of the drug. On the basis of this observation, the authors concluded that 8-aminoquinoline compounds are selectively toxic to sporozoites of *P. gallinaceum* in *Ædes ægypti*.

From the results obtained in the current studies, where each series was repeated 3 to 5 times, it was evident that the three 8-aminoquinoline compounds were toxic to the mosquitoes in doses of 1·0 per cent and above, the toxicity being the highest with pamaquin. Further, the mortality was proportionately higher with 0·1 per cent than 0·01 and 0.001 per cent as would be expected. Development of oöcysts was unhampered under all the series and in all concentrations.

The size and number of oöcysts in the experimental series were comparable to those in the comparison groups.

In all, but one, series sporozoites appeared by the +7 to +10 day, and the extrinsic incubation periods, in both experimental and the comparison group, did not vary widely. With 0·1 and 0·01 per cent pamaquin naphthoate preparation, the delay in the appearance of sporozoites in the experimental group was by two days as compared to the comparison series. In the other series (pentaquin or primaquine), sporozoites appeared at the same time in both groups.

In all cases, whenever sporozoites were detectable they were found to be viable. The prepatent periods in the fowls inoculated with these sporozoites varied between 7 and 10 days both in the experimental as well as in the control series.

In view of these observations, the authors were not able to support the contention of Terzian *et al.* (1951) that the drugs of the 8-aminoquinoline series were toxic to the sporozoites. Whether concentrations above 1·0 per cent would be effective or not, is difficult to conjecture as such concentrations would obviously be too toxic to the mosquitoes themselves.

Further, it may be mentioned that while Covell (1953) has recently reported about the effect of proguanil and pyrimethamine against the sporogony cycle, that of the 8-aminoquinolines was not indicated in this Table.

SUMMARY.

The present paper describes the effect of different concentrations of pentaquin and pamaquin and primaquine (8-aminoquinolines) on the sporogony cycle of *P. gallinaceum* in the body of the mosquito *Aëdes aegypti*.

ACKNOWLEDGMENT.

The authors express their thanks to Dr. U. P. Basu, Director, Bengal Immunity Research Institute, Calcutta, for the supply of pamaquin base.

REFERENCES.

Covell, G. (1953) ... ... *Brit. Med. J.*, August 1, pp. 258-259. Abstract in *Trop. Dis. Bull.*, **50**, pp. 118-119.

Jaswant Singh, Narayandas, M.G. and Ray, A. P. (1953) ... ... *Ind. J. Mal.*, **7**, pp. 33-39.

Johnson, H. A. and Akins, K. (1948) ... *J. Nat. Mal. Soc.*, **7**, pp. 144-147.

Terzian, L. A. (1947) ... ... *Science*, **106**, pp. 449-450.

Terzian, L.A., Stahler, N. and Weathersby, A. B. (1949) ... ... *J. Inf. Dis.*, **84**, pp. 47-55.

Terzian, L. A., Ward, P. A. and Stahler, N. (1951) ... ... *Amer. J. Trop. Med.*, **31**, pp. 682-697.

Indian Journal of Malariology, **8**, 2, *June* 1954.

SUMMARY REVIEW ON THE SINGLE DOSE TREATMENT WITH ANTIMALARIALS.

BY

P. N. GUPTA.

(*Malariologist, Station Hygiene Organization, Ramgarh Cantt.*)

June 1, 1954.

THE progress in chemotherapy of malaria during the last decade has been immense. As a result of this and due to very effective insecticides, malaria has vanished from some countries and has been greatly reduced in others.

The newer synthetic antimalarials which were discovered during recent years are proguanil (paludrine), chloroquine, resochin (avalon, nivaquine, avloclor), amodiaquin (camoquin, cam-aqi) and pyrimethamine (daraprim).

Initially most of these drugs were recommended to be administered in repeated doses lasting from 3 to 10 days as is done with quinine or mepacrine. Subsequently some workers carried out studies on the effectiveness of the single dose treatment.

Proguanil.—Single doses of 100 mg. and 300 mg. have been tried in India by Afridi *et al.* (1947) and Chaudhuri (1948*a*). Relief of clinical symptoms after treatment with 100 mg. was attained in 55 to 72 per cent of cases within a period of 72 hours in respect of *P. vivax* while it was found to be about 75 per cent in *P. falciparum* infection. During the same period, parasite clearance was observed in 55 to 81 per cent of cases in *P. vivax* or *P. falciparum* infection. The rate was observed to be somewhat faster with 300 mg., but as in most cases recrudescence occurred within 7 to 10 days, the single dose regime did not prove ultimately of much value.

Chloroquine.—The dosage range tried was from 250 to 1500 mg. While the range of 1500 mg. proved effective in relieving clinical symptoms due to *P. vivax* and *P. falciparum* within 24 and 48 hours, respectively, adverse side symptoms were invariably encountered (Chaudhuri, 1948*b*). At the other extreme when 250 mg. was used, although most of the cases reacted well there were two cases of *P. falciparum* which did not respond to the drug (Goodwin, 1952). On the other hand,

doses of 400 to 600 mg. were found to be quite satisfactory (Jaswant Singh, Ray and Misra, 1953 ; Bruce Chwatt, 1950). Jaswant Singh, Ray and Misra (*loc. cit.*) commented that the best results could be attained in 600 mg. dosage.

Amodiaquin.—It has been tried in 250 mg. to 600 mg. dosage. Simeons and Chhatre (1947) observed that while 250 mg. had comparatively slow action, a single dose of 10 mg./kg. was most effective. Relief of clinical symptoms was attained within 12 to 36 hours in most cases, and within 48 hours in 100 per cent of cases. Similar observations were reported by Jaswant Singh, Ray and Misra (*loc. cit.*). Relapses occurred in fewer cases and at longer intervals (Chaudhuri *et al.*, 1948 ; Jaswant Singh, Ray and Misra, *loc. cit.*).

Pyrimethamine.—The drug has been tried by most workers in 20, 25 or 50 mg. single dose (Jaswant Singh, Ray, Basu and Misra, 1952 ; Jaswant Singh, Ray, Misra and Basu, 1952 ; Field *et al.*, 1952 ; Gilroy ,1952 ; Hay Arthur, 1952 and Srivastava *et al.*, 1953). Some workers like Chaudhuri (1953) and Laha, Singhal and Navani (1953) have also tried doses ranging from 25 to 100 mg.

From these studies it is evident that in most cases of *P. vivax* and *P. falciparum*, clearance of parasites and relief of clinical symptoms are attained within 72 to 96 hours. However, a few cases of *P. vivax* and a larger number of *P. falciparum* cases either proved refractory or recrudescence occurred early. A dose of 75 to 100 mg. did not accelerate the speed of action ; on the contrary adverse side symptoms were often encountered.

From the above data, it would be clearly evident that action of proguanil and pyrimethamine under the single dosage regimes was not only slow but in many cases unreliable. Recrudescence occurred too soon and too frequently in many cases. On the other hand, both amodiaquin and chloroquine proved highly satisfactory in 500 to 600 mg. dosage. Relief of clinical symptoms was attained quickly and relapses were few and far between.

In view of these, Chaudhuri (1953) and Jaswant Singh, Ray and Misra (*loc. cit.*) have rightly observed that these drugs are ideal under Indian rural conditions.

REFERENCES.

Afridi, M. K., Ramakrishnan, S.P., Goswami, A. and Menon, M. K. (1947) ...	*Ind. J. Mal.*, **1**, p. 221.
Bruce Chwatt, L. J. (1950)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **44**, p. 363.
Chaudhuri, R. N. (1948*a*)	*Ind. Med. Gaz.*, **83**, p. 225.
Idem (1948*b*)	*Report Sci. Advis. Bd. Indian Research Fund Assoc.*, p. 23.
Idem (1953)	*Ind. J. Mal.*, **7**, p. 329.
Chaudhuri, R. N., Rai Chaudhuri, M. N. and Chakravarty, N.K. (1948) ...	*Ibid.*, **2**, p. 1.
Field, J. W., Edison, J. F. B. and Wilson, T. (1952)	Quoted by Goodwin, L. G. (1952) *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 485.
Goodwin, L. G. (1952)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 485.
Gilroy, A. (1952)	Quoted by Goodwin, L. G. (1952).
Hay Arthur, J. (1952)	*Ibid.*
Jaswant Singh, Ray, A. P., Basu, P. C. and Misra, B. G. (1952)	*Ind. J. Mal.*, **6**, p. 435.
Idem (1953)	*Brit. Med. J.*, **1**, p. 1269.

Jaswant Singh, Ray, A. P. and Misra, B. G. (1953)	*Ind. J. Med.*, **7**, p. 19.
Jaswant Singh, Ray, A. P., Misra, B.G. and Basu ,P. C. (1952)	*Ibid.*, **6**, p. 141.
Laha, P. N., Singhal, R. N. and Navani, H. (1953)	*Ibid.*, **7**, p. 339.
Simeons, A. T. W. and Chhatre, K. D. (1917)	*Ind. Med. Gaz.*, **52**, p. 255.
Srivastava, R. S., Chakrabarti, A. K. and Mukherjee, S. K. (1953)	*Ind. J. Mal.*, **7**, p. 5.

Indian Journal of Malariology, **8**, 2, *June* 1954.

WING GRADE COMPOSITION AS AN INDEX FOR JUDGING THE EFFECTIVENESS OF RESIDUAL TOXICITY OF INSECTICIDE DEPOSITS.*

BY

S. R. BHOMBORE,

N. L. SITARAMAN

AND

K. S. NANJUNDIAH.

(*Malaria Investigation Centre, Saklespur, Mysore State.*)

March 30, 1954.)

PREVALENCE of malaria in an area is dependent upon a number of factors such as the presence of adequate number of gametocyte carriers, suitable climatic conditions for the development of parasite in the mosquito, critical density and the longevity of life of the vector species. Of these, longevity of mosquitoes is of the highest importance. Unless mosquitoes live long enough for the malaria parasite to develop to the sporozoite stage, they will not be able to transmit the disease. Vector species must live at least nine to ten days after taking an infected feed to be able to become infective.

Large scale malaria control with residual insecticides is based on the principle of interception to transmission. The span of life of mosquitoes is reduced below ten days so that they are unable to become infective. D.D.T. as residual insecticide being used for control of malaria applied on the walls, loses its toxicity after sometime and has to be applied again before its toxic effect is completely worn off. The duration of the residual toxicity seems to vary in different parts of India and has very often to be determined so as to repeat the application after the first application wears off. There is no doubt that chemical estimation of D.D.T. on the wall gives some idea about the amount of D.D.T. present, but it is

*The studies reported in this paper were conducted by the Bureau of Malariology, Department of Public Health, Government of Mysore, in collaboration with the Division of Medicine and Public Health of The Rockefeller Foundation.

well known that it loses its efficiency biologically much earlier than its disappearance chemically. The steady build-up of mosquito population would also indicate that the insecticide is beginning to lose its potency. The mosquito density is ordinarily studied by either leaving a few separate villages unsprayed for comparison or by leaving a few houses untreated by the side of treated houses. Age composition studies of anopheline populations collected in sprayed or unsprayed houses would, therefore, serve as an important guide in judging the waning of the residual effects of the insecticide.

In studying the effect of limewash on the residual activity of D.D.T. in an irrigated village in Mysore State (Bhombore *et al.*, 1954), a few houses were left unsprayed. In order to study whether D.D.T. spraying in the adjoining houses would serve as a barrier for the influx of mosquitoes into these unsprayed houses, entomological data were collected separately for the sprayed and unsprayed houses. Immediately after spraying, the mosquito density in sprayed houses dropped down to zero, whereas in the unsprayed houses a certain number still remained. It was considered desirable to determine the age composition of the anopheline populations in these unsprayed houses with a view to compare them with those in the untreated comparison villages.

AREA OF STUDIES.

Two villages, Anegola and Goodehosahalli, selected for these studies, comprised a few unsprayed houses in the midst of sprayed houses. These villages lie on the Mysore-Channarayapatna Road, in Kikkeri Hobli, Krishnarajapet Taluk, Mandya District. They are a part of the series of irrigated villages, fed by the North Ramadevara Channel of the Hemavathi River. The irrigation season in the area commences from June and extends to subsequent January, the main cultivated crops being paddy and sugarcane. The average annual rainfall for the past ten years in the area is 28 inches 45 cents. The people are typical agriculturists and their economic condition is poor. The houses are all mixed dwellings, with mudwalls (plastered or unplastered), country-tile roofing, poorly lighted and ill-ventilated except for a central opening in the inner courtyard.

The two comparison villages, which were left unsprayed, were Kattehosahalli and Marasettihalli, about five miles distant from Anegola and Goodehosahalli. The unsprayed villages also lie in the irrigated area and are identical in all respects with the study villages.

MATERIALS AND TECHNIQUE.

Special daytime anopheline collections were carried out once a month in the sprayed and unsprayed houses in the treated villages and also in the comparison unsprayed villages. Perry's criteria for evaluating the probable age of the adult anophelines from their external appearance (Perry, 1912), Christophers' stages of ovarian development (Christophers, 1911) and Viswanathan's classification of the degrees of ingestion and assimilation of blood (Viswanathan and Rao, 1943) were followed in describing the status of each anopheline female.

The mosquitoes collected were arranged in groups based on Perry's wing condition, on Christophers' stages of ovarian follicular development, and on the condition of the abdomen in respect of the gonotrophic cycle.

The mosquitoes were first examined under a dissecting microscope, identified and conditions of wing and abdomen noted. They were then dissected in saline and the stages of ovaries development recorded. For each specimen, all three sets of observations were recorded. Mosquitoes were brought in Barraud cages, examined and dissected within two hours of collection. Observations in both sets of villages were carried out on the same day.

RESULTS AND ANALYSIS OF DATA.

The number of mosquitoes in each stage of the three types of classification was reduced to percentages of the total population collected in the villages. When the data were analyzed, it was found that the three types of classifications could not be correlated to give a common basis for deducing the age of the mosquito. Each of the methods presented some limitations. The wear and tear of the wing depends to a certain extent on weather and temperature. Similarly, ovarian development and the condition of the abdomen depend upon temperature but the number of feeds taken or the number of gonotrophic cycles undergone, is not known. It was evident, therefore, that these criteria tended to discriminate between individual mosquitoes at the same time and place. Taking all the factors into consideration, in spite of its limitations, Perry's classification can be followed to arrive, to a certain extent, at the probable age of the mosquito.

Two series of observations were carried out during these studies, one series from December 1951 to March 1952 and the other from August to October 1952. Results are summarized in Table I, and Chart I represents these graphically.

TABLE I.

Age composition studies.

Per cent population according to wing-grades.

Months.	Unsprayed houses in sprayed villages.				Unsprayed comparison villages.			
	Per cent population.				Per cent population.			
	W_1	W_2	W_3	W_4	W_1	W_2	W_3	W_4
December, 1951	11·4	58·8	29·4	1·2	24·3	48·5	27·1	0·0
January, 1952	4·8	78·4	12·8	4·8	24·0	72·0	4·8	0·0
February, 1952	12·0	28·0	20·0	42·0	2·2	37·4	30·8	30·8
March, 1952	4·8	26·4	50·4	16·8	0·0	10·4	70·2	22·1
August, 1952	4·2	51·5	42·0	2·1	9·6	59·2	28·9	2·5
September, 1952	43·2	25·2	16·2	16·2	36·9	25·2	27·0	12·6
October, 1952	44·0	32·0	24·0	0·0	10·5	55·5	34·5	0·0

From a study of Table I, it is evident that the age composition of the anopheline population in unsprayed houses in treated villages is very similar to that in untreated comparison villages.

DISCUSSION.

In sprayed houses, collections during the period of study were either negative or negligible. The low density of mosquitoes in these houses and the fact that the few specimens collected were either in W_1 or W_2 condition, would seem to indicate the persistence of the toxicity of the residual film. Since no mosquitoes showing W_3 or W_4 condition were encountered, it is possible that the young mosquitoes, entering the treated houses, pick up the insecticide, get knocked down and are incapable of further development. The presence of anophelines in the unsprayed houses amidst sprayed ones and the fact that the composition of the age-groups is about the same as found in the collections from unsprayed comparison villages, would indicate the inadvisability of leaving even a single house unsprayed in an otherwise sprayed village and the possibility of having a comparison area in a sprayed village itself, instead of having one far off.

During December, January, August, September and October, when the irrigation season was in progress and there was a continuous supply of water in the canals, conditions were very favourable for breeding and a large proportion of the mosquito population, both in the unsprayed houses amidst sprayed houses and in the unsprayed comparison villages, consisted of young ones. During February and March, when the irrigation season was at an end and there was a fall in opportunities for continuous breeding, older mosquitoes predominated.

WING INDEX.

As a consequent of these studies on the age composition of anopheline populations in sprayed and unsprayed villages, the following scheme seems to give some help in judging the effective residual toxicity of insecticidal deposits in the field.

Mosquitoes collected from a village are first arranged in groups according to Perry's wing grades. These groups are then reduced to a single figure by weighing, as in the method for calculating average enlarged spleen figures. Thus the number of individual in Wing Group I (youngest mosquitoes) is retained as it is, but that in Wing Group II is multiplied by that factor, 3-condition mosquitoes by three and 4-condition mosquitoes by four (oldest mosquitoes). These products are added up and divided by the total number of anophelines caught in each village, yielding the "Wing Index" for that village. If the insecticidal deposit is active, the "Wing Index" in that village will be low (there is a shift to the left), for older mosquitoes will have had increasing opportunities for achieving lethal contact with the insecticides. As the insecticide begins to lose its potency, the "Wing Index" will rise (there is a shift to the right).

CHART-2.

AGE COMPOSITION STUDIES

WING-INDEX

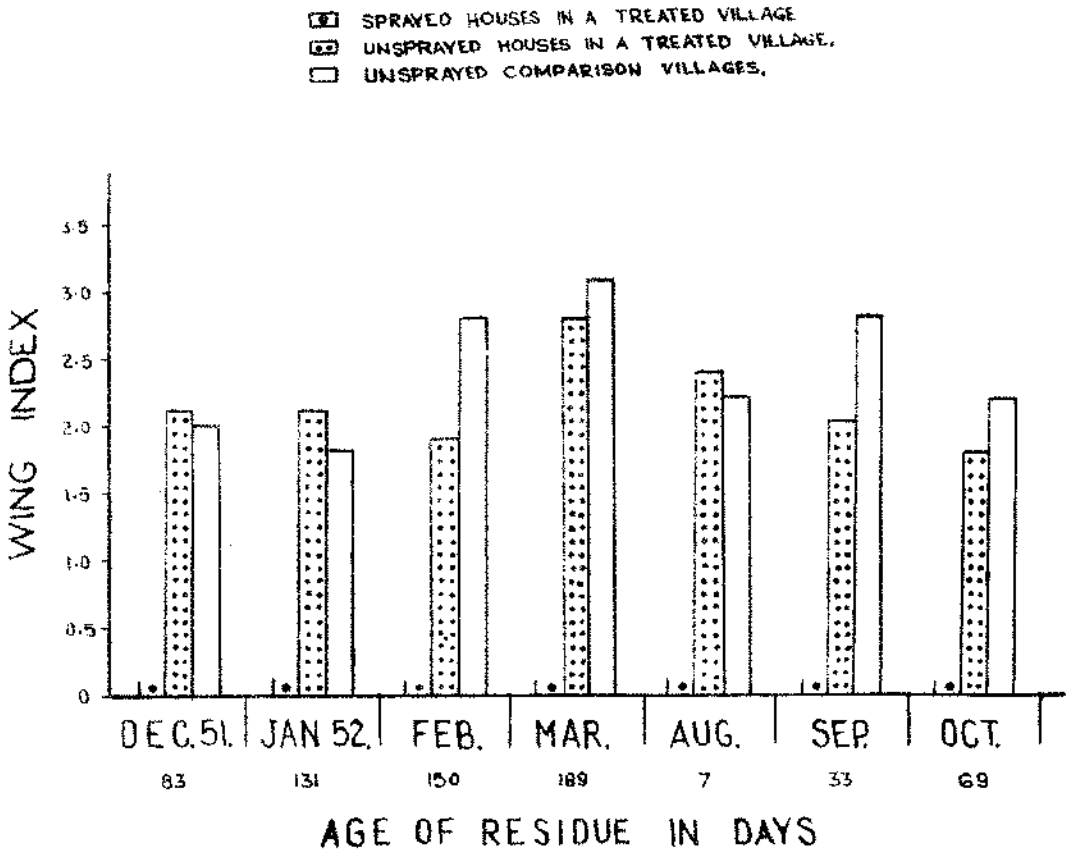


TABLE II.

Age composition studies presented as "Wing Index".

(A) Sprayed houses in a treated village. (B) Unsprayed houses in a treated village.
(C) Unsprayed comparison villages.

Date of spray.	Date of observations.	Age of residue in days.	(A) Number of adult anophelines captured.	(A) Wing-grade findings in sprayed houses in a treated village. W_1	W_2	W_3	W_4	(A) Wing Index	(B) Number of adult anophelines captured.	(B) Wing-grade findings in unsprayed houses in a treated village. W_1	W_4	W_2	W_4	(B) Wing Index.	(C) Number of adult anophelines captured.	(C) Wing-grade findings in unsprayed comparison villages. W_1	W_4	W_3	W_4	(C) Wing Index.
Sep. 19, 1951	Dec. 12/15, 1951	83	0	0	0	0	0	0·0	121	11	82	28	0	2·1	140	31	74	35	0	2·0
Sep. 19, 1951	Jan. 30/31, 1952	131	0	0	0	0	0	0·0	61	3	47	8	3	2·1	21	5	15	1	0	1·8
Sep. 19, 1951	Feb. 18/20, 1952	150	0	0	0	0	0	0·0	50	21	14	10	5	1·9	46	1	17	14	14	2·8
Sep. 19, 1951	Feb. 28/30, 1952	189	0	0	0	0	0	0·0	41	2	11	21	7	2·8	79	0	8	54	17	3·0
	Total		0	0	0	0	0	0·0	273	37	154	67	15	2·2	286	37	114	104	31	2·4
Jul. 28/29, 1952	Aug. 4/5, 1952	7	0	0	0	0	0	0·0	92	1	49	40	2	2·4	239	23	141	69	6	2·2
Jul. 28/29, 1952	Sep. 1/2, 1952	33	0	0	0	0	0	0·0	56	24	14	9	9	2·0	113	14	28	30	41	2·8
Jul. 28/29, 1952	Oct. 7, 1952	69	0	0	0	0	0	0·0	25	11	8	6	0	1·8	67	7	37	23	0	2·2
	Total		0	0	0	0	0	0·0	173	36	71	55	11	2·1	419	44	206	122	47	2·4

Seasonal fluctuations in the natural age-composition of anopheline populations of the region need not be a hindrance to the use of " Wing Index " as a means of appraising residual toxicity of insecticides if either of the following circumstances can be achieved :—

(*i*) The presence of unsprayed villages for comparative purposes ; or

(*ii*) The presence of villages sprayed at different times, so that deposits in some villages are appreciably older than in others.

During these studies, two sets of observations were carried out, selecting two treated villages with a few unsprayed houses and two entirely unsprayed villages. The wing-grade findings in the sprayed houses in a treated village were nil, no anophelines being encountered. The " Wing Index " was therefore 0·0. The average " Wing Index " in unsprayed houses in a treated village was 2·2 in one instance and 2·1 in the other. These indices compared favourably with those for the untreated comparison villages, *viz.*, 2·4 in both observations (Table II).

SUMMARY.

1. A study of the age composition of anopheline populations, using wing-condition as the criterion, showed that the age-composition of mosquitoes in unsprayed houses amidst sprayed houses in a sprayed village was similar to that in the unsprayed comparison villages.

2. The possibility of having a comparison area in a sprayed village itself is indicated, by leaving some houses untreated.

3. The importance of spraying every house in a village in malaria control programmes, is revealed.

4. The " Wing Index " is proposed as a simple entomological method for judging the effective residual activity of insecticidal deposits in the field.

ACKNOWLEDGMENT.

The authors are indebted to the authorities of The Rockefeller Foundation, to Dr. T. Chandrasekharaiya, Director of Public Health with the Government of Mysore, and to Dr. B. Ananthaswamy Rao, Superintendent, Bureau of Malariology, Department of Public Health, Bangalore, for their constant encouragement and guidance.

This paper is published with the permission of the Director of Public Health in Mysore, Bangalore.

REFERENCES.

BHOMBORE, S. R., SITARAMAN, N. L. and NANJUNDIAH, K. S. (1954) ... ... *Ind. J. Mal.*, **8**, pp. 61-67.
CHRISTOPHERS, S. R. (1911) ... ... *Paludism.*, No. 2, pp. 73-88.
PERRY, E. L. (1912) ... ... *Ibid.*, No. 5, pp. 32-40.
VISWANATHAN, D. K. and RAO, T. R. (1943) ... *J. Mal. Inst. Ind.*, **5**, pp. 255-260.

Indian Journal of Malariology, **8**, 3, *September* 1954.

STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

III. Morphology and transmission.

BY

LT.-COLONEL JASWANT SINGH, M.B., ch.B.(Edin.), D.P.H.(Eng.), D.T.M. & H.(London),

C. P. NAIR, D.M.S. (Madras), M.P.H. (Johns Hopkins)

AND

A. P. RAY, M.B.B.S.

(Malaria Institute of India, Delhi.)

(June 29, 1954.)

MIXED plasmodial infections are known to occur in nature in oriental monkeys, *M. irus*, the crab eating monkey of Malaya and Burma (Sinton, 1934). Sinton and Mulligan (1932) isolated a pure strain of *P. knowlesi* from this species of monkey by inoculating the infected blood into brown monkey of Northern India, *S. rhesus* (*Macaca mulatta mulatta*) and serially subpassaging thereafter. This strain on account of its great pathogenicity to *S. rhesus* monkey, formed a valuable material for various researches in India as well as in other parts of the world. In recent years, this strain maintained at the Malaria Institute of India, was found to be losing its virulence gradually (Jaswant Singh *et al.*, 1949 : 1950). Attempts to procure the strain from other research centres outside India did not meet with any success. With a view to isolating a virulent strain again, a specimen of the blood from an infected Malayan monkey, received through the courtesy of Dr. Edison, Institute for Medical Research, Malaya, was injected into *rhesus* monkeys in 1951 but only *P. cynomolgi* and *P. inui* could be isolated (Jaswant Singh *et al.*, 1951). Another attempt was made in early 1953 by Dr. Edison who inoculated the blood of an infected *Kra* monkey (*M. irus*) into young *S. rhesus* monkeys sent for this purpose by the Malaria Institute of India. The infection caused in these monkeys was very heavy and resembled *P. knowlesi* infection. So samples of blood, obtained from two of these *S. rhesus* monkeys (Malaria Institute of India Monkeys 3052 and 3056), were flown to Delhi and since then the strain has been maintained in the Institute at Delhi. The *Kra* monkey from which this new strain was isolated was obtained from Nuri Valley, near Tampin and hence

the strain is named, as desired by Dr. Edison (Edison, 1953) as Nuri strain of *P. knowlesi* (Jaswant Singh, *et al.*, 1953 ; Edison and Davey, 1953). In this paper, an account is given of the morphological features and the results of the preliminary trials on the transmission of this new strain of *P. knowlesi* (Nuri strain).

METHODS AND MATERIAL.

Apart from the study made in more than 200 *rhesus* monkeys inoculated for the purpose of maintaining the strain, detailed investigation was made in four additional monkeys, two inoculated soon after receiving the strain and the other two four months after that date, by which time the strain had already been serially passaged 35 times in quick succession.

Weight of the monkeys used for the present study ranged from 2·5 to 5 kg. body weight. They were all inoculated intravenously with five million parasitized erythrocytes per kg. body weight from donors showing very heavy infection.

Throughout the course of infection, blood smears were collected at 8 a.m. and 5 p.m. daily from most of the monkeys and in four at three-hourly intervals.

The stain used in all the cases was J.S.B. (Jaswant Singh and Bhattacharji, 1944). The stages of the parasites enumerated in thin films were categorised under seven headings; (1) rings, (2) early trophozoites (trophozoites Stage I), (3) medium sized trophozoites (Stage II trophozoites), (4) fully grown trophozoites (Stage III trophozoites), (5) early schizonts with less than five chromatin particles (Stage I schizonts), (6) grown up schizonts with five or more chromatin divisions including the fully matured ones, and (7) gametocytes.

The methods adopted for the transmission experiments were similar to that described by Jaswant Singh *et al.*, (1950). *A. annularis* mosquitoes were bred from larvæ collected in the field in the neighbourhood of Delhi and *A. stephensi* from the colony maintained at the Institute. Overnight starved mosquitoes were fed the following day on monkeys showing gametocytes in peripheral blood. Fully fed ones were sorted out and kept in Barraud cages and maintained on glucose and raisin till used for dissection.

MORPHOLOGY.

Host cell.—Host cell is not usually enlarged but some smears of the first few serially subpassaged monkeys occasionally showed slight enlargement and the same was not, however, confined to any particular stage of the parasite (Plate VIII, Figs. 19, 20, 22, 23 and 31). Parasites generally invade mature cells but during the peak period of parasitæmia, even some of the immature red blood corpuscles, such as those with cabbot's ring and basophilic stippling, are invaded (Plate VIII, Figs. 33 and 34).

The parasites have a remarkable property of altering the shape of the host cells. This occurred mostly in the form of irregular crenation (Plate VIII, Figs. 37 to 42) and was more evident during the ring and trophozoite stages than in the schizont stage. From Table I, it can be seen that 2 to 40 per cent of rings,

3 to 57 of trophozoites and zero to 40 of schizonts had this change in the host cell on the fourth day of infection.

Other distortions of the host cell included oval or pear or triangular or angular shape and fimbriated ends (Plate VIII, Figs. 20, 25 to 30). All these distortions combined, formed only a small percentage (less than 1 to 16 per cent) of the infected cells. In some smears, these distortions were practically absent or required a search of many parasites before they could be observed.

Many smears stained with J.S.B. stain showed stippling of the infected cells. These were not confined to older parasites but appeared also in young trophozoites. They resemble sometimes Maurer's dots (Plate VII, Fig. 17 ; Plate VIII, Fig. 37) ; occasionally Schuffner's dots (Plate VII, Fig. 19; Plate VIII, Fig. 31). More often, the stippling appeared different to either Maurer's or Schuffner's dots, being irregular in distribution and size and varying in numbers and intensity of staining (faint to pink or even red) (Plate VII, Figs. 20, 21 and 30 and Plate VIII, Figs. 21, 23, 24, 28, 29, 32, 35 and 36).

STAGES.

Rings.—Rings exhibited various features comparable to *P. falciparum*. They have been classified for convenience into three categories, (1) early fine hair like rings, occupying about 1/5 to 1/6th of the red blood cell, (2) slightly grown-up forms measuring 1/4th to 1/3rd the R.B.C. (upto six hours growth) with the thickening of the cytoplasm opposite to the chromatin, (3) still older forms, upto 11 hours growth (Plate VII, Figs. 13 and 14), occupying about 1/3rd to 1/2nd the area of the infected red blood corpuscle and containing dense solid cytoplasm. Sometimes this stage resembles rings with tail like processes.

Chromatin appears in different forms. Usually it is either round or oval or dot-like (Plate VII, Figs. 5 and 6). Quite often it takes a rod-like or an elongated appearance (Plate VII, Figs. 2, 3 and 4). The percentages of the different stages of the parasite showing morphological variations are given in Table I. From that it will be seen that 1 to 17 per cent of the rings had this elongated chromatin. Many rings contained two chromatin particles (1 to 17 per cent). These dots, when present, were located either side by side or at opposite poles of the ring (Plate VII, Figs. 8 and 9). Presence of accessory chromatin dots during ring stage is another characteristic feature (Plate VII, Figs. 5 and 10). The situation of these accessary chromatin dots varied and was found sometimes on the cytoplasmic ring and at other times either in the centre of the ring or adjacent to the cytoplasm. One to 20 per cent of the rings counted contained the accessory chromatin dot (Table I). On rare occassions, two accessory chromatin dots in one ring, in addition to the usual single chromatin (0·8 per cent in one instance), or one accessory chromatin dot and two normal chromatin particles in the same ring, were also recorded. The classical bird's eye form (Plate VII, Fig. 1) with the chromatin in the centre, were quite common and the same occurred in 2 to 28 per cent of the rings counted. Regarding the position of the ring in the R.B.C., the applique or accole forms formed zero to nine per cent of the total (Table I). Of these the applique forms were more often observed than the accole forms.

EXPLANATION OF PLATE VII.

1	Birds eye form.
2-4	Rings with rod-like or elongated chromatin.
5	Signet ring appearance of the parasite with accessory chromatin dot.
6	Ring with oval shaped chromatin.
7	Four rings in one R.B.C.
8-9	Rings with double chromatin.
10	Two rings, one with an accessory chromatin dot in a host cell.
11	Three rings in one R.B.C.
12	Two rings in one R.B.C.
13–14	Early (Stage I) trophozoites.
15–21	Medium sized (Stage II trophozoites).
	(17) Host cell with stippling.,
	(18) Two trophozoites in one R.B.C.
	(19-21) Two trophozoites in one R.B.C. showing stippling.
	(20) Three trophozoites in one R.B.C. showing stippling.
22–28	Fully developed trophozoites.
29–35	Early schizonts.
	(30) R.B.C. shows stippling.
36–42	Fully developed schizonts.
43, 44, 46 and 48	Female gametocytes.
45, 47 and 49	Male gametocytes.

EXPLANATION OF PLATE VIII.

1–6	Trophozoites with prominent vacuole.
7–12	Band forms.
13, 16 to 18	Amœboid trophozoites.
14–15	Advanced rings with web like structure of cytoplasm.
19 and 22	Early schizonts showing enlarged host cell.
20	Gametocyte in an enlarged angular shaped host cell.
21	Early schizont in R.B.C. showing stippling.
23	Two trophozoites in an enlarged host cell stippling.
24	Two trophozoites in a cell showing heavy stippling.
25–30	Oval or pear shaped or fimbriated host cell.
	(28-29) Host cells with stippling.
31	Enlargement of R.B.C. and heavy stippling during ring stage.
32	Early schizont in an oval shaped cell showing stippling.
33–34	Rings in immature R.B.C.
35	Early trophozoite showing heavy stippling of R.B.C.
36	Two rings in one R.B.C. showing heavy stippling.
37–42	Host cells showing irregular crenation.
	(37) Stippling of host cell.

PLATE VII.

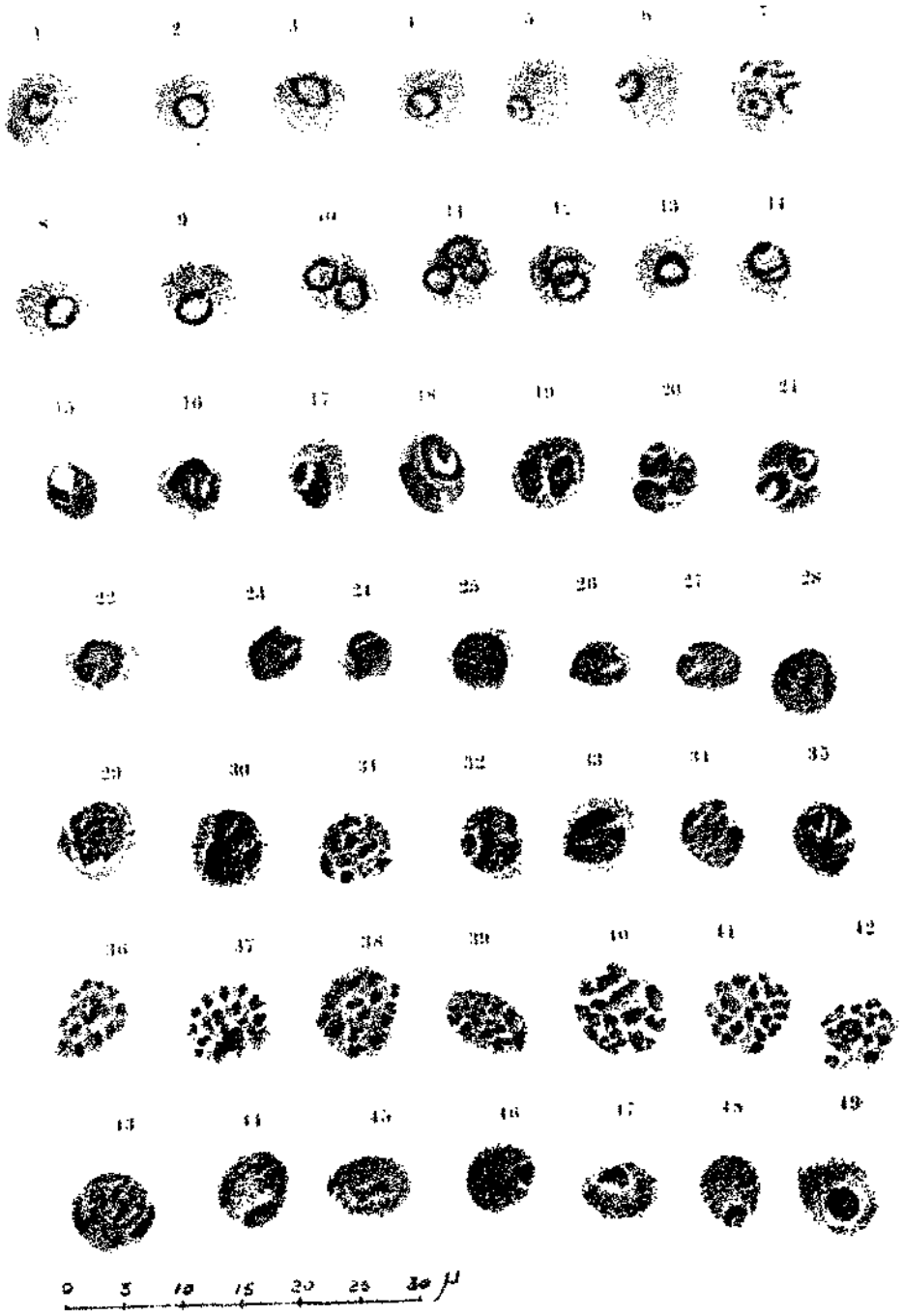

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
24
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
0 5 10 15 20 25 30 μ

PLATE VIII.

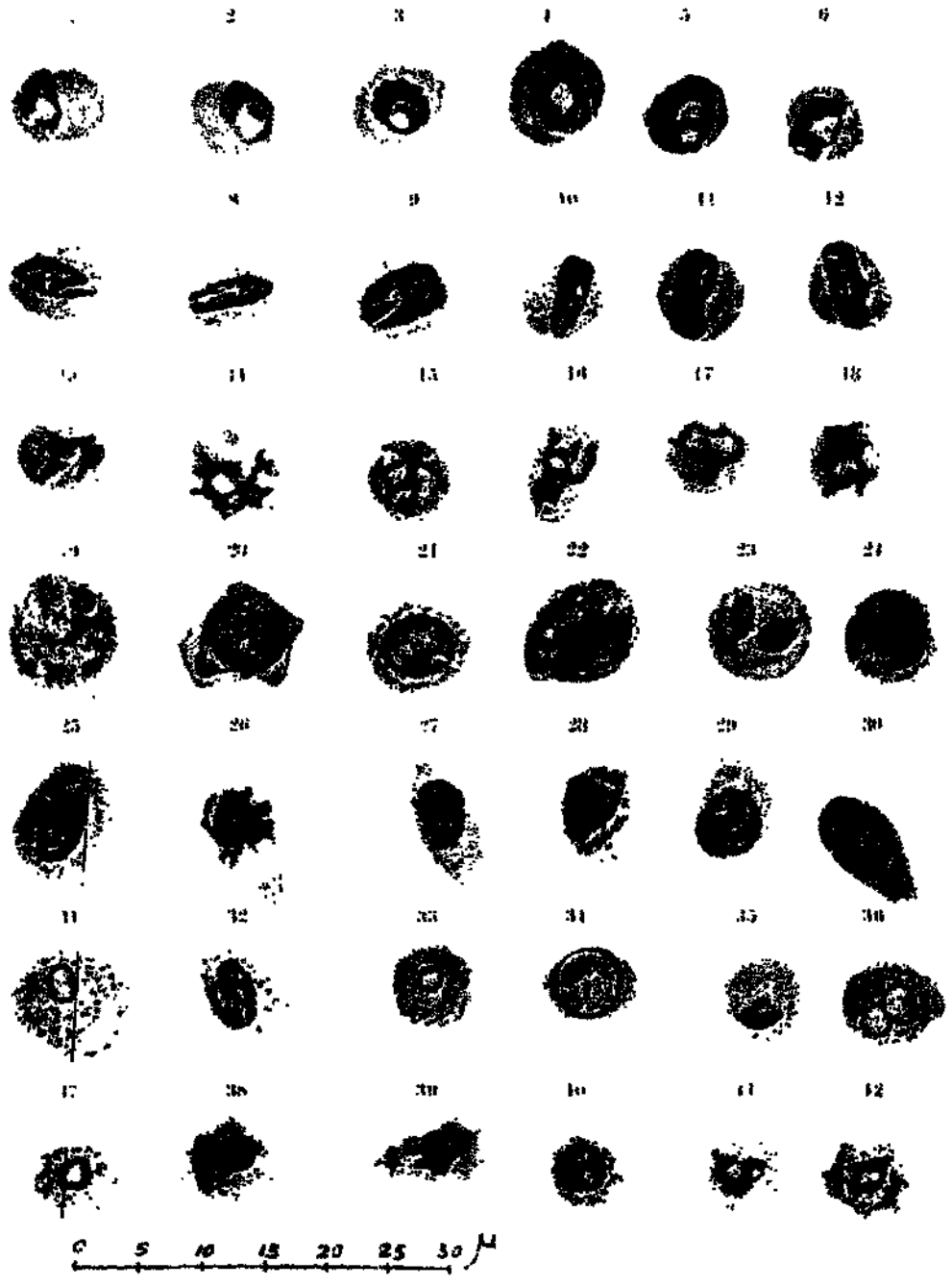

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
0 5 10 15 20 25 30 μ

TABLE I.

Percentage of the different stages of the parasites showing morphological variations.

Days.	1			2			3			4			5		
	R	T	S	R	T	S	R	T	S	R	T	S	R	T	S
Enlarged host cell (*a*)	...	...	...	...	...	...	...	*1	*1	*1	...	...	...	...	...
Schuffner's dots (*b*)	...	...	...	...	...	...	...	*1	...	...	...	...	...	...	...
Amœboidicity (*c*)	...	...	...	...	...	...	...	*1	...	...	...	...	...	...	...
(*b*) and (*c*) above	...	...	...	...	...	...	...	*1	...	...	...	...	...	...	...
(*a*), (*b*) and (*c*) above ...	...	...	...	...	...	...	...	*1	...	...	...	...	...	...	...
Irregularly crenated R.B.C. ...	...	...	...	*1–43	...	...	1–43	*1–17	...	2–40	3–57	0–40	*1–20	*1–10	...
Distortion of host cell other than crenation	...	...	...	0–1	0–1	...	0–4	0–14	...	*1–16	*1–16	0–11	...	*1–25	0–9
Double chromatin	...	...	...	1–8	...	...	2–14	...	...	2–17	...	...	7–12	...	...
Elongated chromatin	...	...	...	3–4	...	...	1–13	...	...	2–17	...	...	2–4	...	...
Accessary chromatin	...	...	...	2–20	...	...	1–7	...	...	1–7	...	...	1–11	...	...
Double chromatin + one accessary chromatin	...	...	...	*1	...	...	*1	...	...	...	...	...	...	...	...
Birds eye form	2–8	...	...	1–28	...	...	*1–20	...	...	1–10	...	...	10–17	...	...
Applique and accole forms ...	...	...	...	0–2	...	...	*1–9	...	...	*1–5	...	...	*1–2	...	...
Double infection	...	...	...	1–4	*1–6	...	2–17	1–40	...	8–27	3–47	...	4–20	7–18	...
Multiple infection—3 parasites	...	...	...	1–2	0–3	...	*1–12	*1–4	...	*1–9	*1–10	...	4–29	*1–14	...
4 parasites	...	...	...	...	...	...	...	...	...	...	...	...	4–5	...	...
Band form	...	...	...	*1–13	*1–8	...	0–10	1–18	...	*1–23	1–60	...	0–10	1–60	...
Tenue form	...	...	...	...	...	...	0–30	...	...	0–20	...	...	0–25	...	...

R = rings S = schizonts T = trophozoites. * = less than

Multiple infection was a very common feature. Two rings in one cell (Plate VII, Figs. 10 and 12) were encountered quite often and were numerous on the fourth and fifth days of infection, when its density reached 20 to 27 per cent of the rings counted. Infection of cells with three rings (Plate VII, Fig. 11) though not so high during the initial stages, reached a high figure of 29 per cent in some of the smears studied on the fifth day. Infection with four rings (Plate VII, Fig. 7) was seldom seen during the first four days but it suddenly appeared upto a density of four to five per cent on the fifth day.

Trophozoites.—Trophozoites of medium growth ((11 to 15 hours) i.e., Stage II trophozoites occupy about 1/2 to 2/3rd of the R.B.C. and are round or oval or elongated, with an almost regular margin (Plate VII, Figs. 15-21). The cytoplasm is dense and stains deeply. Vacuole is present in almost all cases and is uniformly small (Plate VII, Figs. 15 to 21).

Fully grown trophozoites (Stage III trophozoites 15 to 18 hours) occupy about 3/4th or more of the infected cell (Plate VII, Figs. 22-28). They are solid looking deeply stained parasites with a regular round or oval or almost elongated shape. No vacuole is seen at this stage. The chromatin is located generally towards the margin.

Double and triple infection (Plate VII, Figs. 18, 19 and 21) steadily increased with the successive cycles until on the fourth or fifth day of the infection, cells with two and three parasites appeared in 3 to 47, and up to 14 per cent, respectively, of the total Stages II and III trophozoites combined (Table I).

Schizonts.—Schizont stage starts with the division of the chromatin when the parasite grows to 3/4th the cell or even more (18-21 hours). The cytoplasm at this stage is dense and deeply stained (Plate VII, Figs. 29-35).

In fully developed schizonts (21-24 hours), four to sixteen merozoites are formed (average ten). The parasite occupies the whole of the normal sized R.B.C. (Plate VII, Figs. 36-42). The merozoites are arranged in an irregular or rosette form.

Sexual forms.—Sexual forms (Plate VII, Figs. 43-49) are round and occupy the whole cell. The differences between male and female are just the same as with other species. In female, the chromatin is deeply stained, appears compact and usually situated eccentrically. The cytoplasm is deeply stained. In males, the chromatin is diffuse and generally located in the centre. The cytoplasm is very often light pink or light blue.

Pigments.—Pigments appeared early in the growth of the parasite. In the slightly grown-up rings, cytoplasm appeared slightly granular at times suggesting the presence of very fine pigment dust and in the early trophozoite stage, this pigment dust appeared brownish and could be made out clearly. During Stage II and Stage III trophozoites and early schizonts, they were found scattered over the cytoplasm, becoming coarse and prominent with the advance in growth and appearing darkish brown or darkish green or greenish brown. When schizont is fully matured, pigments appear aggregated into one or two loose or dense clumps or in the form of one or two masses within the parasite. On rare occasions, more than two and even upto six such small masses were seen in one schizont. The colour of the pigment varied and as such appeared black in some and dark-brown

or greenish-brown in others. The pigments in both male and female parasites are scattered irregularly and appear as coarse dark or almost black or dark-brown granules.

Abnormalities.—Occasionally about 20 to 30 per cent of the trophozoites appeared like tenue forms (Stephen, 1914). During the earlier studies, it was not unusual to find in some smears the cytoplasm of parasites during early stages (advanced stage of ring of Stage I trophozoites) having a web like structure with one or more chromatin dots located either in the centre of the web or along the strands of cytoplasm (Plate VIII, Figs. 14 and 15).

Ring stages of the parasite appeared sometimes as band forms from the very first day of infection but their prevalence was maximum on the fourth day when this amounted to less than one to a maximum of 23 per cent.

Occurrence of band forms (Plate VIII, Figs. 7-12) during the trophozoite stage (Stages II and III) was a very characteristic feature. Here again these numbers steadily increased with the advance of infection and finally reached a figure ranging from one to 60 per cent of the total of these two stages studied on the last two days.

In the earlier subpassages, a few variations such as prominent vacuole (Plate VIII, Figs. 1 to 6) and amoebidicity of the cytoplasm (Plate VIII, Fig .13, and 16 to 18) were observed which in the subsequent studies were almost absent.

In addition to the few abnormalities already mentioned, the following two features were also observed during the earlier part of the study : (*a*) presence of stippling like Schuffner's dots and amœboidicity of the parasite in an unenlarged cell, (*b*) stippling like Schuffner's dots and amœboidicity in enlarged cells, resembling almost *P. cynomolgi*. The percentage of these forms was, however, extremely low and beyond easy detection unless many fields or the whole smear was examined.

TRANSMISSION THROUGH INSECT HOST.

In the course of the transmission experiments, 220 *A. annularis* and 261 *A. stephensi* were dissected, out of which oöcysts and sporozoites were detected in three *A. annularis* in one batch which were subsequently confirmed by staining.

DISCUSSION.

But for a few variations, the morphology of the Nuri strain of *P. knowlesi* is very similar to that described by Sinton and Mulligan (1933).

Mulligan (1935) noticed striking abnormalities such as oval, pear shaped fimbriated, triangular or irregularly crenated forms in many of the infected red cells with the old strain. In the absence of the data regarding the relative proportions of these different distortions, it is not possible to compare exactly these with the ones met with in the present studies, but one point appeared apparent that all such distortions, except the irregularly crenated ones, are present only in a minor degree in the new (Nuri) strain (Table I).

One of the remarkable features in the morphology of Nuri strain of *P. knowlesi* is the frequent presence of stippling in the infected cells. This may,

perhaps, be due to the stain itself as stipplings have been recorded in other plasmodia. Ramakrishnan and Satya Prakash (1950) observed that in *P. berghei*, stippling appeared to a great extent in smears stained with J.S.B. than with Geimsa. This was also reported by Jaswant Singh *et al.* (1950) in the non-virulent strain of *P. knowlesi*. Mulligan (1935) found stipplings in schizonts and fully developed trophozoites of *P. knowlesi*, only when stained with a special stain (in " panoptic " film) and not with Geimsa.

Regarding the morphology of the original strain, Brug (1934) observed that nuclei of the young parasites were usually surrounded by a clear, wholly unstained halo. He also noted that erythrocytes with basophilic dots showed an increased incidence of infection as well as an increased degree of infection. These two features were not, however, made out in the studies with the present strain.

According to Mulligan (1935), pigments in fully developed schizonts (in the previous strain) consisted of one or two dark-brown or almost black masses. It is a well-known fact that the appearance of pigments vary considerably with the type of illumination, intensity of staining and the magnification employed. Though it is difficult to judge to what extent those factors would have influenced the present study, it was observed that in the Nuri strain, the pigments in fully developed schizonts appeared either black or dark-brown. In reflected light, they even appeared at times dark-green or greenish-brown. Moreover instead of always being in dense masses as described by Mulligan (1935), they also appeared aggregated into one or two loose or dense clumps. On occasions, more than two such masses or clumps were visible in one schizont and this happened even in cases where there was not even a far remote chance of two or more parasites occupying one cell.

Tenue forms and band forms not included in the description given by Sinton and Mulligan (1932 : 1933), were important features of the present strain. However, band forms were reported earlier by Brug (1934).

Brug (1934) differentiated *P. knowlesi* typicus strain from *P. knowlesi* var. *sintoni* strain, on account of the fact that the latter strain showed frequent presence of rod-shaped pigments in the large parasites, rare occurrence of distorted and fimbriated infected R.B.C. and regular occurrence of red rim and red septa in multinucleated schizonts.

From the few dissimilarities already enumerated in the forgoing paragraphs, it would appear that " Nuri " strain is not identical with either *P. knowlesi* typicus or *P. knowlesi* var. *sintoni*, and hence it is considered desirable to differentiate it by its separate name (Nuri strain) as first suggested by Dr. Edison.

Some smears of the monkeys, during the first few serial passages of the strain, showed a few parasites with morphological variations almost resembling *P. cynomolgi* but such abnormalities were not detected after the subsequent serial passages. It may be recalled in this connection that two monkeys were studied in detail during the second serial passage in the strain maintenance series (after receiving the strain initially from Dr. Edison), and the other two during the 35th serial passage. This indicates that the natural vertebrate host (the " *kra* " monkey), in all probability, harboured at the time of inoculating its blood into the *rhesus* monkey, a mixed plasmodial infection, probably of *P. knowlesi* and *P. cynomolgi*,

but due to the extreme virulence and rapid multiplication of the former in the *rhesus* monkeys, *P. cynomolgi* eventually got rapidly filtered out, resulting thus in the isolation of the present pure strain of *P. knowlesi* (Nuri).

In view of the rapidly growing infection with *P. knowlesi* and its severity, the *rhesus* monkeys die long before mature gametocytes are found in fair numbers in the peripheral circulation. This may perhaps be the reason why sporogony cycle could not be completed by certain workers like Mulligan and Knowles (quoted by Russell, 1942) ; Russell *et al.* (1946) ; Mohan (1949) and Russell and Mohan (quoted by Mohan, 1949). However, Mulligan (1935) observed complete sporogony cycle in a few specimens of *A. annularis* out of many hundreds of mosquitoes of various species that were tried. During the present studies with Nuri strain, the authors were able to detect oöcysts and sporozoites in only three out of 220 *A. annularis* and none in *A. stephensi*. But recently, Hawking and Mellanby (1953) had fair degree of success in this respect by using a special technique involving the administration of para-amino-benzoic acid to monkeys kept on a milk diet.

SUMMARY.

1. Morphology of Nuri strain of *P. knowlesi* was studied in more than 200 *S.rhesus* monkeys.

2. But for the exception of a few variations which are described in detail, the morphology of the parasites is almost identical with those described by Sinton and Mulligan (1932), Mulligan (1935) and Brug (1934) with regard to the previous virulent strain of *P. knowlesi.*

3. Strain difference between the Nuri strain and the other previous strains are discussed.

4. There was some indication of the existence of a mixed infection in the first few serially subpassaged monkeys but subsequent repeated transfer from monkey to monkey, has resulted in the isolation of a pure strain.

ACKNOWLEDGEMENT.

The authors thank Mr. S. K. Ochaney, the Artist at the Institute, for his accurate drawings on Plates VII and VIII.

REFERENCES.

Brug, S. (1934) ... ... *Riv. Malariol.*, **13**, p. 121.
Brumpt, E. (1949) ... ... *Malariology* edited by Boyd, M. F., Vol. I p. 72. W. B. Saunders Co., Philadelphia and London.
Edison, J. F. B. (1953) ... ... *Personal communication.*
Edison, J. F. B. and Davey, D. G. (1953) ... *Trans. Roy. Soc. Trop. Med Hyg.*, **47**, p. 259.
Hawking, F. and Mellanby, H. (1953) ... *Ibid.*, **47**, p. 438.
Jaswant Singh and Bhattacharji, L. M. (1944) ... ... *Ind. Med. Gaz.*, **79**, p. 102.
Jaswant Singh, Ray, A. P. and Nair, C. P. (1949) ... ... *Ind. J. Mal.*, **3**, p. 145.
Idem (1950) ... ... *Ibid.*, **4**, p. 317.
Idem (1953) ... ... *Nature*, **172**, p. 122.
Jaswant Singh, Ray, A. P., Nair, C. P. and Basu, P. C. (1951) ... ... *Ind. J. Mal.*, **5**, p. 433 .

Mohan, B. N. (1949)	*Personal communication.*
Mulligan, H. W. (1935)	*Arch. Prot.*, **84**, p. 285.
Ramakrishnan, S. P. and Satya Prakash (1950)	*Ind. J. Mal.*, **4**, p. 369.
Russell, P. F. (1942)	*Final Report of the Madras Malaria Investigation Project.* International Health Division of The Rockefeller Foundation, New York.
Russell, P. F., West, L. S. and Manwell, R. D. (1946)	*Practical malariology.* W. B. Saunders Co., Philadelphia and London.
Sinton, J. A. (1934)	*Current Science*, **3**, p. 48.
Sinton, J. A. and Mulligan, H. W. (1932)	*Rec. Mal. Surv. Ind.*, **3**, p. 357.
Idem (1933)	*Ibid.*, **3**, p. 381.
Stephens, J. W. W. (1914)	Quoted by Brumpt, E. (1949).

Indian Journal of Malariology, **8**, 3, *September* 1954.

STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

IV. Periodicity.

BY

LT.-COLONEL JASWANT SINGH, M.B., Ch.B.(Edin.). D.P.H.(Eng.), D.T.M. & H.(London),

C. P. NAIR, D.M.S. (Madras), M.P.H. (Johns Hopkins)

AND

A. P. RAY, M.B.B.S.

(Malaria Institute of India, Delhi.)

(July 20, 1954.)

A NEW strain of *P. knowlesi* (Nuri strain), highly pathogenic, to *M. mulatta mulatta* of Northern India, was recently isolated (Jaswant Singh, Ray and Nair, 1953 ; Edison and Davey, 1953). Morphological characters and transmission with this strain have recently been reported by Jaswant Singh *et al.* (1954). The present paper records observations on the periodicity of this Plasmodium.

MATERIAL AND METHODS.

Of the four monkeys utilized to determine the periodicity, three (Monkeys 3111, 3988 and 4010) were inoculated intravenously with five million parsitized erythrocytes per kg. body weight, while the fourth (Monkey 3160) received a dose of fifty million parasites per kg. body weight.

Blood smears were taken at intervals of one, two, three and four hours from Monkeys 4010, 3988, 3160 and 3111 respectively, from the time of inoculation till death of the animals, except in the case of Monkey 4010 from which smears were collected only for 48 hours commencing from the third day of patent infection.

J.S.B. stain was used in all cases. Parasites were classified and enumerated under seven different stages, namely, (1) rings, (2) early trophozoites (Stage I), (3) medium sized trophozoites (Stage II), (4) fully grown trophozoites (Stage III), (5) early schizonts with less than five chromatin divisions (Stage I), (6) grown up schizonts with five or more chromatin divisions including the fully

matured ones, and (7) gametocytes. Total parasite counts were determined in terms of percentages of red blood carpuscles infected. Likewise counts of the different stages of the parasite were expressed as percentages of total parasites. Ehrlich's eyepiece adjusted to count about 100 red blood corpuscles was used in the counts. When infection was very heavy, the eyepiece was adjusted to include 25 red blood corpuscles. In early stages of the infection when only few parasites could be detected in the thin films, the whole smear was searched. Later, from the time infections became moderate, at least a minimum of 10,000 red blood corpuscles were counted to determine the percentage of cell infection. For differential parasite counts, at least a hundred parasites were examined.

When schizonts were present in fairly large numbers, at least a hundred were counted, but if they were scanty in number, a large area of the thick film was examined.

OBSERVATIONS.

The parasitized R.B.Cs. were seen in peripheral circulation on the same day of inoculation but proportionately in larger numbers in Monkey 3160 which had received an inoculum of fifty million parasites.

The total parasite counts of the four monkeys (Charts 2, 4, 6 and 8) indicate daily acute rise, followed by decline in parasite density, finally resulting in sudden increase in parasitæmia by fifth or sixth day, when cell infection reached about 98 per cent. At this stage, taking into consideration the multiple infection so common in this species, all the monkeys died, with an overall parasitæmia of about 143 per 100 R.B.Cs.

The course of events in the asexual cycle of the four monkeys are given in Charts 1 to 9. Daily peaks within 24 hours in total parasite counts, are evident. A similarity in the curves of the differential parasite counts at different stages can be traced in all the four experiments (Charts 1, 3, 5 and 7). Period taken for the completion of asexual cycle as calculated from the peaks of the different stages of the parasites and the total parasite population, are recorded in Table I. It may be observed that even though 16 to 28 hours were recorded for the completion of cylces by the different stages, the overall average comes to 24·3 hours or, for all practical purposes, 24 hours.

The peaks of differential and total parasite counts are given in Table II. It seems that the peaks of the schizonts (Stage II) occurred in the case of Monkey 3111 (from which four-hourly smears were taken) at 4 p.m. on the second day, 8 a.m. on the third and fourth days and at 12 noon for the following two days. Thus the cycle was accelerated by about eight hours during the second day of infection. On the third day, the cycle was 24 hours but on the fourth day it was retarded by four hours.

In the case of Monkey 3160 (3 hourly smear series), the peaks occurred at 11 a.m. regularly during the second, third and fourth days after inoculation whereas during the first day after inoculation and the last day of infection, the same happened at 2 p.m. Thus the cycle was accelerated by three hours during the first day, and retarded by a similar period on the last occasion.

TABLE I.

Duration of the asexual cycle.

Stages	Cycle 1 (Hours).				Cycle 2 (Hours).				Cycle 3 (Hours).				Cycle 4 (Hours).				Cycle 5 (Hours).				Average.
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	
Rings	24	21	20	...	20	24	24	...	28	21	28	...	20	27	24	24	28	21	...	...	23·6
Trophozoites																					
Stage I	28	21	22	...	20	24	26	...	24	24	24	...	24	27	...	23	24	...	...	...	23·93
Stage II	28	27	24	...	20	24	24	...	20	21	26	...	28	27	...	24	...	...	...	...	24·41
Stage III	28	27	22	...	20	21	28	...	20	24	26	...	28	27	...	26	...	...	...	...	24·75
Schizonts																					
Stage I	28	24	24	...	24	24	22	...	24	24	28	...	24	24	24	26	...	...	...	...	24·6
Stage II	16	21	22	...	24	24	22	...	28	24	30	...	24	27	26	25	...	...	...	...	24
Total parasites	20	24	26	...	28	24	24	...	28	30	24	...	24	21	24	30	20	...	...	...	24·8

Average=24·3 or, say, 24.

M1=monkey number 3111, M2=monkey number 3160, M3=monkey number 3988, and M4=monkey number 4010.

TABLE II.

Time of differential and total parasites count peaks.

Day after inoculation.	Monkey number.	Interval between successive smears (in hours).	Differential parasite count peaks.							Total parasite-count peaks.
			All multi-nucleated stages.	Schizonts, Stage I.	Schizonts, Stage II.	Rings.	Tropho-zoites, Stage I.	Tropho-zoites, Stage II.	Tropho-zoites, Stage III.	
0–1	3160	3	...	...	...	8 p.m.	11 p.m.	11 p.m.	2 a.m.	...
1–2	3988	2	9 a.m.	9 a.m.	11 a.m.	5 p.m.	9 p.m.	1 a.m.	5 a.m.	3 p.m.
	3160	3	8 a.m.	8 a.m.	2 p.m.	5 p.m.	8 p.m.	2 a.m.	5 a.m.	2 p.m.
	3111	4	...	...	...	4 p.m.	8 p.m.	12 Night	4 a.m.	8 p.m.
2–3	3988	2	9 a.m.	9 a.m.	9 a.m.	1 p.m.	7 a.m.	1 a.m.	3 a.m.	5 p.m.
	3160	3	8 a.m.	8 a.m.	11 a.m.	5 p.m.	8 a.m.	2 a.m.	2 a.m.	2 p.m.
	3111	4	4 a.m.	4 a.m.	4 p.m.	4 p.m.	12 Night	4 a.m.	4 a.m.	4 p.m.
3–4	4010	1	...	...	...	12 Noon	9 p.m.	12 Night	4 a.m.	4 p.m.
	3988	2	7 a.m.	7 a.m.	7 a.m.	1 p.m.	9 p.m.	1 a.m.	7 a.m.	5 p.m.
	3160	3	8 a.m.	8 a.m.	11 a.m.	2 p.m.	8 p.m.	11 p.m.	2 a.m.	2 p.m.
	3111	4	8 a.m.	8 a.m.	8 a.m.	12 Noon	8 p.m.	12 Night	4 a.m.	8 p.m.
4–5	4010	1	10 a.m.	7 a.m.	10 a.m.	12 Noon	8 p.m.	12 Night	4 a.m.	10 p.m.
	3988	2	1 p.m.	11 a.m.	1 p.m.	5 p.m.	9 p.m.	3 a.m.	9 a.m.	5 p.m.
	3160	3	8 a.m.	8 a.m.	11 a.m.	5 p.m.	11 p.m.	2 a.m.	5 a.m.	8 p.m.
	3111	4	8 a.m.	8 a.m.	8 a.m.	4 p.m.	8 p.m.	12 Night	4 a.m.	12 Night
5–6	4010	1	11 a.m.	9 a.m.	11 a.m.	...	...	...	...	...
	3988	2	3 p.m.	11 a.m.	3 p.m.	5 p.m.	... ...	...	...	5 p.m
	3160	3	11 a.m.	8 a.m.	2 p.m.	11 a.m.	2 p.m.	...	...	5 p.m.
	3111	4	8 a.m.	8 a.m.	12 Noon	12 noon	8 p.m.	4 p.m.	8 a.m.	12 Night
6	3111	4	12 Noon	8 a.m.	12 Noon	4 p.m.	8 p.m.	...	...	8 p.m.

From a similar study of Monkey 3988, it was observed that the peak occurred at 11 a.m. on the first day, 9 a.m. on the second, 7 a.m. on the third, 1 p.m. on the fourth and at 3 p.m. on the fifth day. So a 22-hour segmentation is indicated on the second and third days, 30 hours on the fourth and 26 hours on the fifth day of infection.

Peaks in the schizont percentages of Monkey 4010 (from which one-hourly smears were studied) indicate that the parasites divided synchronously at 10 a.m. on the fourth day, and 11 a.m. on the fifth day of infection.

In general, peaks of early schizonts occurred generally between 7 and 11 a.m. every day, and of the more advanced ones between 7 a.m. and 3 p.m., but with a greater frequency at 11 a.m. Monkeys 3111 and 3160 showed a progressively increasing count of Stage II schizont on each succeeding day of infection (Charts 1 and 3) but this phenomenon was not, however, well demonstrated in Monkey 3988 (Chart 5). On the fourth day of infection, Monkey 4010 from which hourly smears were collected, showed a greater percentage of schizonts than what was recorded in others.

From Chart 9 in which all multinucleated schizonts are recorded separately in each case without making any distinction between early and late schizonts, a clear cut single brood of infection is evident in Monkeys 3111, 3160 and 4040, but the same was not found fully applicable to Monkey 3988. Here the curves suggest the possibility of the existence of two broods, with the second or minor brood closely following the major and attaining the peak at 3 p.m., 9 a.m., 11 a.m., and 9 p.m. on the first, second, third and fourth day of infection, respectively. Thus it would appear that the minor brood aligned itself with the major brood on the second day by following an eighteen hour cycle and thereafter it multiplied at 26 and 34 hours intervals on the third and fourth day, respectively.

The average number of merozoites per schizont per day differed during the whole course of infection. Their standard errors are recorded for the different monkeys in Table III. They are, in majority of the cases, about 0·2 or less. The merozoite means throughout the infection were found to be $10\pm0{\cdot}4$, $10{\cdot}7\pm0{\cdot}11$, $9{\cdot}6\pm0{\cdot}15$ and $11{\cdot}5\pm0{\cdot}17$ in the case of Monkeys 3111, 3160, 3988 and 4010 respectively, and were in general higher during the earlier part of the infection *i.e.*, up to the third cycle as compared to the subsequent cycles. The overall average, for the entire period of infection in the four monkeys, worked out to be $10{\cdot}25\pm0{\cdot}095$.

Period of liberation of rings by segmenters is tabulated in Table IV. Irrespective of the day of infection, rings were liberated over a period of nine hours (11 a.m. to 8 p.m.) in Monkey 3160. But in Monkeys 3111 and 3988, this period was found to be twelve hours commencing at 8 a.m. in the former, and at 7 a.m., 9 a.m. and 11 a.m. on the three successive days in the latter. In the fourth monkey (4010), the same was found extended over a maximum period of 13 hours (8 a.m. to 9 p.m.). In general, rings appeared in the peripheral blood of these monkeys from nine to thirteen hours with the peak count between 2 p.m. and 5 p.m.

Taking into account the number of rings and the merozoite means as adopted by Taliaferro and Taliaferro (1949), the day to day rate of increase of parasites and the percentage parasite survival have been calculated and recorded in Table V.

CHART I.

PERIODICITY OF *P. KNOWLESI* (NURI STRAIN)

Differential parasite count of 4-hourly smears. Monkey 3111 *inoculated with 5 million parasitized cells per kilogram body weight at* 16·00 *hours on* January 22, 1953.

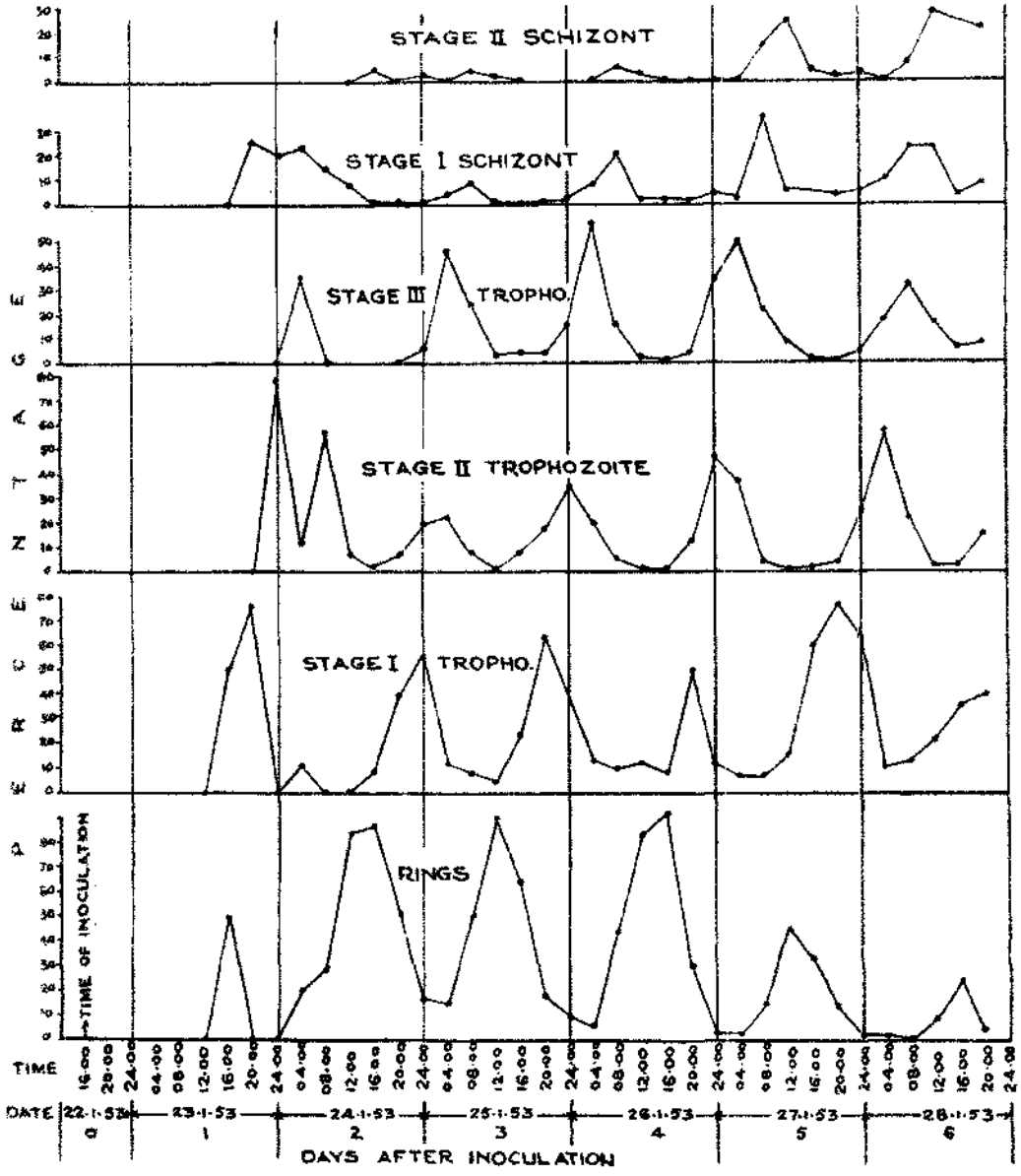


CHART 2.

COURSE OF BLOOD INDUCED *P. KNOWLESI* (NURI STRAIN) INFECTION

Monkey 3111 *inoculated with* 5 *million parasitized cells per kilogram body weight at* 16·00 *hours on* January 22, 1953.

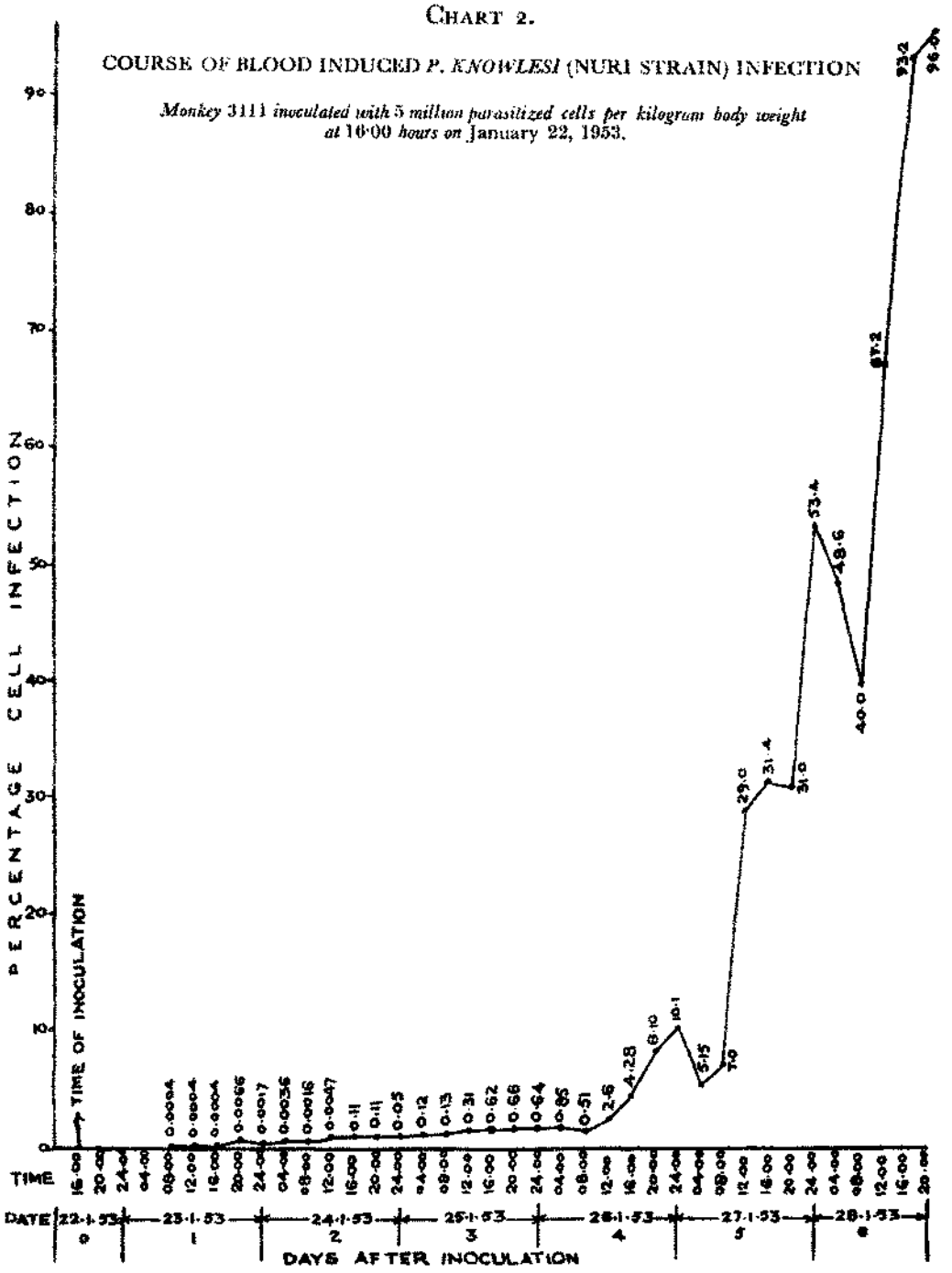


CHART 3.

PERIODICITY OF *P. KNOWLESI* (NURI STRAIN)

Differential parasite count of 3 hourly smears. Monkey 3100 *inoculated with* 50 *million parasitized erythrocytes per kilogram body weight at* 18.00 *hours on* February 11, 1953.

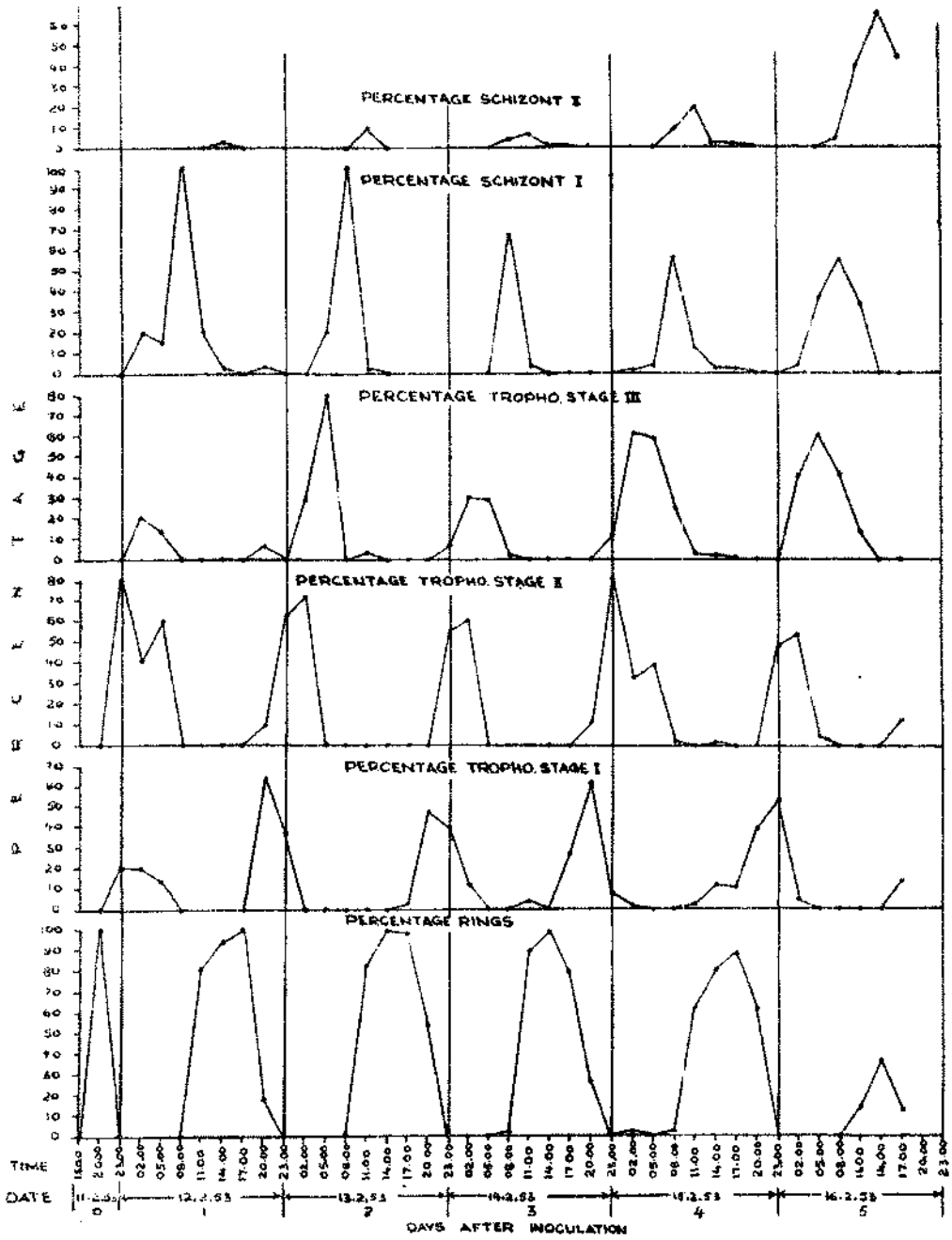
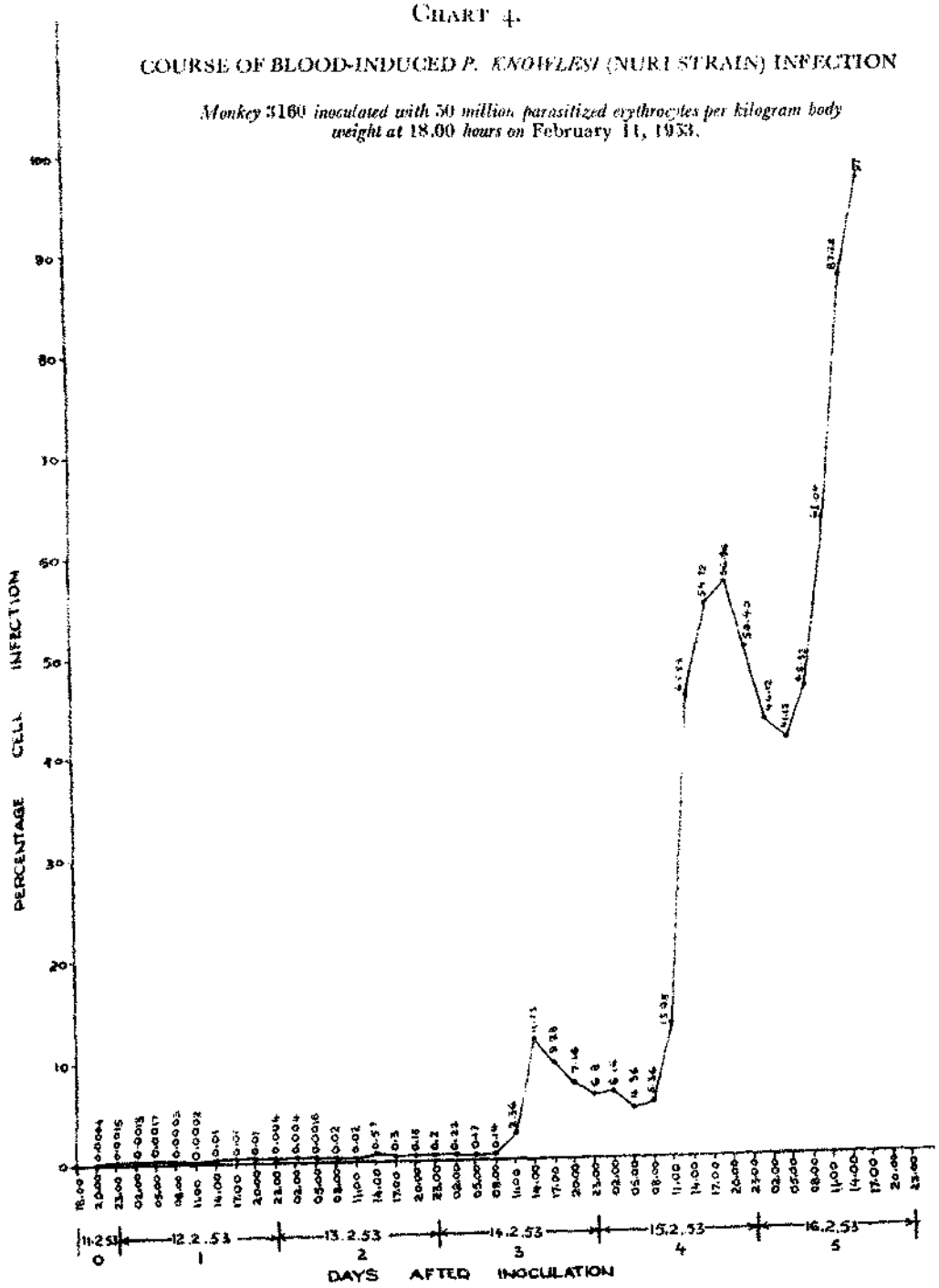


CHART 4.

COURSE OF BLOOD-INDUCED *P. KNOWLESI* (NURI STRAIN) INFECTION

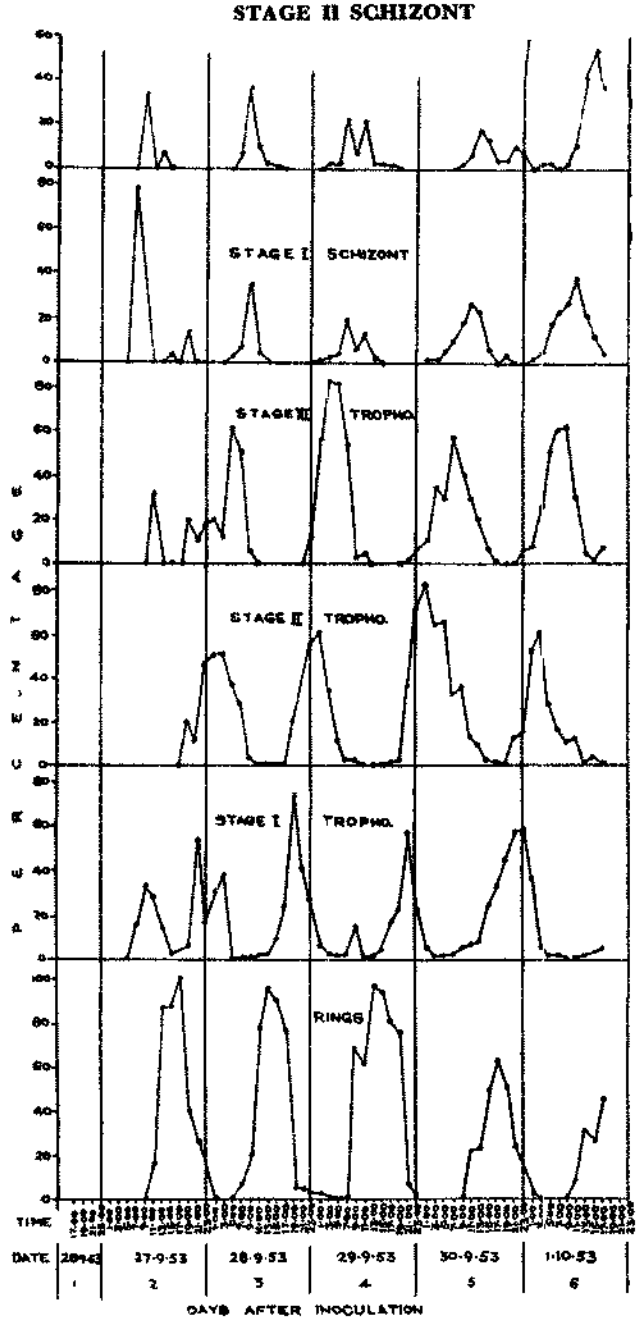
Monkey 3160 *inoculated with* 50 *million parasitized erythrocytes per kilogram body weight at* 18.00 *hours on* February 11, 1953.



GRAPH 5

PERIODICITY of *P. KNOWLESI* (NURI STRAIN).

Differential parasite count of 2 hourly smears. Monkey 3988 *inoculated with 5 million parasitized erythrocytes per kg. body weight at* 17·00 *hours on* September 26, 1953.



GRAPH 6.

Course of blood-induced P. Knowlesi *(nuri strain) infection. Monkey* 3988 *inoculated with* 5 *million parasitized cells per kilogram body weight at* 17·00 *hours on* September 28, 1953.

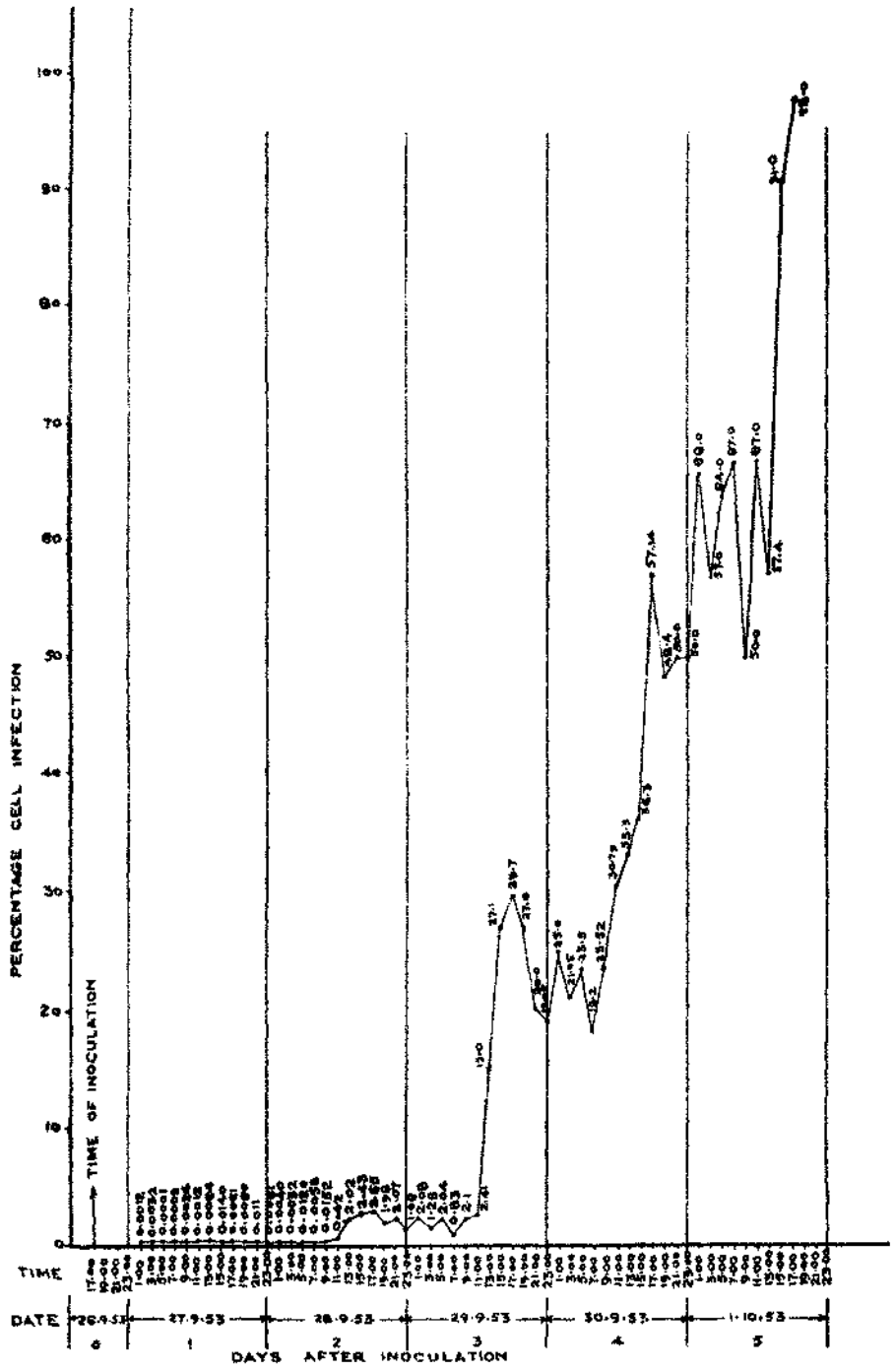


CHART 7.

PERIODICITY of *P. KNOWLESI* (NURI STRAIN).

Differential parasite count of one-hourly smears. Monkey 4010 *inoculated with* 5 *million parasitized cells per kg. body weight at* 17.00 *hours on* October 7, 1953.

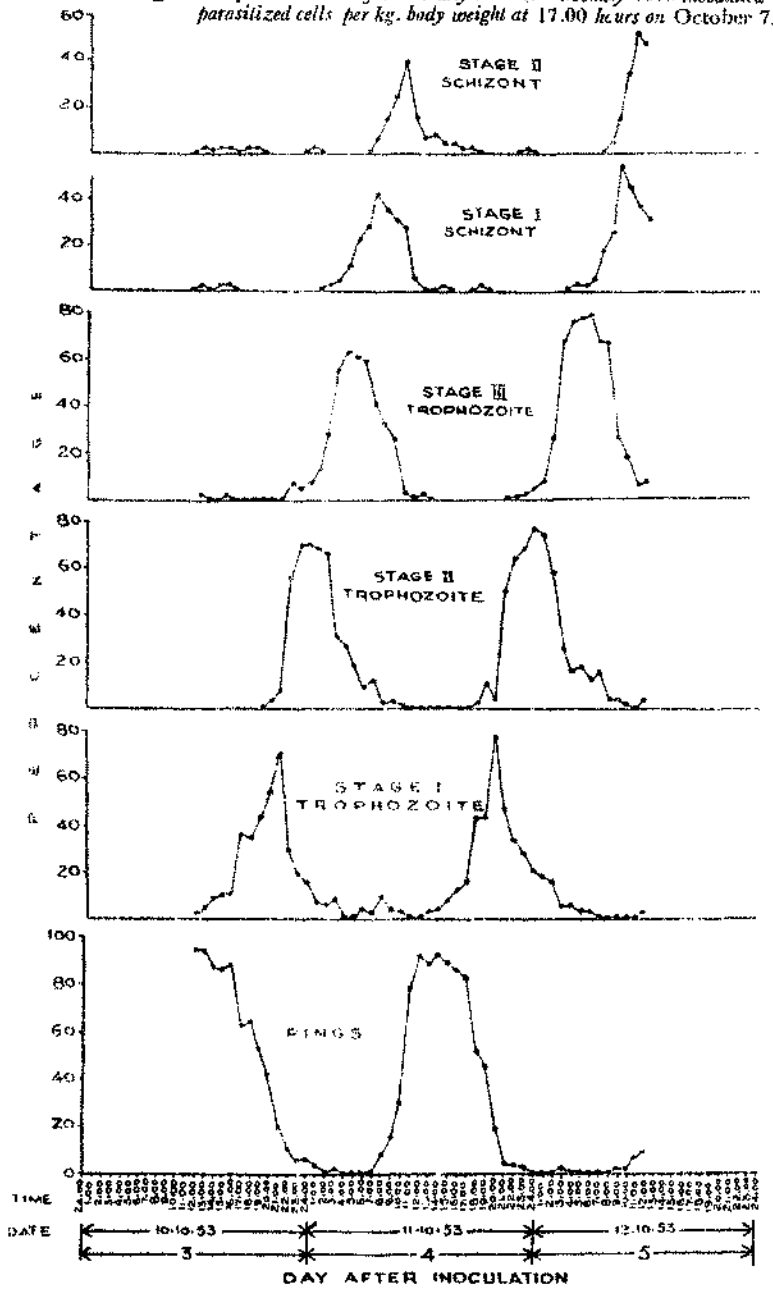


CHART 8.

Course of blood-induced P. knowlesi *(Nuri strain) infection. Total parasite count of Monkey* 4010 *inoculated with* 5 *million parasitized cells per kilogram body weight at* 17.00 *hours on* October 7, 1953.

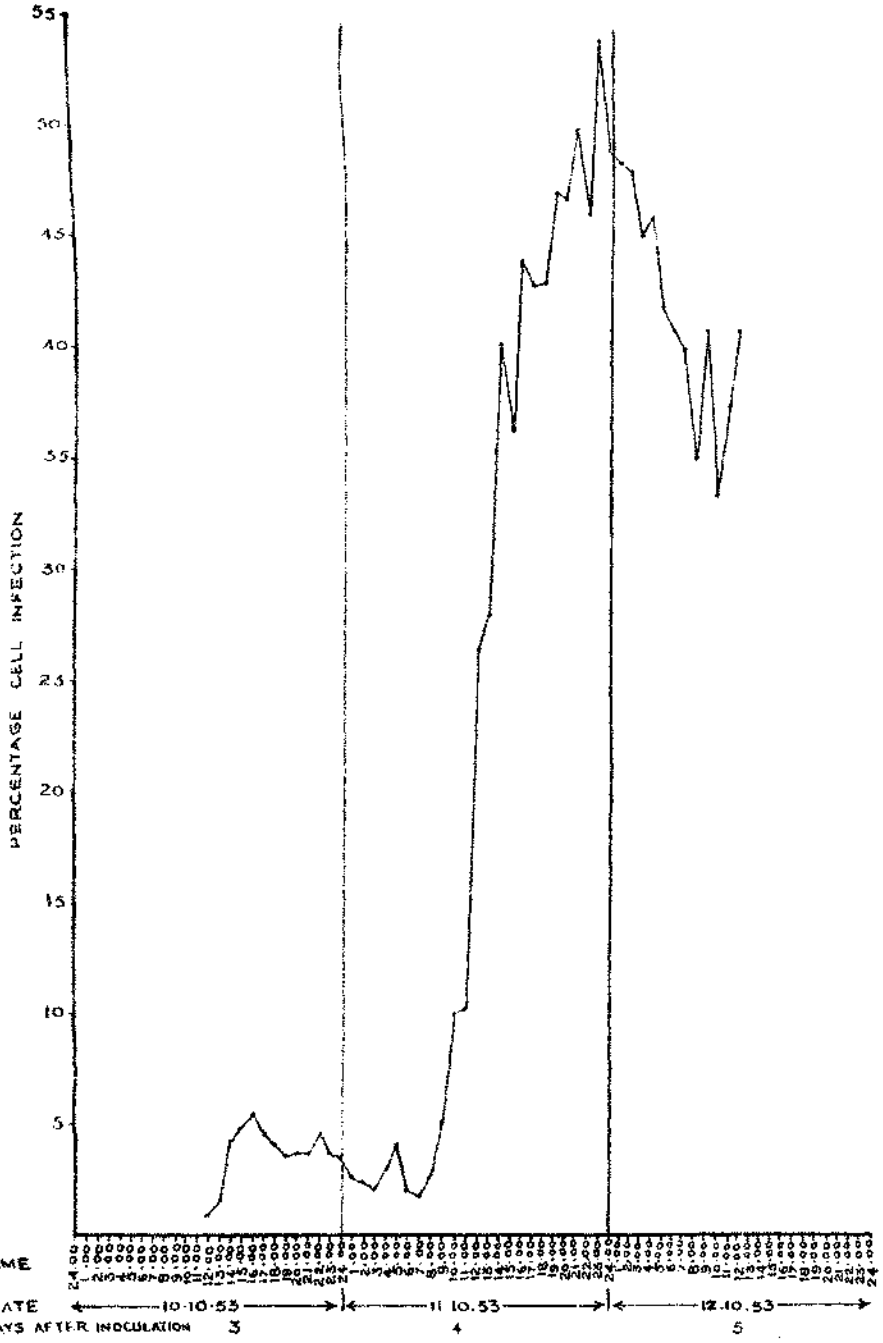


CHART 9.

PERIODICITY OF *PLASMODIUM KNOWLESI*
Count of multinucleated stages of Monkeys 4010, 3988, 3160 *and* 3111 *from smears collected at hourly, two hourly, three hourly and four hourly interval respectively.*

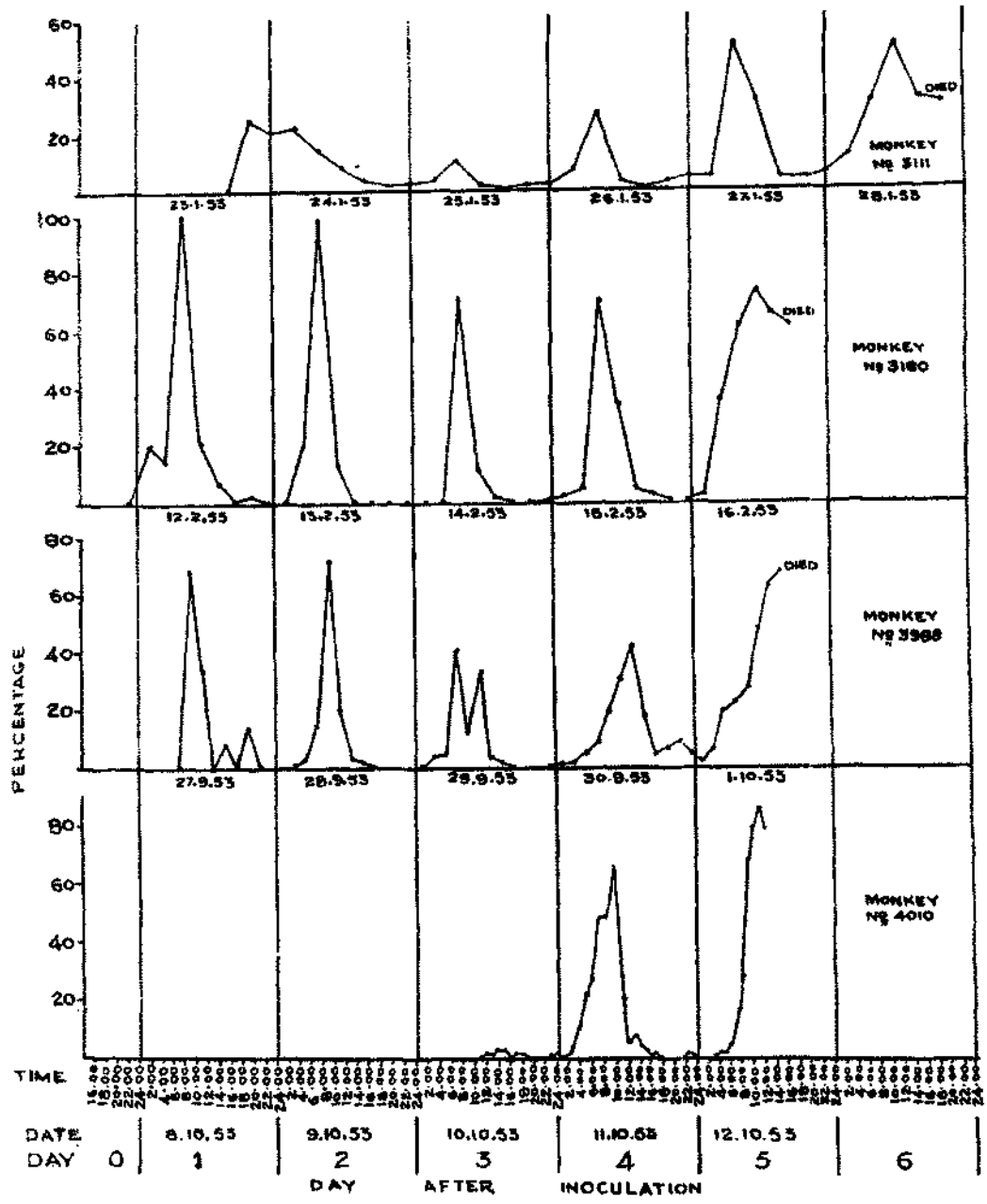


TABLE III.

Average number of merozoites per schizont per day during the course of P. knowlesi *(Nuri strain) infection.*

Monkey number.	Dose of inoculum (million parasites per kg. body weight of animal).	Interval at which smears were collected (hours).	Total number of schizonts counted.	Days after inoculation.							Total period for all monkeys
				1	2	3	4	5	6	Total period.	
3111	5	4	34	...	10	10±0·6	13±0·7	12·3±0·9	9±0·3	10·3±0·4	
3160	50	3	330	12±1·4	14	14±0·6	9·5±0·12	12±0·22	...	10·7±0·11	
3988	5	2	472	...	14·5±0·8	13·6±0·18	7·1±0·12	9·4±0·18	...	9·6±0·15	
4010	5	1	125	...	...	11·2±0·14	11·7±0·18	9·9±0·29	...	11·5±0·23	
All (3111, 3160, 3988, 4010)	5 to 50	1 to 4	970	12±1·4	14·2±0·73	13·2±0·18	9·1±0·11	11·1±0·17	9±0·3	...	10·25±0·095

TABLE IV.

Period of liberation of rings by segmenters.

Monkey number.	Time.											
	Day after inoculation.											
	1		2		3		4		5		6	
	Range.	Peak.	Range.	Peak.	Range.	Peak.	Range.	Peak.	Range.	Peak.	Range.	Peak.
4010	...	...	...	...	...	...	8 a.m.-9 p.m.	2 p.m.	...	...	...	...
3988	11 a.m.-11 p.m.	5 p.m.	7 a.m.-7 p.m.	1 p.m.	9 a.m.-9 p.m.	1 p.m.	11 a.m.-11 p.m.	5 p.m.	...	...	...	...
3160	11 a.m.-8 p.m.	5 p.m.	11 a.m.-8 p.m.	2 p.m.	11 a.m.-8 p.m.	2 p.m.	11 a.m.-8 p.m.	5 p.m.	11 a.m.	2 p.m.	...	...
3111	...	4 p.m.	...	4 p.m.	8 a.m.-8 p.m.	12 noon	8 a.m.-8 p.m.	4 p.m.	8 a.m.-8 p.m.	12 noon	12 noon-8 p.m.	4 p.m.

TABLE V.

Overall survival of parasites per segmentation.

Days after inoculation.	1				2				3				4				5				6
Monkey number	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1
Average ring count per 100 parasites during the day	0·0003	0·005	0·00004	...	0·03	0·24	0·4085	...	0·16	3·3	11·06	1·92	1·52	18·5	15·68	14·75	5·1	17·5	18·6	...	7·4
Rate of increase	...	...	...	...	100	45	10,000	...	5·3	13·7	27·2	...	9·5	5·6	1·4	7·7	3·4	0·95	1·2	...	1·5
Merozoite mean	...	...	...	...	10	14	14·5	...	10	14	13·6	11·2	13	9·5	7·1	11·7	12·3	12	9·4	9·9	9
Survival percentage	...	...	...	...	> 100	> 100	> 100	...	53	98	> 100	...	73	58	19·7	74·3	27·6	8	12·8	...	14·4

M1 = monkey number 3111, M2 = monkey number 3180, M3 = monkey number 3988, M4 = monkey number 4010.
> = more than.

Though the rate of increase during the first two days of patent infection was found to be more than what can be accounted for, on the basis of the merozoite means only, this rate of increase during the rest of the period was, however, within the limits of the observed merozoites means. After the first one or two days of patent infection, the survival rate of the parasites was in markedly decreasing order on each of the subsequent days. Thus the observed final survival rate on the last day of infection was found to be as low as 8 to 14 per cent (Table V).

The period of growth of the different stages of the parasite as studied from the four monkeys, is recorded in Table VI. Even though these periods varied within some range in the different monkeys, the average came to six hours for the growth of rings, five hours for early trophozoites, four hours for Stage II trophozoites, and three hours each for the other three stages (grown-up trophozoites, early and developed schizonts).

TABLE VI.

Period of growth of the different stages of the parasite.

Monkey number.	Rings (R-T_1) (hours.)	Trophozoites, Stage I (T_1-T_2) (hours.)	Trophozoites, Stage II (T_2-T_3) (hours.)	Trophozoites, Stage III (T_3-S_1) (hours.)	Schizonts, Stage I (S_1-S_2) (hours.)	Schizonts, Stage II (S_2-R) (hours.)
4010	8·5	3·5	5·0	3·0	2·8	2·0
3988	5·5	5·0	4·5	3	2·0	4·4
3160	4·0	4·5	2·2	4·5	4·2	3·6
3111	6·0	4·8	4·8	2·4	4·0	3·2
Average	6·0	4·5	4·1	3·2	3·4	3·3
		(5·0)	(4·0)	(3)	(3)	(3)

R = Rings T_1 = Stage I trophozoites T_2 = Stage II trophozoites T_3 = Stage III trophozoites.
S_1 = Schizonts, Stage I S_2 = Schizonts, Stage II

Gametocytes were first detected in Monkey 3111, thirty-six hours after, and in Monkeys 3160 and 3988 twenty hours after the appearance of asexual forms. In Monkeys 3111 and 3160, this stage persisted throughout the course of infection, in the former in a density of less than one to a maximum of nine per cent of the total parasites counted, and in the latter in 0·2 to 2·0 per cent. The highest number recorded in both was just before the death. In the initial stages when the asexual parasitæmia was very low, sexual forms in Monkey 3988 formed about 5 to 15 per cent of the total parasites. Subsequently, these stages were seen only occasionally and their count never exceeded one to two per cent. In Monkey 4010 also, the gametocytes were scanty and never exceeded one per cent in any of the counts.

DISCUSSION.

Nuri strain of *P. knowlesi* in *S. rhesus* monkeys is found to be very highly virulent, death of monkeys occurring within five to six days after the intravenous inoculation of 5 to 50 million parasitized erythrocytes per kg. body weight of the animal. Parasite multiplication in these monkeys was very rapid, and before death, its density reached about 94 to 98 per cent of the total R.B.Cs., which in terms of the actual number would work out to about 143 per 100 R.B.Cs., excluding about an equal number of merozoites seen extra-cellularly at this stage.

Taliaferro and Taliaferro (1949) while working with the original strain observed that in approximately 60 per cent of the monkeys, progressive acute infection occurred, and in the remainder, the infection was only intermittingly fatal. Belding (1952) indicated that *P. knowlesi* (original strain) produced a severe infection in *S. rhesus* with 65 per cent of the erythrocytes parasitized, and caused death in six to twelve days. McKee and Geiman (1948) found that by fasting monkeys for 24 to 48 hours, parasite infection caused by *P. knowlesi* (original strain) was strikingly ameliorated but similar result could not be obtained by Jaswant Singh, Nair *et al.* (1953) in their studies with Nuri strain of *P. knowlesi.* In view of these and certain morphological differences observed in the present strain (Jaswant Singh *et al.,* 1954), it is believed that it has some special characteristics of its own, quite different from the original strain.

In the four monkeys studied, the total parasite counts revealed a progressive parasitæmia which, according to Taliaferro and Taliaferro (1949), is typical of every synchronously reproducing plasmodium. The method followed for the study of schizogony cycle was the same as adopted by Mulligan (1935). Schizonts were present on all days during the course of infection and this formed a valuable guide to indicate each asexual cycle.

Segmentation occurred generally at every 24 hours' interval, but early or delayed or prolonged segmentation during the course of infection was common, which eventually caused aberrations in the asexual cycle. The changes, in the time of segmentation of the parasites, were obviously affected as a result of those brought about in the survival and death rate of the parasites by the different actions of innate and acquired immunity. To speak of one instance, in Monkey 3988, there was shortening of the cycle during the early two days, and lengthening during the subsequent days (Table VI). Corresponding to these, the merozoite means (Table V) showed a high figure during the former (13·6 to 14·5), and lower one during the latter period (9·4 to 7·1). These conditions explain to some extent the very high rate of increase in parasitæmia observed on second and third days, and a very contrasting low rate of increase during the subsequent days, as a result, most probably, of the action of innate and acquired immunity.

The rate of increase in the ring counts (Table V) during the earlier part of the infection was found to be 45 to 10,000 fold. This is rather unexplainable as the maximum number of merozoites, ever recorded in any of the monkeys on any day, was not more than 16. Similar sudden rise in parasitæmia at the beginning of infection has also been recorded by Brug (1934), Afridi (1938) and Taliaferro and Taliaferro (1949). Brug (1934) tried to explain this on the hypothesis that the plasmodia remained and multiplied in the internal organs, and the blood later

on was periodically flooded with these forms. Afridi (1938), however, did not find any significant difference in the parasite density in spleen and peripheral blood. According to Taliaferro and Taliaferro (1949), the parasite counts are not reliable in the beginning because the infection is only just appearing and moreover there is differential retention of large forms of the parasite in the internal organs, which is especially the greatest at the beginning of the infection. Perhaps these two factors may answer, to some extent, the discrepancies observed in the rate of multiplication in the early part of the infection.

The duration of asexual cycle was found to be of 24 hours in all the monkeys. In three of the monkeys (3111, 3160 and 4010), there was no indication of the existence of more than one brood, though from the curve of Stage II schizont of Monkey 3111 (Chart 2), it would lead one to speculate whether there could possibly be two broods, but that this is perhaps not so, is indicated from Chart 9 in which the peaks, caused by all multinucleated schizonts, are recorded. In the fourth monkey (3988), the schizont curves do not fully satisfy the existence of only one brood. Taliaferro and Taliaferro (1949) are of the opinion that extra broods do not occur in species that have a 24 hour cycle. Even though this statement may be found applicable in many cases of *P. knowlesi* infection, mainly due to the rapid multiplication of the parasites and brief period of infection in the host as a result of high pathogenicity of the parasites to the host, it is hard to say whether this general rule can be made applicable in all cases and under all conditions. This aspect requires further detailed study.

Gametocytes were found in good numbers in the first two monkeys (3111 and 3160) that had received inoculation as second and fifth passage since the Nuri strain was first isolated, but in the other two (56th and 64th serial passages), these numbers were found very much reduced. Whether this is as a result of the blood forms of the parasite undergoing repeated serial sub-passages in the successive vertebrate host, is an aspect that requires further study, as such occurrences, with other strains of plasmodia, are not unknown.

SUMMARY.

1. Periodicity of Nuri strain of *P. knowlesi* was studied in four *S. rhesus* monkeys.

2. Periodicity was observed to be 24 hours in all cases. A clear cut single brood infection was observed in three monkeys but in the fourth, there was suspicion of the possibility of the existence of a minor brood closely following the main one and later merging into the latter.

3. Time of segmentation and appearance of other stages in the peripheral blood, are recorded.

4. Merozoites per schizont ranged from four to sixteen with an average of ten. During the first two days of the infection, the count in all the four monkeys remained high as compared to the remaining period.

5. Calculations of the rate of increase of rings between two successive cycles and the daily merozoite means showed that excepting the first two days of the

infection, during the rest of the period the rate of increase was within the limits of the observed merozoite means.

6. A reduction in the gametocyte density in the circulating blood was observed in Monkeys 3988 and 4010 as compared to the figure obtained for 3111 and 3160. This reduction is presumed to be the outcome of repeated subinoculation from one monkey host to another.

REFERENCES.

Afridi, M. K. (1938)	*J. Mal. Inst. Ind.*, **1**, p. 355.
Belding, D. L. (1952) ...	*Text book of clinical parasitology.* Century Crofts. Inc., New York.
Brug, S. (1934)	*Riv. Malariol.*, **13**, p. 121.
Edison, J. F. B. and Davey, D. G. (1953) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, p. 259.
Jaswant Singh, Nair, C. P., Ramakrishnan, S. P. and Ray, A. P. (1953) ...	*Ind. J. Mal.*, **7**, p. 253.
Jaswant Singh, Nair, C. P. and Ray, A. P. (1954)	*Ibid.*, **8**, p. 155.
Jaswant Singh, Ray, A. P. and Nair, C. P. (1953)	*Nature*, **172**, p. 122.
McKee, R. W. and Geiman, Q. M. (1948) ...	*Parasite infections in man* edited by Harry Most. Columbia University Press.
Mulligan, H. W. (1935)	*Arch. Protist.*, **84**, p. 285.
Nair, C. P., Ray, A. P. and Jaswant Singh (1953)	*Ind. J. Mal.*, **7**, p. 371.
Sinton, J. A. and Mulligan, H. W. (1932)	*Rec. Mal. Surv. Ind.*, **3**, p. 357.
Taliaferro, W. H. and Taliaferro, L. A. (1949)	*J. Inf. Dis.*, **85**, p. 107.

Indian Journal of Malariology, **8**, 3, *September* 1954.

STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

V. Acquired resistance to pyrimethamine.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., Ch.B.(Edin.), D.P.H.(Eng.), D.T.M. & H. (London),

C. P. NAIR, D.M.S. (Madras), M.P.H. (John Hopkins)

AND

A. P. RAY, M.B.B.S.

(*Malaria Institute of India, Delhi.*)

(July 20, 1954.)

WORKING with an avirulent strain of *P. knowlesi*, Jaswant Singh, Ray *et al.* (1952) were able to demonstrate acquisition of high degree of resistance to proguanil and a cross resistance to pyrimethamine. Subsequently, Jaswant Singh, Nair *et al.* (1953) reported that high degree of resistance to pyrimethamine could be produced in a strain of *P. cynomolgi* which was also cross resistant to proguanil.

In the present paper, the authors record their observations on the development of acquired resistance to pyrimethamine in a recently isolated highly virulent strain of *P. knowlesi* (Jaswant Singh, Ray and Nair, 1953*b* ; Edeson and Davey, 1953) maintained by blood-passage in *M. mulatta mulatta* (*M. rhesus, S. rhesus*). The morphology, course of infection, periodicity etc., have already been reported earlier (Jaswant Singh *et al.*, 1954*a* : 1954*b*).

MATERIALS AND METHODS.

Monkeys, negative to tuberculin test (Nair and Ray, 1954), weighing 2·5 to 5·0 kg. were used. The dose of infective inoculum was 5×10^6 parasitized erythrocytes and administered intravenously in all cases. Successive passages were made when infection was found to be patent.

J.S.B. stain (Jaswant Singh and Bhattacharji, 1944) with the modified technique (Jaswant Singh, Ray and Nair, 1953*a*) was adopted for parasitological studies. For parasite count, Ehrlich's eye-piece was used and the technique adopted was similar to that reported earlier (Jaswant Singh, Ray *et al.*, 1952).

The doses of antimalarials were calculated in terms of mg. of the base per kg. of the body weight of animals. Drug was administered orally with the help of a rubber catheter attached to a syringe. The M.E.D. (Class II effect) of pyrimethamine, proguanil and quinine against this strain of plasmodium, were previously assessed as 0·05, 0·2 and 30 mg., respectively (Nair *et al.*, 1953).

OBSERVATIONS.

Although the M.E.D. for Class II effect for pyrimethamine was calculated to be 0·05 mg. at the commencement of the present study, treatment for three days was begun in a monkey (3521) with a sub-effective dose of 0.00001 mg./kg. Parasitæmia increased progressively similar to that in untreated series and the animal died. Two more passages were carried out with the same dose and the results were identical. During subsequent passages, the dose was increased to 0·000025 mg. and two similar sub-inoculations were made. Thereafter the procedure was continued in a similar manner and the dose was increased gradually to either double or two-and-a-half times the previous dose (Chart I) till one mg./kg. was reached. Up to this stage, the monkeys behaved in the same way as the untreated group. But when the dose was increased to two mg. in the forty-second sub-passage series, there was temporary clearance of parasites (Class II effect) for the first time. In view of this, in the subsequent few passages the dose was brought down again to 0·75 mg. and 1·0 mg., but treatment was continued in both cases for 14 days. During further subpassages, the doses were increased to 2, 4, 8, 12, 16 and 20 mg./kg. and treatment was continued for periods varying from 7 to 14 days.

Any further increase in the dose of pyrimethamine was not feasible as practically all the monkeys died on account of toxic effects. Even doses of 16 and 20 mg., proved somewhat toxic to many monkeys.

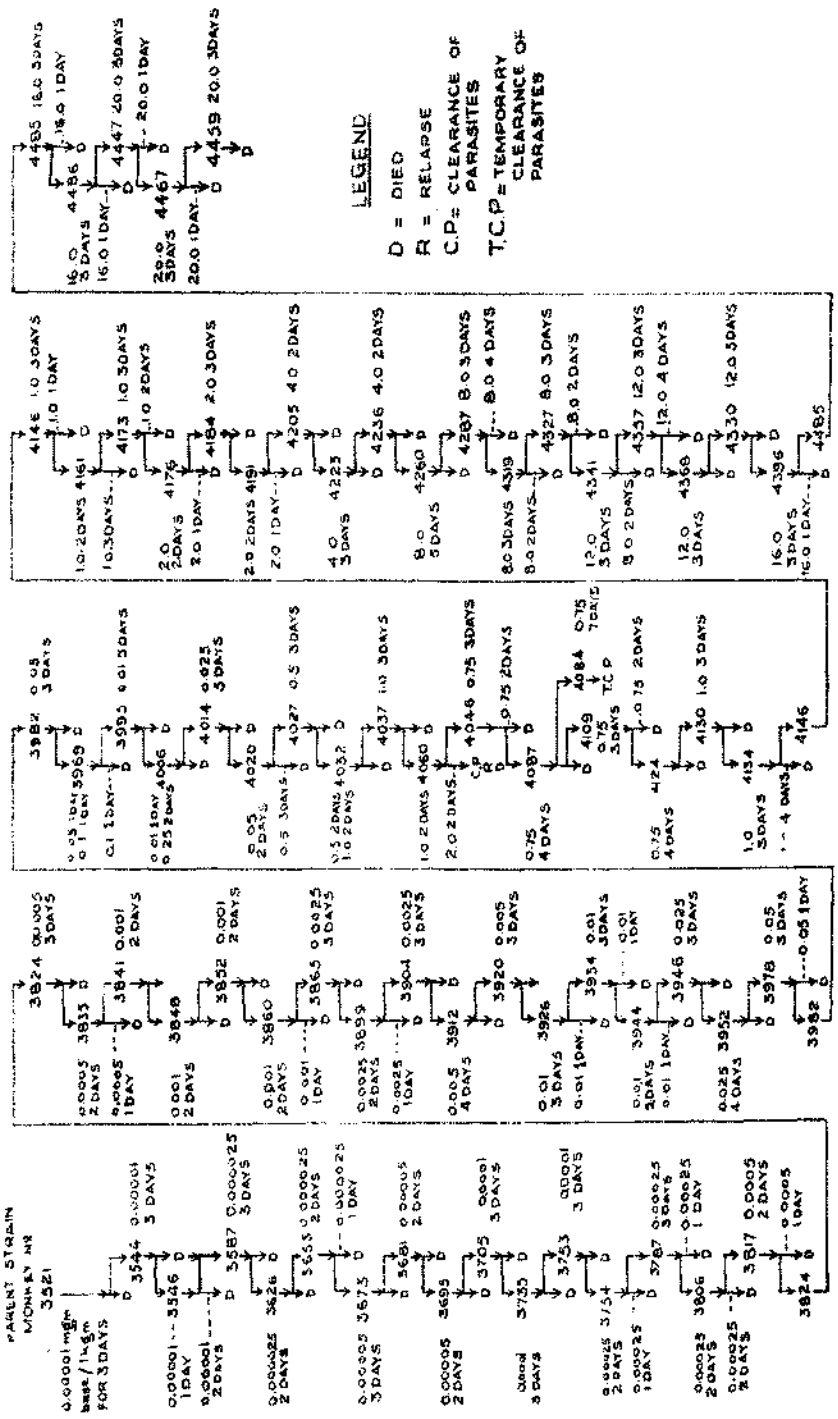
At various stages, resistance developed was cross-checked and compared to the parent strain. During the final stage, two groups of monkeys were inoculated with 5×10^6 parasitized erythrocytes, one with the parent while the other with the resistant strain. The animals inoculated with resistant strain were treated with 20 mg./kg., while those infected with parent strain received 0·0001 mg. It was observed that while there was progressive rise in parasitæmia, infection was controlled in those infected with parent strain.

PERSISTANCE OF THE ACQUIRED RESISTANCE.

In order to ascertain the stability of the resistance acquired, the resistant strain, after passaging it serially in 24 monkeys over a period of $2\frac{1}{2}$ months during which period it was not exposed to pyrimethamine, was tested again with 20 mg. of the drug. The strain remained insensitive to treatment and the monkey died on the 5th day of drug administration with very high parasitæmia.

CHART I

Acquired resistance to daraprim in Nuri strain of P. knowlesi. Serial passages and dosage schedules.



SENSITIVITY TO OTHER ANTIMALARIAL DRUGS.

Sensitivity of the resistant strain was tested against quinine, mepacrine, 4-aminoquinolines, proguanil and its active metabolite, bromoguanide and its active metabolite and pamaquin. In each series two groups of animals were placed, one was inoculated with parent strain and the other received the pyrimethamine resistant strain. The results are shown in Table I from which it would be observed that :—

(*a*) Quinine, mepacrine and 4-aminoquinolines were equally effective against both strains of the plasmodium. As regards pamaquin, the resistant strain was not in any way less sensitive than the parent strain.

(*b*) *Sulphadiazine.*—No appreciable difference in the sensitivity to the drug in the two strains was observed.

(*c*) While a dose of 0·2 mg. of proguanil was effective against parent strain, against the resistant strain even 30 mg. proved ineffective.

(*d*) *Active metabolite of proguanil.*—A dose of 0·5 mg. controlled parasitæmia in a monkey infected with parent strain whereas even 30 mg. proved ineffective. However a dose of 40 mg. was able to control the infection in the same way as 0·5 mg. against the parent strain.

(*e*) *Bromoguanide.*—Bromoguanide, when given in 0·1 mg. dose against the parent strain, was effective in clearing the parasites from the peripheral blood of a monkey before the termination of treatment but a dose as high as 20 mg., was found insufficient to save the life of the animal infected with the resistant strain. 30 mg. dosage on the other hand showed some activity against the resistant strain but proper assessment could not be made as the monkey died on account of its toxic effect.

(*f*) *Active metabolite of bromoguanide.*—Parasite clearance was effected in one monkey with 1·5 mg. of the drug in the case of the parent strain, whereas with the resistant strain the infection proved refractory to treatment even in doses as high as 20 to 40 mg.

(*g*) *M.* 3349.—Parent as well as resistant strain responded well to 20 mg. dosage, whereas 10 mg. dosage proved inactive in both the cases.

DISCUSSION.

At the initial stage of the investigation, it was observed that the Nuri strain of *P. knowlesi* was sensitive to the extent that even as small a dose as 0·0001 mg. of pyrimethamine caused Class I effect. With 0·05 mg., complete clearance of parasites could be obtained and this was established previously as the M.E.D. for Class II effect. In this respect, it may be mentioned that the M.E.D. (Class II) of pyrimethamine was observed to be 0·005 mg./kg. against the non-virulent strain of *P. knowlesi* (Jaswant Singh *et al.*, 1951). Thus it would appear that the present strain is ten times less sensitive to pyrimethamine than the non-virulent strain.

During the final check up, it was observed that even a dose of 20 mg. of pyrimethamine was ineffective against the Nuri strain of *P. knowlesi* which was

TABLE I.

Course of parasitæmia in monkeys infected with the parent and pyrimethamine resistant strains of P. knowlesi *(Nuri strain) on days following the first day of treatment.*

Strain.	Monkey number.	Drug.	Dosage mg/base kg. weight.	Average daily parasite count per 10,000 R.B.C. on days following the commencement of treatment or second day of patent parasitæmia										Remraks.
				1	2	3	4	5	6	7	8	9	10	
P	4477	...	...	6	1010	2050	5200	D	...	...	...	...	...	
P	3374	Pyrimethamine	0·0001	125	255	230	320	175	7	neg.	neg.	neg.	I	Relapsed on fourth day
R	4459	,,	20	380	1250	D	...	...	...	...	...	...	...	
R	4503	Proguanil	20	525	840	1000	2550	6400	D	...	...	...	...	
R	4541	,,	30	37	150	640	4400	5000	D	...	...	...	...	
R	4763	,,	30	200	1460	D(T)	...	...	...	...	...	...	...	
P	4384	Proguanil metabolite	0·5	390	100	12	neg.	neg.	neg.	neg.	neg.	neg.	neg.	
R	4434	,, ,,	20	1050	4000	D	...	...	...	...	...	...	...	
R	4572	,, ,,	30	9	9	500	1300	3000	6000	D	...	...	...	
R	4570	,, ,,	40	9	2	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	Died eleven days after, due to relapse.
P	4538	Bromoguanide	0·1	5	2	<1	neg.	neg.	neg.	ne.g	neg.	neg.	neg.	Relapse eight days after
R	4534	,,	20·0	130	55	255	1150	2500	3750	D	...	...	...	
R	4452	,,	30·0	205	25	1	D(T)	...	...	...	...	...	...	
P	4569	Bromoguanide metabolite	1·0	7	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	Relapsed five day after.
R	4537	,,	20·0	8	65	460	2050	5000	...	...	...	...	...	
R	4582	,,	30·0	185	350	925	3400	D	...	...	...	...	...	
R	4627	,,	40·0	56	250	2000	3900	D	...	...	...	...	...	
P	4643	Sulphadiazine	0·25	12	3	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.	

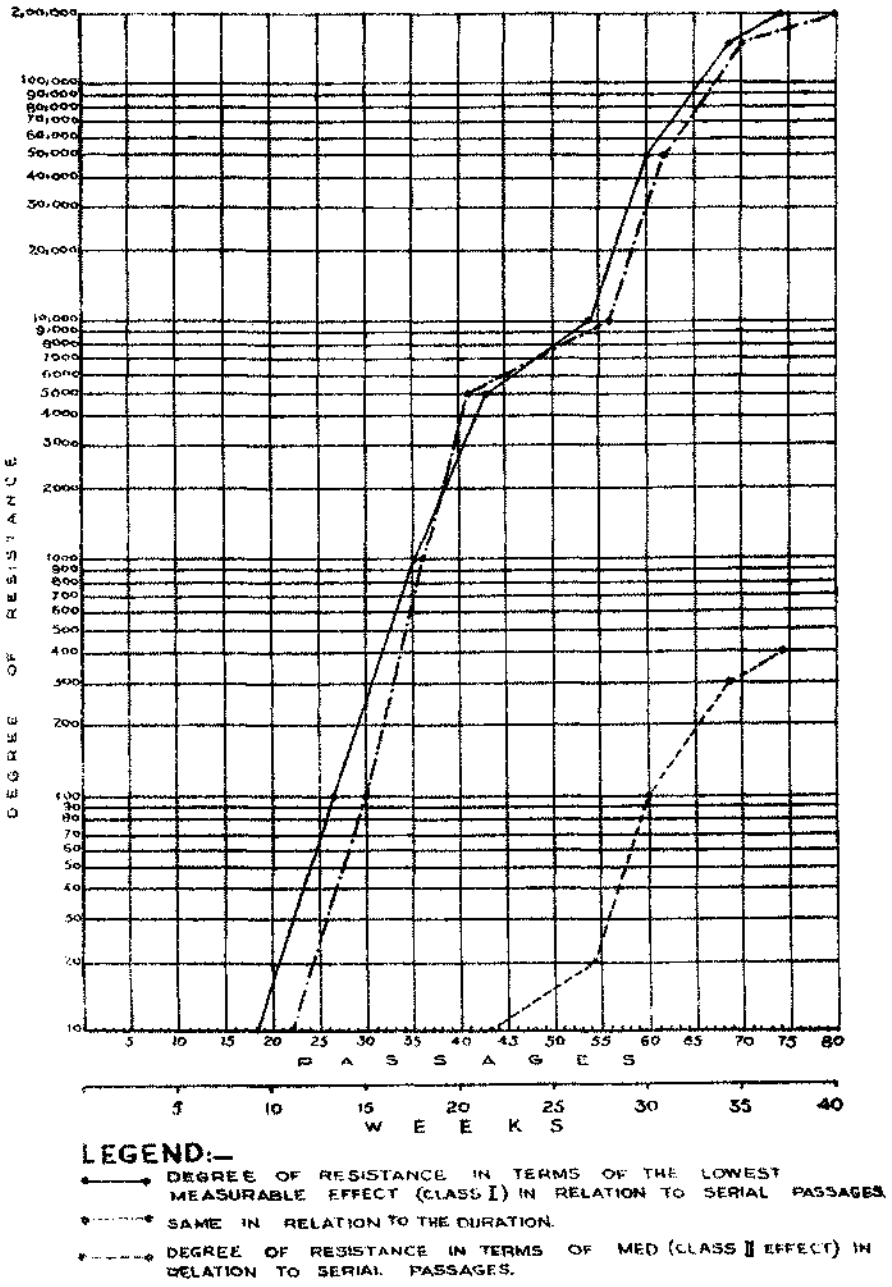
TABLE I. (*Concld.*)

Strain.	Monkey number.	Drug.	Dosage mg/base kg. weight.	Average daily parasite count per 10,000 R.B.C. on days following the commencement of treatment or second day of patent parasitæmia										Remarks.
				1	2	3	4	5	6	7	8	9	10	
R	4756	Sulphadiazine	0·25	52	2	1	neg.	neg.	neg.	neg.	2	8	100	
R	4413	,,	1·0	43	35	23	5	18	9	5	10	925	1000	Died five days after.
R	4469	,,	1·0	35	33	170	185	13	55	4	103	485	4852	Died next day.
R	4684	,,	2·5	325	525	161	neg.	neg.	neg.	neg.	neg.	neg.	neg.	Relapsed two days after.
P	X121	M 3349	10	225	900	3400	8000 (D)	...	...	...	...	...	...	
P	412D	,,	20	5	1	neg.	neg.	neg.	neg.	neg.	...	...	...	
R	4621	,,	15	300	2650	3300	(D)	...	...	...	...	...	...	
R	4577	,,	20	200	950	25	neg.	neg.	neg.	neg.	neg.	neg.	neg.	Relapsed five days after.
R	4490	Quinine	30	5	2	1	<1	<1	neg.	neg.	neg.	neg.	neg.	Relapsed second day.
P	5208	Pamaquin	0·5	450	1225	1400	2000	750	205	300	150	300	1600	Passed on to acute infection.
R	4813	,,	0·5	27	<1	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	Relapsed fifth day.
P	4557	Mepacrine	7	9	1	<1	neg.	neg.	neg.	neg.	neg.	neg.	neg.	Died ten days after.
R	4602	,,	7·0	430	8	<1	<1	,,	,,	,,	,,	,,	,,	Relapsed four days after.
P	4556	Amodiaquine	3·5	25	<1	<1	neg.	,,	,,	,,	,,	,,	,,	
R	4509	,,	3·5	413	30	<1	,,	,,	,,	,,	,,	,,	,,	Relapsed two days after.
P	4521	Chloroquine	2·0	18	neg.	neg.	neg.	,,	,,	,,	,,	,,	,,	
R	4442	,,	2·5	430	40	3	1	,,	,,	,,	,,	,,	,,	Relapsed three days after

P = Parent strain, R = Resistant strain, D = Died, D(T) = Died due to toxic effect, neg. = negative.

CHART 2.

Resistance in P. knowlesi *to pyrimethamine. Degree of resistance acquired and its relation to the serial passages and time taken for the development.*



reared initially with small but gradually increased doses of pyrimethamine for prolonged period. Thus the resistance developed to pyrimethamine in this strain was in the region of 20×10^5 fold and over, if the M.E.D. for Class I effect (0·0001 mg.) is taken into account. Compared to M.E.D. for Class II effect (0·05 mg.) against the parent strain, the acquired resistance was built up to 400-fold and over. Progress of resistance acquired with successive passages is graphically presented in Chart 2. It would be noted that the rate of increase was rapid up to the parasites developing 5000-fold resistance, the serial passages and duration required being only on an average of 8·7 passages and 3·6 weeks, respectively, for every 10-fold rise from the time resistance was first indicated to have developed (after nine sub-passages within a period of seven weeks). This is in contrast to the low increase thereafter as is evident from the average figures of 24·5 passages and 7·5 weeks required for every such 10-fold rise.

The interesting features of the present studies were concomitant development of cross resistance in the pyrimethamine resistant strain of *P. knowlesi* to proguanil, bromoguanide and their active metabolites, but not to sulphadiazine, quinine, mepacrine, 4-aminoquinolines, pamaquin and M-3349.

Schmidt and Genther (1953) and Jaswant Singh, Nair *et al.* (1953) demonstrated that similar resistance to pyrimethamine could be developed in *P. cynomolgi* as well and that the resistant strain was cross-resistant to proguanil and proguanil derivatives. Similar observation was also made by Thurston (1953) in *P. berghei* and it was shown that a pyrimethamine resistant strain was cross-resistant to, (1) C TW 6, 2 : 4-diamino-5-p-chlorophenyl-pyrimidine which differed from pyrimethamine only in the absence of the ethyl group on the 6-position of the pyrimidine ring (Chase, Thurston and Walker, 1951) ; (2) Active metabolite of proguanil (Carrington, *et al.*, 1951 ; Crowther and Levi, 1953) ; (3) Pteridines 0/97 [(6 : 7-diisoprophyl)-2 : 4-diamino pteridines] ; and (4) Pteridine 0/164 [6 : 7-(1-ethylindolo)-2 : 4 diamino-pteridines] (Collier and Waterhouse, 1950 : 1952).

In view of the normal tardy reaction in the plasmodia to proguanil, triazines and the diaminopyrimidines and that they acquire resistance to them easily, Ryley (1953) suggested that they exerted their effects on the anabolic rather than catabolic system of the parasites.

Josephson *et al.* (1953) have also put forward a hypothesis that antimalarials like sulphonamides, metalinamides, diaminopyrimidines, triazines, etc., which kill the parasites at the presegmental stages, are probably analogues of normal metabolites and that parasites can acquire resistance to them readily unlike those antimalarials which exert their action against all stages.

Whatever be the explanation ; the question on the use of antimalarials like proguanil, pyrimethamine, etc., on a mass scale for prolonged period, as in suppressive treatment, should be reviewed in the light of the data available, particularly now, when similar resistance and cross-resistance have been observed in *P. vivax* as well (Hernandez *et al.*, 1953).

SUMMARY.

P. knowlesi (Nuri strain) was passaged 73 times in *M. rhesus* monkeys in the presence of gradually graded doses of pyrimethamine over a period of 40 weeks which eventually resulted in the acquisition of more than 20×10^5 fold resistance by the strain to this drug.

The sensitivity of the resistant strain to various drugs was determined by the standard therapeutic test as followed in the laboratories of the Malaria Institute of India against simian malaria, and found that proguanil, bromoguanide and their active metabolites were highly cross-resistant to this resistant strain, whereas drugs of 4-aminoquinolines, pamaquin, mepacrine, quinine, and M-3349 were all as effective as against the parent strain.

The resistance developed was found to be of a permanent nature as much as the strain remained insensitive to pyrimethamine even after two and a half months, during which period it was passaged 24 times in the absence of the drug.

REFERENCES.

Garrington, H.C., Growther, A.F., Davey, D.G., Levi, A. A. and Rose, F. L. (1951)	*Nature*, **168**, p. 1080.
Chase, B. H., Thurston, J. P. and Walter, J. (1951)	*J. Chem. Soc.*, **p.** 3439.
Collier, H. O. J. and Waterhouse, P. D. (1950)	*Ann. Trop. Med. Parasit.*, **44**, p. 156.
Idem (1952)	*Brit. J. Pharmacol.*, **7**, p. 161.
	Ibid., 8, p. 93.
Crowther, A. F. and Levi, A. A. (1953) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, p. 259.
Gideon, J. F. B. and Davey, D. G. (1953) ...	
Hernandez, T., Myatt, A. V., Coatney, G. R. and Jeffery, G. M. (1953) ...	*Amer. J. Trop. Med. Hyg.*, **2**, p. 797.
Jaswant Singh and Bhattacharji, L. M. (1944)	*Ind. Med. Gaz.*, **79**, p. 102.
Jaswant Singh, Misra, B. G., Ray, A. P., Basu, P. C. and Bami, H. L. (1951) ...	*Ind. J. Mal.*, **5**, p. 531.
Jaswant Singh, Nair, C. P. and Ray, A. P. (1954*a*)	*Ibid.*, **8**, p. 155.
Idem (1954*b*)	*Ibid.*, **8**, p. 165.
Jaswant Singh, Nair, C. P., Ray, A. P. and Misra, B. G. (1953)	*Ibid.*, **7**, p. 357.
Jaswant Singh, Ray, A. P., Basu, P. C. and Nair, C. P. (1952)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 639.
Jaswant Singh, Ray, A. P. and Nair, C. P. (1953*a*)	*Ind. J. Mal.*, **7**, p. 267.
Idem (1953*b*)	*Nature*, **172**, p. 122.
Josephson, E. S., Taylor, D. J., Greenberg, J. and Coatney, G. R. (1953) ...	*J. Inf. Dis.*, **93**, p. 257.
Nair, C. P. and Ray, A. P. (1954) ...	*Ind. J. Tubercul.*, **1**, No. 2, p. 1.
Nair, C. P., Ray, A. P. and Jaswant Singh, (1953)	*Ind. J. Mal.*, **7**, p. 371.
Ryley, J. F. (1953)	*Brit. J. Pharmacol.*, **8**, p. 424.
Schmidt, L. H. and Genther, G. S. (1953)...	*J. Pharm. Expt. Therap.*, **107**, p. 610.
Thurston, J. P. (1953)	*Parasit.*, **43**, p. 246.

Indian Journal of Malariology, **8**, 3, *September* 1954.

NEED FOR A FRESH APPROACH ON RELAPSE MECHANISM IN MALARIA.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., Ch.B.(Edin.), D.P.H.(Eng.), D.T.M. & H. (London)

AND

S. P. RAMAKRISHNAN, M.B.B.S., D.P.H.

(Malaria Institute of India, Delhi.)

(July 1, 1954.)

THE precise cause for relapse in malaria has been and continues to be a subject of speculation, although in recent times some of the lacunæ towards further understanding of the problem have been filled. At the commencement of 20th century, Grassi (1900) and Schaudinn (1902) considered that relapses were caused by parthenogenesis or regressive schizogony of the macrogametocytes. That theory has not stood the test of time as it is now known experimentally that gametocytes do not give rise to a relapse (Boyd, 1949).

Bignami (1910, quoted by Corradetti and Verolini, 1950) considered that acquired immunity in malaria is transitory and that when it attenuates after a lapse of time, the parasites reappear giving rise to a relapse. Hackett (1937) reviewed the position and discussed at length the various theories for the causation of relapse on the basis that renewal of disease signifies a removal of immunity. The host factor is the one that has generally been considered liable to be put out of action by a variety of causes as fatigue, heat, dietetic indiscretions, inter-current diseases, chilling, drugs, blood letting, trauma, parturition, and surgical operation. The parasite itself has not been given sufficient consideration although Schilling (1934) conceived of the possibility of parasites becoming resistant to host immunity in order to explain his observations on the incubation period, toxic manifestations, the initial febrile period and relapses.

Recently Alving quoted by Sapero (1947) has expressed that both length of the prepatent period and the tendency to relapse in malarial infections, vary with dosage of sporozoites. When the inoculum is small, the prepatent period is

prolonged and the liability to a relapse greatly diminished. The converse is true when the inoculum is large.

The discovery of a heretofore cryptic life cycle in *vivax* malaria by Shortt *et al.* (1948) and Shortt and Granham (1948) has brought out a new concept on the mechanism of relapses. Corradetti and Verolini (1950) reopened the speculation on the origin of relapse in malaria and as a result of observations carried out on blood-induced infections of *P. malariæ* in man and *P. cynomolgi* in monkeys, concluded that it is difficult to support the theory that relapses are exclusively dependant on the presence of exo-erythrocytic forms. Whether it be, that the pre-erythrocytic parasites or their persistent forms release erythrocytic parasites into the blood continuously or periodically, it is still necessary to assume that the acquired immunity becomes low at certain periods for a patent relapse to manifest itself. In other words, the exo-erythrocytic parasites may truly act as a reservoir but it still has to be explained why renewed patent activity of the erythrocytic parasites takes place at specific periods.

The relapse pattern in malaria appears to be characteristic for each species and even to some of the known sub-species or strains (Coatney and Coopper, 1948 ; Young *et al.*, 1949). Usually the relapse episodes have a well defined periodicity. These features would favour relapses being more a function of the parasites than any alteration of immunity in a large number of people at the same time (Hackett, *loc. cit.* ; Ramakrishnan, Satya Prakash and Krishnaswami, 1951). Injection of adrenalin, glucose, exposure to cold, fatigue, starvation, etc., as means of precipitating relapse, yield the most inconsistent results in experimental malariology (Russell, West and Manwell, 1946 ; Bianco *et al.*, 1947). These factors may act concomitantly during a relapse but not causatively.

It would appear that a fresh approach is necessary to elucidate the precise mechanism precipitating relapse in malaria. The authors consider it conceivable that relapse in malaria is precipitated by parasites which have become resistant to acquired host immunity. Parasites existing in small numbers, when the infection is latent, would necessarily have to be resistant to the existing degree of host immunity. When the degree of resistance is greater than that of current immunity, increased multiplication of parasites manifests itself in the form of patent relapse. Such a conception would appear to adequately explain all that there is so far known about the natural history of the disease. There is nothing revolutionary in the assumption that parasites can acquire resistance to immunity. It is well known that pathogens acquire resistance to many of the modern therapeutic agents like antibiotics and drugs which act by affecting the growth and/or multiplication. The tenability of such a hypothesis on a theoretical basis in considered here.

The erythrocytic invasion by parasites of the three common species of human malaria, in most cases, gives rise to an initial period of continuous non-remittant fever, and after a time each of the species assumes its characteristic periodicity. By the time the parasite establishes its periodicity, several minor broods maturing at different times are considered to have been eliminated either by the gradual development of immunity or otherwise. It would appear that the parasites which give rise to fever at specific intervals are those which survive the low degree of immunity developed during the initial infection.

The parasites responsible for each bout of remittant fever stimulate the host antigenically, and at the culmination of each bout the majority of parasites causing the bout are destroyed. The few survivors multiply, further stimulate the host antigenically and once again the majority of them get destroyed. It is conceivable that, at each successive bout the relevant parasite population is exposed to increasingly adverse circumstances to which it gradually acquires increasing resistance. At the end of the primary episode, during which the terminal bouts are of progressively decreasing intensity, the resistance acquired by the parasite is not sufficient to overcome the immunity, and the result is a latent infection.

Parasitæmia in latent infections is sub-patent in most cases. This can be proved by subinoculation of blood from such cases to susceptible hosts. The sub-patent parasite population can be maintained by continuous propagation of the erythrocytic parasites and/or by either continuous or periodic replenishment from the reservoir, namely the exo-erythrocytic stages. But the very fact that there is a sub-patent population in the presence of a developed immunity necessarily follows that these parasites are resistant to immunity. The host-parasite relationship is not static as parasites during latency are continuously boosting the host immunity and concurrently acquiring a greater resistance to it. If at intervals a small proportion of the parasites have acquired sufficient resistance to overcome the existing degree of immunity, a relapse can occur. The relapse is almost always of shorter duration and lower intensity than the primary episode (Talliaferro, 1949). The increased multiplication of the resistant parasites even during the period of relapse results in an increased antigenic stimulation whereby the immunity is further boosted overcoming the brief relapse episode. Such periodic episodes continue to happen but they are of progressively lower intensity till the immunity is of a much higher order and the parasites are eliminated.

P. falciparum at one extreme is a parasite which has the largest number of merozoites per schizont and consequently is a fast reproducing species resulting in maximum numbers. A maximum antigenic stimulus would appear to be inevitable resulting in the production of acquired immunity to a greater degree as well as in a shorter space of time than in the case of the other two species. The least tendency to relapse in *falciparum* malaria is probably due to the fact that the parasites have less chances of acquiring any resistance to the acquired immunity which is developed quickly and to a high degree.

At another extreme is *P. malariæ* which has the least number of merozoites per schizont and is the slowest multiplying parasite. The parasite density is never high in this infection, resulting in low antigenic stimulus, thereby affording maximum chances for the parasites to acquire resistance to the slowly developing immunity. *P. vivax* can be said to occupy a status between the above two.

From the clinical point of view, a relapse would connote a re-activation of disease. From the parasitological point of view, it connotes a renewed increase in the multiplication of parasites despite the immunity acquired by previous antigenic stimulation. From the epidemiological point of view the "carrier state" is prolonged periodically by the formation of fresh gametocytes concurrently with each relapse. Underlying all these different points of view, there would appear to be a single biological principle that relapse episodes are manifestations of the attempt by parasites to survive against a continuously changing environment

which is progressively more and more adverse to it. Only the adverse environment is partly due to the activities of the parasite itself. It would appear logical that the parasite must necessarily become more and more resistant to the increasing immunity till such a time the latter is stepped up to a sufficient potent degree to destroy all the parasites which had acquired resistance to smaller amounts of the same immunity.

The hypothesis would appear to do away with the necessity for the arbitrary distinction between recrudescences and relapses. The distinction is based on the time interval between the primary and the recurring episodes (James, 1931). The hypothesis would explain the difference logically, as it is clear that during the phase when immunity has not reached the highest degree, namely, immediately after the primary epidsode, there are greater chances for the parasites to have acquired sufficient resistance to the existing level of immunity and such parasites tend to increase their multiplication at less frequent intervals. It must also be remembered that the parasite population is maximum during the primary episode, affording greater chances for selective survival of resistant forms. Each recrudescence due to increased antigenic stimulus, results in a higher degree of immunity against which the surviving parasites take a longer time to acquire resistance. Consequently the relapse occurs at longer intervals.

The hypothesis is not applicable to abnormal events like loss of blood due to wounds, surgical intervention and parturition precipitating a relapse. The relapse occurring under such conditions is more likely due to a sudden removal, quantitatively of appreciable amounts of circulating immunity, and is comparable to relapse induced by splenectomy in experimental malariology.

Chemotherapeutic studies on malaria have often yielded rich results in unravelling immunity problems, behaviour of different strains and physiology of parasites. Josephson *et al.* (1953) in their rationalization of acquired resistance of malaria parasites to drugs have shown that antimalaria drugs, irrespective of their chemical and biological properties, can be classified into two groups, namely, one which acts on all the asexual stages and the other which acts on the dividing erythrocytic stages only. Many drugs which have little or no action *in vitro* act *in vivo* through their metabolites, and they seem to belong to the second group, and to which parasites acquire resistance easily and quickly.

It is also well-known that the mode of action of many synthetic drugs is through their antagonism to normal nutrients essential to the parasite. In cases where resistance to a drug is acquired, the parasite probably adapts itself either to do away with the particular nutrient or to produce it in excess to overcome its partial destruction by the drug.

By analogy it would appear conceivable that parasites can acquire resistance to developing host immunity. Another point of resemblance between acquired resistance to drug and host immunity is inferred from the observation by Josephson *et al.* (*loc. cit*) referred to above, that drugs affecting the asexual reproduction of the parasite lend themselves more easily for the acquirement of resistance to them by the parasites. Acquired immunity has been shown by Talliaferro and Talliaferro (1934) to affect the asexual reproduction and that merozoite number in the crisis forms, is always small. It is well known that ribonucleic acid is essential

for the division of nucleus. Bringmann and Holtz (1953) have shown that acquired immunity in *toxoplasmosis* caused the disappearance of ribonucleic acid from the cytoplasm of the organisms when exposed to immune serum. They offer this as an explanation of the failure of *toxoplasma* to stain when mixed with immune serum for the Sabin-feldman's test.

Work of Zuckerman (1945) indicates that one of the serological components of malaria immunity is chiefly in the nature of an opsonin which renders the parasites more amenable to phagocytosis. If the hypothesis is tenable, then it would appear that a selective breeding of parasites, resistant to the current opsonin titre, takes place at different levels of immunity.

Members of the 8-aminoquinoline group of drugs and others which have any influence at all on the relapse rate are those which act on the pre-erythrocytic forms or their persistent stages. This is in no way contrary to the hypothesis, as it has been stated earlier that the exo-erythrocytic phase acts as a true reservoir of parasites and constantly replenishes the peripheral blood with parasites some of which become resistant to the immunity and multiply selectively.

The hypothesis seems applicable to all infectious diseases with a tendency to relapse, whether they be of treponemal, bacterial, protozoal or helminthic origin. The fundamental pattern of the natural history of these diseases is not dissimilar. Indeed, Schilling (quoted by Hackett, *loc. cit.*) believed that immunisation in malaria is a reciprocal reaction between organism and host in which each is protected from the other as in ordinary " carrier " state of diseases. Such a trend of thought would be in keeping with the recent concept that hosts utilize their comparatively few defence mechanisms with little modification against all varieties of infectious agents (Culbertson, 1951).

The hypothesis as stated in this paper takes into consideration all the known facts regarding the natural history of malaria. Direct experimental proof for it, however, is difficult. If it can be established experimentally that parasites at different stages of infection of the host exhibit different degrees of susceptibility to a standard immune serum, then the hypothesis can be considered safe for further work. The intracellular nature and difficulty of isolating the different growing stages of malaria parasites are some of the obstacles to prove the hypothesis by experimental malaria. *Trypanasome* infection of rats runs a course not very dissimilar to rodent malaria caused by *P. berghei*. Experiments are now in progress to attempt to test the hypothesis experimentaly in *trypanosomiasis* of rats.

REFERENCES.

Bianco, A. A., Saunders, G.M., Levine, A. S. and Cohan, R. (1947)	Quoted by Coatney, G. R. and Cooper, W. C. (1948) : *Proceedings of the fourth international congress on tropical medicine and malaria*, **1**, p. 637. Department of State, Washington, D.C.
Boyd, M. F. (1949)	*Malariology*, **1**, p. 67. W. B. Saunders Company, Philadelphia and London.
Bringmann, G. and Holtz, J. (1953) ...	*Zschr. f. Hyg. u. Infec.*, **138**, p. 151. Abstracted in *Trop. Dis. Bull.*, **51**, p. 636 (1954).
Coatney, G. R. and Cooper, W. C. (1948)...	*Proceedings of the fourth international congress on tropical medicine and malaria*, **1**, p. 637. Department of State, Washington, D.C.

Corradetti, A. and Verolini, F. (1950) ...	*J. Nat. Mal. Soc.*, **9**, p. 327.
Culbertson, J. T. (1951)	*Parasitic infections in man* edited by Harry Most, p. 19. Columbia University Press, New York.
Grassi, B. (1900)	Quoted in *Malariology* edited by Boyd, M. F. Volume I, p. 67. W. B. Saunders Company, Philadelphia and London, 1949.
Hackett, L. W. (1937)	*Malaria in Europe*, p. 152. Oxford University Press, London.
James, S. P. (1931)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **24**, p. 177.
Josephson, Edward S., Taylor, D. J., Greenberg, J. and Coatney, G. R. (1953) ...	*J. Inf. Dis.*, **93**, p. 257.
Ramakrishnan, S. P., Satya Prakash and Krishnaswami, A. K. (1951) ...	*Ind. J. Mal.*, **5**, p. 447.
Russell, P. F., West, L. S. and Manwell, R. D. (1946)	*Practical Malariology*, p. 329. W. B. Saunders Company, Philadelphia and London.
Sapero, J. J. (1947)	*Amer. J. Trop. Med.*, **27**, p. 271.
Schaudins, F. (1902)	Quoted in *Malariology*, edited by Boyd M. F. Volume I, p. 67. W. B. Saunders Company, Philadelphia and London.
Schilling, Claus (1934)	*Zent. f. Bakt.*, **131**, p. 25. Abstract in *Trop. Dis. Bull.*, **31**, p. 702.
Shortt, H.E., Garnham, P.C.C., Covell, G. and Shute, P. G. (1948)	*Brit. Med. J.*, **1**, p. 547.
Shortt, H. E. and Garnham, P. C. C. (1948)	*Ibid.*, **1**, p. 1225.
Talliaferro, W. H. (1949)	*Malariology*, edited by Boyd, M. F. Volume II, p. 938. W. B. Saunders Company, Philadelphia and London.
Talliaferro, W. H. and Talliaferro, L. G. (1934)	*Amer. J. Hyg.*, **20**, p. 1.
Young, M. D., Eyles, D. E. and Burgess, R. W. (1949)	*J. Nat. Mal. Soc.*, **7**, p. 171.
Zuckerman, A. (1945)	*J. Inf. Dis.*, **77**, p. 28.

Indian Journal of Malariology, **8**, 3, *September* 1954.

LIVER ENLARGEMENT DURING ACUTE INFECTION IN SIMIAN MALARIA.

BY

A. P. RAY, M.B.B.S.

AND

C. P. NAIR, D.M.S. (Madras), M.P.H. (Johns Hopkins).

(*Malaria Institute of India, Delhi.*)

(July 20, 1954.)

SPLEEN index forms one of the most important guides in a malaria survey as this organ is invariably enlarged in malarial infections. With regard to the liver, although it is generally agreed that it is somewhat enlarged in acute malaria, precise informations regarding the degree of enlargement are lacking.

Coggeshal (1937) and Afridi (1938) have shown that spleen is enlarged in *P. cynomolgi* or *P. knowlesi* infection in *M. mulatta mulatta* (*S. rhesus*, *M. rhesus*). But experimental studies of Taliaferro and Cannon (1936) did not show significant enlargement of the liver in *P. brasilianum* infection in black howler (*A. palliata equatorialis*) or white-throated (*Cebus capucinum*) monkeys. But in brown howler (*A. palliate trabeata*) and spider monkeys (*Ateles dariensis* and *A. geoffroyi*), small statistically significant increases were noted. Taliaferro and Mulligan (1937) had never seen great enlargement of liver in *P. cynomolgi* and *P. knowlesi* infections in *M. mulatta*. But the procedures adopted for measurement of liver were not indicated by them.

During the present studies, an attempt has been made to assess the degree of enlargement of liver, if any, in *M. mulatta mulatta* due to acute infections with *P. knowlesi* and *P. cynomolgi*.

MATERIALS AND METHODS.

Host.—Observations were made in 248 *M. mulatta mulatta* out of which 29 were normal, 196 infected with *P. knowlesi* and 23 with *P. cynomolgi*.

The normal monkeys were not sacrificed specifically for the purpose. During catching operations or transportation, a few monkeys had injuries like fracture of the limbs or skull or got involved in some such accidents. As, such animals would not have served any useful purpose, their internal organs, including the liver, were examined on autopsy.

P. knowlesi infection being fatal to all monkeys, it had been possible to make observation on a large number of animals, though analysis were made from a random sample (every fourth monkey) of 49 out of a population of 196.

A strain (Nuri) of *P. knowlesi* recently isolated (Jaswant Singh, Ray and Nair, 1953 ; Edeson and Davey, 1953) was used for these studies. The strain is highly virulent and irrespective of the dose of inoculum and the route of inoculation, death occurs in all monkeys within four to six days after patent infection is established. At the peak of infection, shortly before death, parasitæmia may reach as high as 98 per cent (Jaswant Singh, Nair and Ray, 1954).

The strain of *P. cynomolgi* used, was originally isolated by Sinton and Mulligan (1932). Rarely the animals die during peak of infection but occasionally a few odd monkeys die soon after crisis, which is usually between 8 to 15 days after patent infection is established.

The volume (in c.c.) of the liver, removed soon after death in each case, was measured by displacement of water in a measuring cylinder. Side by side, weight of the liver was taken in all cases.

RESULTS.

The volume of liver observed in each monkey was classified according to the weight of the animals as shown in Table I from which it would be observed that the total number of normal monkeys were 29 and the mean liver volume in c.c. was 107. The mean liver volume of the 49 monkeys (random sample) which died of *P. knowlesi* infection was 156 c.c., while that of 23 monkeys which died of *P. cynomolgi* infection, was 172 c.c.

TABLE I.

Mean liver volume of normal monkeys and of those infected with P. knowlesi *and* P. cynomolgi.

Weight group of monkeys in kg.	Number of normal monkeys.	Mean liver volume in c.c.	Number of monkeys infected with *P. knowlesi*.	Mean liver volume in c.c.	Number of monkeys infected with *P. cynomolgi*.	Mean liver volume in c.c.
1–2	13	46	6	75	6	81
3–4	8	136	23	134	8	138
5–6	6	183	14	200	7	202
7–8	2	182	6	233	2	251
Total	29	107	49	156	23	172

Thus increase in the volume of liver in monkeys dying of *P. knowlesi* infection was by 45 per cent while in the case of *P. cynomolgi* it was 60 per cent.

The results were further analysed statistically, and significance (X^2) tests were carried out from data plotted in Tables II and III.

TABLE II.

Observed and expected mean liver volume of normal monkeys and of those infected with P. knowlesi.

Weight group of monkeys in kg.	Observed mean liver volume in c.c. (in monkeys infected with *P. knowlesi*).	Expected mean liver volume in c.c. (in normal monkeys).
1–2	75	46
3–4	134	136
5–6	200	183
7–8	233	182
Total ...	642	547

$X^2 = 16{\cdot}5$

TABLE III.

Observed and expected mean liver volume of normal monkeys and of those infected with P. cynomolgi.

Weight group of monkeys in kg.	Observed mean liver volume in c.c. (in monkeys infected with *P. cynomolgi*).	Expected mean liver volume in c.c. (in normal monkeys).
1–2	81	46
3–4	138	136
5–6	202	183
7–8	251	182
Total ...	672	547

$X^2 = 28{\cdot}5$

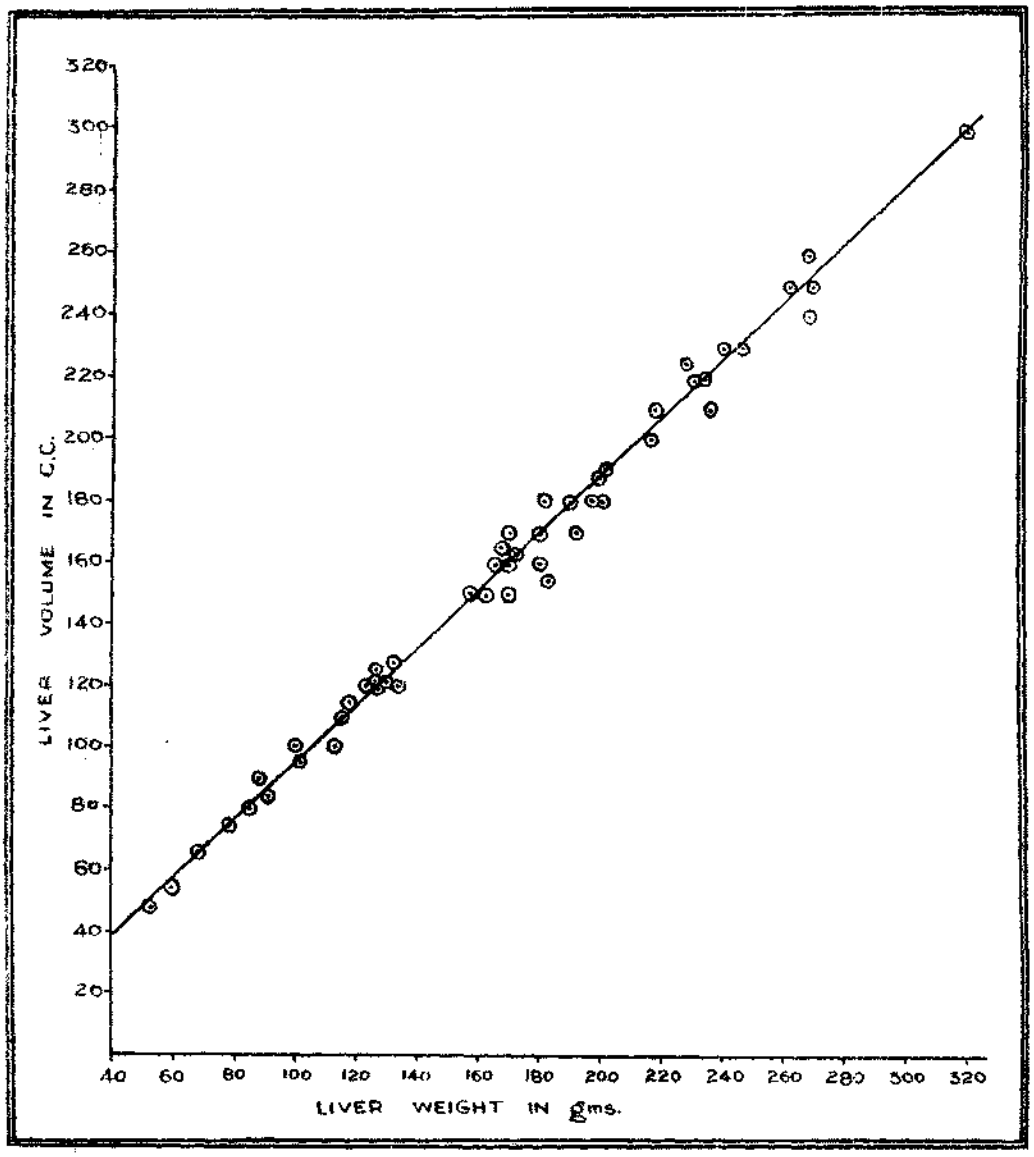
In *P. knowlesi* infections, the probability of error by chance occurrence is about 0·01 while in *P. cynomolgi* it is in the region of 0.005.

The liver volume and weight in all groups were proportional as would be seen from Graph 1, showing volume and weight of the liver in monkeys which

died of *P. knowlesi* infection. Similar correlations were observed both in the normal as well as in those which died of *P. cynomolgi* infection, and therefore not shown.

GRAPH. 1

Liver volume in c.c. and weight in gms. in fatal P. knowlesi *infection.*



DISCUSSION.

In human malaria most of the informations which are available, come from clinicians or from autopsy reports, but only few workers have recorded their observations on the condition of the liver at the time of routine survey. Hughes and Srivastava (1927) reported that in some groups of cases, there existed concomitant enlargement of liver along with splenomegaly. Williams (1940) also observed in African children that hepatomegaly and splenomegaly were common in children suffering from malaria. In an hyperendemic village in Madhya Bharat, Jaswant Singh, Ray *et al.* (1954) observed that while spleen rate was 64 per cent, hepatomegaly was present in 20 per cent of the children all of whom had Class III to V spleen (Hackett). Similar observations were made by Ray (1954*a*) who while conducting malaria survey in a village in Manipur noticed that the spleen rate was 56 per cent, and 35 of those showing enlarged spleen of Class II-V type, had hepatomegaly. Recently, Black (1954) reported that in certain malarious areas there was concomitant enlargement of liver and spleen, and that the highest incidence of liver enlargement and the largest livers were noticed in hyper- and holo-endemic areas. Orlina (1941) and Russell *et al.* (1946) believe that liver enlargement is noticed as often with *P. vivax* as due to *P. falciparum* infection.

During the present experimental studies, ample light has been thrown to show that in acute infections of *P. cynomolgi* and *P. knowlesi* there was considerable enlargement of liver. In the former case, it was enlarged by 60 per cent while in the latter by 45 per cent. Thus it would be obsreved that in *P. cynomolgi* infection the degree of enlargement was higher than in *P. knowlesi* infection. Statistically also, the enlargement observed, was found to be highly significant.

Taliaferro and Mulligan (1937) believed that enlargement of liver in malaria was "due partly to acute congestion, partly to distension of the sinusoides with macrophages and other cells and partly to swelling of the hepatic cells themselves". Recently, Ray (1953 : 1954*b*) has reported that while in *P. cynomolgi* infection there was occasional swelling of the parenchyma cells, in *P. knowlesi* there was extensive fatty infiltration and centrilobular necrosis.

It has also been reported that when monkeys kept on low protein diet were infected with *P. cynomolgi*, there was extensive fatty infiltration of the liver which became worse after repeated infections (Ray, 1953). In view of these observations, it is considered desirable that during future malaria surveys, details of the liver enlargement are also recorded along with other observations, particularly in highly malarious rural areas in India where majority of the population live on a diet deficient of proteins of high biological value (Patwardhan, 1952).

SUMMARY.

Volume and weight of livers were recorded in 29 normal *M. mulatta mulatta*, 196 infected with *P. knowlesi* and 23 with *P. cynomolgi*.

In both infections, the volume of liver was found to be increased ; by 60 per cent in *P. cynomolgi* and by 45 per cent in *P. knowlesi*. Statistical analysis also showed highly significant results.

The authors consider that liver survey should also be carried out during future malaria surveys as the liver is invariably involved in malaria.

ACKNOWLEDGEMENT.

The authors wish to thank Dr. A. K. Chakrabarti, Assistant Director, Malaria Institute of India, Delhi, for his assistance in confirming the statistical findings.

REFERENCES.

Afridi, M. K. (1938) .. ... *J. Mal. Inst. Ind.*, **1**, p. 355.
Black, R. H. (1954) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, p. 226.
Coggeshall, L. T. (1937) ... ... *Amer. J. Trop. Med.*, **17**, p. 605.
Edeson, J. F. B. and Davey, D. G. (1953) *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, p. 259
Hughes, T. A. and Srivastava, D. L. (1927) *Ind. J. Med. Res.*, **15**, p 427.
Jaswant Singh, Nair, C. P. and Ray, A. P. (1954) ... ... *Ind. J. Mal.*, **8**, p. 165.
Jaswant Singh, Ray, A. P., Misra, B. G. and Nair, C. P. (1954) ... ... *Ibid.*, **8**, p. 127.
Jaswant Singh, Ray, A. P. and Nair, C. P. (1953) ... ... *Nature*, **172**, p. 122.
Orlina, M. M. (1941) ... ... *Trans. -Kuibysher Milit. Med. Acad. Red. Army*, **5**, p. 29. Abstract in *Trop. Dis. Bull.*, **39**, p. 519 (1942).
Patwardhan, V. N. (1952) .. ... *Ind. J. Med. Sci.*, **6**, p. 135.
Ray, A. P. (1953) ... ... *Ind. J. Mal.*, **7**, p. 271.
Idem (1954*a*) ... ... Unpublished report.
Idem (1954*b*) .... *Bull. Nat. Soc. Ind. Mal. Mosq. Dis.*, **2**, p. 25.
Russell, P. F., West, L. S. and Manwell, R. D. (1946) ... ... *Practical malariology*, W. B. Saunders, London and Philadelphia. p. 587.
Sinton, J. A. and Mulligan, H. W. (1932) *Rec. Mal. Surv. Ind.*, **3**, p. 357.
Taliaferro, W. H. and Cannon, P. R. (1936) *J. Inf. Dis.*, **59**, p. 72.
Taliaferro, W. H. and Mulligan, H. W. (1937) ... ... *Ind. J. Med. Res. Memoir* No. **29**.
William, C. D. (1940) ... ... *Lancet*, March 9, p. 441.

Indian Journal of Malariology, **8**, 3, *September* 1954.

ANTIMALARIAL ACTION OF THE ACTIVE METABOLITE OF BROMOGUANIDE AGAINST *P. CYNOMOLGI*.

BY

A. P. RAY, M.B., B.S.,

C. P. NAIR, D.M.S. (Madras), M.P.H. (Johns Hopkins),

M. K. MENON, M.B.B.S.

AND

B. G. MISRA, L.M.P., L.T.M. (Cal.),

(Malaria Institute of India, Delhi.)

(July 18, 1954.)

In a quantitative evaluation of the antimalarial activity of chloroquine, nivaquine, amodiaquin and avloclor against blood-induced infections of *P. cynomolgi*, it was observed that while the efficacy of chloroquine and nivaquine was almost the same, avloclor was 1·5 times and amodiaquin 0·43 times as active as the other two (Jaswant Singh *et al.*, 1953). Subsequently, Nair *et al.* (1953) tested proguanil, pyrimethamine and bromoguanide against the same species and reported that whereas bromoguanide was only 0·68 times as active, pyrimethamine and proguanil were 1·36 and 1·5 times as active as chloroquine. The present paper records the report of a similar study with the active metabolite of bromoguanide, 1-p-bromophenyl-2 : 4-diamino-6 : 6-dimethyl-1 : 6-dihydro-1 : 3 : 5-triazine (Bami, 1953 : 1954). The results were also compared against those obtained after treatment with M3349 and mepacrine.

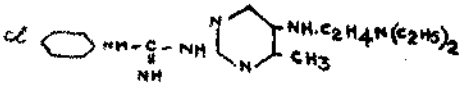
MATERIALS AND METHODS.

In the present study, 63 normal *M. rhesus* monkeys, each weighing between 2·5 and 6·0 kg. body weight, were used. The technique adopted for testing, like selection of monkeys, dosage of infective inoculum, time and duration of drug administration, etc., for activity were similar to those described by Jaswant Singh *et al.* (1953).

The criterion for activity of the drug was the clearance of parasites from the peripheral blood at least within 24 hours after the administration of the last dose of the drug (Class II effect of Shannon). The minimum amount of the drug required to produce this effect in a group of three to five monkeys used for such purposes, was considered as the minimum effective dose of the drug. In case there was only deceleration in parasitæmia in experimental group as compared to the comparison series, and not actual clearance within the specified time, the effect was taken as Class I effect. Class III effect was judged by the efficacy of the drug in completely sterilizing the blood-induced infection following the initial Class II effect. In such cases, smears were examined for a period of 30 days before and after splenectomy. Finally the ultimate comparison of the effectiveness of these drugs was based on calculating the quinine equivalent.

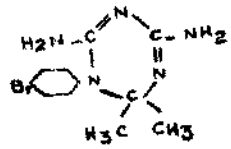
Chemical structure and formula of M3349, mepacrine and active bromoguanide metabolite.

M 3349



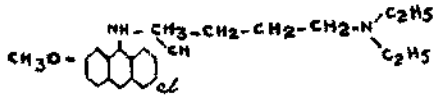
2—p-chlorophenyl guanidino-4β-dithylamino-ethylamino-6-methylpyrimidine.

Active metabolite of bromoguanide



1—p-bromophenyl-6 : 6-dimethyl-2 : 4-diamino-1 : 6-dihydro-1 : 3 : 5-triazine.

Mepacrine



RESULTS.

The results obtained with the different schedules of mepacrine are tabulated in Table I. With a dose of 2 mg./kg., the course of infection remained unchanged, whereas 4 to 8 mg. doses gave a measurable effect inasmuch as either Class I or Class II effect was produced. In a few, Class III effect also was observed. Of these, the minimum dosage required to clear the parasites temporarily in all monkeys tried, within 24 hours after the cessation of treatment, was found to be 5·0 mg./kg. which was, therefore, taken as the M.E.D. of mepacrine. On the other hand doses of 16 and 24 mg. were more active and resulted in Class III effect in majority of the animals.

Action of M3349 is shown in Table II. Up to 2 mg. dosage, no changes could be affected in the course of infection similar to that observed under mepacrine. Doses of 5·0 and 7·5 mg. were active to the extent of producing Class I effect in some of the monkeys tried whereas with 10 and 15 mg. doses, Class II effect was produced in some but not in all. However, with 20 mg. dosage, temporary clearance of parasites from the blood was effected in all the three monkeys within 72 to 120 hours from the commencement of treatment. This dose was, therefore, considered to be the M.E.D. of M3349.

TABLE I.

Effect of mepacrine against blood-induced P. cynomolgi *infection.*

Dosage base mg./kg. weight.	Number of monkeys used.	Activity							Remarks.
		Inactive	Class I	Class II			Class III		
				Number	Parasite clearance (hours)	Relapse (days).*	Number	Clearance (hours).	
2	3	3	...	...	...	...	...	...	...
4	3	1	1	1	120	12	...	...	...
5	3	...	...	1‡	96	...	2	60, 72	M.E.D.
6	4	...	...	3†	60, 84, 96	7	1	48	
8	3	...	...	3	96, 120, 141	17†	...	...	
16	3	...	...	1‡	96	...	2	72, 72	
24	3	...	...	2†	96, 96	...	1	96	

*Relapsed since the cessation of treatment.
†Two died during observation period.
‡One died during observation period.

TABLE II.

Effect of M. 3349 *against blood-induced* P. cynomolgi *infection.*

Dosage base mg/kg. weight.	Number of monkeys used.	Activity							Remarks.
		Inactive.	Class I.	Class II			Class III		
				Numbers	Parasite clearance (hours).	Relapse (days).	Number.	Clearance (hours).	
1	2	2	...	...	...	...	...	...	
2	2	2	...	...	...	...	...	...	
5	2	1	1	...	...	...	...	...	
7.5	2	1	1	...	...	...	...	...	
10	4	...	2	2	84, 168	15, 16	...	...	
15	3	...	1	2	72, 120	13, 15	...	...	
20	3	...	...	3*	72, 108, 120	6, 10	...	...	M.E.D.

*One died during the observation period.

Twenty-three monkeys were treated with bromoguanide metabolite, the doses varying from 0·1 to 1·5 mg. Doses of 0·1 and 0·2 mg. were ineffective in 50 per cent of the monkeys. With 0·3 to 0·4 mg., Class I effect was attained in all cases. Though Class II effect was seen in one animal treated with 0·6 mg., the same was not evident in both the monkeys treated with 0·8 mg. In comparison to these results, parasite clearance was effected in four out of five monkeys treated with 1·0 mg. dose, and five out of five monkeys treated with 1·2 mg. dose, usually before completion of the course. Of these nine, with the exception of one that died during the observation period, all relapsed during the follow-up period. The M.E.D. for Class II effect was thus established to be 1·2 mg./kg.

Table III.

Effect of active metabolite of bromoguanide against blood-induced P. cynomolgi *infection.*

Dosage base mg/kg. weight.	Number of monkeys used.	ACTIVITY							Remarks.
		Inactive.	Class I.	Class II			Class III.		
				Number.	Parasite clearance (hours).	Relapse (days).	Number.	Clearance (hours).	
0·1	2	1	1		...	...	...	...	
0·2	2	1	1		...	...	...	...	
0·3	2	...	2		...	...	...	...	
0·4	2	...	2	...	...	...	...	...	Indication of early resistance to drug in one monkey.
0·6	2	...	1	1	120	6	...	...	"
0·8	2	1	1						
1·0	5	...	1	4*	48, 108, 144, 168	7, 13, 13	...	...	
1·2	5	...	...	5	96, 96, 144, 168, 168	2, 7, 12, 12	...	...	
1·5	1	...	...	1	96	...	...	...	

*One died during the observaiton period.

DISCUSSION.

From the results obtained during these studies, it would be observed that the M.E.D. for metabolite of bromoguanide, M3349 and mepacrine is 1·2, 20·0 and 5·0 mg./kg., respectively. As the M.E.D. of quinine against the same strain of *P. cynomolgi* was established to be 20 mg. (Jaswant Singh *et al.*, 1953), obviously the Q.E. (quinine equivalent) of the three compounds now tested are 16·7, 1·0 and 4.0.

It is interesting to note that the quinine equivalent of mepacrine was observed to be the same in *P. cynomolgi* as in *P. gallinaceum* in chicks (Jaswant Singh *et al.*, 1952).

Nair *et al.* (1953) had observed earlier that M.E.D. of bromoguanide and proguanil against the same strain of plasmodium was 2·2 and 1·0 mg., respectively, and their quinine equivalent 0·09 and 20. Thus the active metabolite of bromoguanide was found to be about twice as active as the parent compound. Further, though M3349 is considered to be proguanil precursor, it was 20 times less active than proguanil. It would, therefore, be evident that in both cases the compounds of metabolite of bromoguanide and proguanil are more active than their parent or precursor compounds. Similar observations were reported by Josephson *et al.* (1951) who reported that against *P. gallinaceum,* a metabolite of pamaquin was 16 times more active than the parent compound.

These observations, therefore, throw sufficient indications that unless one is working with radically different chemicals, there is considerable scope of further advancement in the production of better antimalarials if sufficient attention is paid to the study of degradation products of established antimalarials.

SUMMARY.

Mepacrine, M3349 and the active metabolite of bromoguanide were tested in 63 *S. rhesus* monkeys infected with the asexual forms of *P. cynomolgi.* The minimal effective doses of these three drugs were found to be 5·0, 20·0 and 1·2 mg., respectively, of the base per kg. body weight of the animal. The quinine equivalent of these drugs, therefore, is 16·7 for metabolite of bromoguanide, 4 for mepacrine and 1 for M 3349.

REFERENCES.

Bami, H. L. (1953) ... ... *Ind. J. Mal.*, **7**, p. 283.
Idem (1954) ... ... *Curr. Sci.*, **25**, p. 124.
Jaswant Singh, Basu, P. C. and Ray, A. P. (1952) ... ... *Ind. J. Mal.*, **6**, p. 145.
Jaswant Singh, Nair, G. P. and Ray, A. P. (1953) ... ... *Ibid.*, **7**, p. 239.
Josephson, E. S., Taylor, D. J., Greenberg, J. and Ray, A. P. (1951) ... ... *Proc. Soc. Exp. Biol. Med.*, **76**, p. 700.
Nair, C. P., Ray, A. P. and Jaswant Singh (1953) ... ... *Ind. J. Mal.*, **7**, p. 351.

Indian Journal of Malariology, **8**, 3, *September* 1954.

A NOTE ON THE MALARIA PROBLEM IN BHUJ, KUTCH STATE.

BY

SATYA PRAKASH, B.SC., M.B.B.S.

(Malaria Institute of India, Delhi.)

(July 1, 1954.)

A MALARIA survey of Kutch State was carried out by Afridi *et al.* (1938) who made detailed recommendations for the control of the disease. Pal (1946) during a resurvey noted that little of the recommendations had been given effect to and consequently the malaria situation had deteriorated as compared to the previous survey.

Since the partition of India in 1947, the State of Kutch has grown in importance. Karachi, which was till then holding importance both as sea and air port, had to be replaced. Due to the geographical location of Kutch and the scope it presented for expansion for rehabilitation of displaced people as well as international air and sea-port facilities, Bhuj is being developed as an air port and Kandla as a sea port.

The note records briefly the results of malaria surveys in the State carried out in 1949, 1950 and 1951 and the main recommendations for the control of malaria. The first survey was carried out in July 1949, before the commencement of malaria transmission during the year ; the second in November-December of 1951 at the end of the transmission season.

Kutch State has a total area of over 7,600 sq. miles. It is practically an island with sea on the west and south, and is cut off from the mainland on the north and east by vast salt marshes, known as the Rann of Kutch. Bhuj is located in a hilly area (340 feet above sea level) in central Kutch. The population of this city was 20,000 in 1941 and has since gone up enormously due to influx of people since partition of the country. The 1951 census recorded a population of 30,000 for the city of Bhuj.

TABLE I.

Malaria cases in Bhuj.

Month/Year.	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950
January ...	236	359	584	1,302	572	746	458	423	506	833
February ...	335	385	576	960	509	527	462	379	360	618
March ...	619	402	569	818	616	576	474	441	356	598
April ...	604	494	609	856	788	426	407	418	357	658
May ...	441	431	826	939	988	392	385	386	360	781
June ...	289	443	497	960	855	390	314	357	328	661
July ...	371	463	376	805	1,360	480	348	381	317	876
August ...	473	466	814	912	709	458	272	431	512	1,131
September ...	495	439	636	1,404	964	615	382	484	476	1,645
October ...	500	847	861	815	1,100	552	468	410	2,118	2,548
November ...	514	1,097	2,701	1,256	979	547	737	386	3,395	2,171
December ...	364	733	2,366	1,102	713	398	637	428	1,364	
Yearly total ...	5,241	6,577	11,406	12,129	10,153	6,107	5,344	4,924	10,448	12,620

About 5,000 to 13,000 cases of malaria are treated every year in the hospital at Bhuj. Table I records the number of cases treated monthly from 1941 to 1950. It will be seen from the table that 1941, 1942, 1946, 1947 and 1948 were the years during which the number of cases treated was low while the figures for the years 1943, 1944 ,1945, 1949 and 1950 are considerably high. It was not possible to elucidate any relevant causes for the difference.

Analysis of the monthly hospital figures reveals that in the years of low incidence (1946, 1947 and 1948), there does not appear to be any month during which the incidence is markedly higher than others. But during the years of high incidence (1943, 1944, 1945, 1949 and 1950), there appears to be a marked increase in the number of cases during March to May as well as August to December. It would, therefore, seem that in the area there are two well defined malaria seasons during a year, namely March to May and August to December, which are favourable for increased malaria transmission during some years and not so during others. The plausible causes for such an occurrence are not clear from the survey, but they are possibly dependent on rainfall and other meterological conditions.

The spleen rate and the average enlarged spleen in the different sectors of Bhuj City varied from 18 to 35 per cent, and 10 to 7 c.m., respectively. These figures are compared with those obtained in 1938 by Afridi *et al.* (*loc. cit.*) in Table II. Between 1938 and 1950-51, both the indices have shown an increase.

TABLE II.

Results of spleen surveys of Bhuj.

Locality.	Spleen rate, per cent.			Average enlarged spleen in cm.*		
	1938	1950	1951	1938	1950	1951
Kumbar Bara ...	28·0	48·21	44·0	11·0	9·5	10·0
Bhangi Colony ...	...	35·71	25·0	...	8·0	9·2
Bhilwada Sadar ...	10·0	18·75	33·0	11·0	9·0	9·5
Divita Street (central)	6·0	32·35	34·0	9.0	8·5	8·0
Waniawad School ...	...	36·70	31·0	...	8·5	7·8
Patwadi Gate School	20·0	39·60	35·0	9·7	9·0	8·7
Bhid Gate School ...	...	41·38	39·0	11·0	8·6	9·0

*Determined by the method of Christophers and Khazan Chand (1924).

TABLE III.

Parasite rate in Bhuj.

Date.	Locality examined.	Number of smears examined.	Per cent positive smears.
July, 1949	Bhuj City	197	1·0
November-December 1950	Kumber Bara	29	58·6
	Bhangi Colony	7	14·3
	Bilwada Sadar	10	40·0
	Divita Street Central	36	36·1
	Waniawad Gate School	76	23·7
	Patwadi Gate School	100	21·0
	Bhid Gate School ...	101	17·8
	Total for 1950 ...	359	25·6

Table III records the parasite rates determined in 1949 and 1950 from the different sections of Bhuj City. Before the commencement of autumnal transmission, the parasite rate was one per cent while towards the end of the transmission period it rose to 25·6 per cent and varied from 17 to 59 per cent in the different parts of the city. Both *P. falciparum* and *P. vivax* were encountered and the former was considerably more predominant than the latter.

Seven species of adult Anopheles mosquitoes were caught from houses and cattlesheds during the survey. They were :

A. annularis
A. barbirostris
A. culcifacies
A. fluviatilis
A. stephensi
A. subpictus and
A. turkhudi

A large number of culicines including *Ædes Ægypti* were found.

During the survey under report, 171 specimens of female *A. culicifacies*, 195 of *A. stephensi*, 188 of *A. subpictus* and 27 of *A. annularis* were dissected, but no gut or gland infections were encountered. However, Afridi, *et al.* (*loc. cit.*) reported 3·2 per cent infection rate in *A. stephensi*.

Mosquito breeding places in Bhuj fall under the following categories—tanks including ornamental water collections, storm water channels, soakage pits, borrowpits, domestic water collections like storage tanks and cisterns, surface wells in use as well as those abondoned. Both anopheline and culicine mosquitoes were found breeding freely in all the above types of breeding places. Among the tanks and garden ponds, however, a few which were stocked with *gambusia* fish, were free from mosquito larvæ. 472 wells were examined during the survey and in most of them *Culex* and *A. stephensi* larvæ were found.

The construction works at Kandla which are in progress have given rise to a large number of potential mosquito breeding places. A rapid reconnaisance survey of the Kandla port area gave the impression that malaria is a considerable problem in the area.

The State budget for the year 1951-52 was Rs. 35,000/- for malaria control. With the limited resources, it was only possible to commence malaria control in the city of Bhuj and its immediate surroundings. Based on an approximate cost of eight annas per head per year, it is estimated that Rs. 2·5 lakhs a year will be required for a state-wide malaria control.

Under the National Malaria Control Plan, one half of a standard malaria unit has been sanctioned for the State in 1953-54 and the operations have commenced. It has already been stated that Kutch is virtually an island. It will, therefore, be of interest to explore the possibilities of total eradication of malaria from the State.

The author is grateful to Dr. K. T. Tasker, Chief Medical Officer of the State, for his help during the survey.

REFERENCES.

AFRIDI, M. K., MAJID, S. A. and SINGH, J. (1938) ... ... *J. Mal. Inst. Ind.*, 1, p. 187.
PAL, RAJINDAR (1946) ... ... *Malaria in Kutch.* Unpublished report.
CHRISTOPHERS, S. R. and KHAZAN CHAND (1924) ... ... *Ind. J. Med. Res.*, 11, p. 1065.

Indian Journal of Malariology, **8**, 3, *September* 1954.

FIELD TRIALS OF NIVAQUINE, CAMOQUIN AND RESOCHIN AGAINST HUMAN MALARIA.

BY

C. P. DUTTA,

(*State Malaria Officer, Assam*),

J. PRADHAN

AND

S. K. BHATTACHARJEE.

(*Malaria Institute, Shillong.*)

(September 7, 1954.)

PLAN OF TRIALS.

NIVAQUINE and camoquin were supplied to different dispensaries in the districts of Sibsagar and Goalpara for trials on patients suffering from malaria and were administered by the Medical Officer in charge of the respective dispensaries under the instructions of the authors, whereas resochin was tried on patients either stationed at or coming in Shillong and was conducted at the Malaria Institute, Shillong, under the supervision of the authors.

Blood smears were taken from all the fever cases, and those showing malaria parasites in their blood were administered different antimalarials in different doses according to their age, weight, etc. Thereafter blood smears were taken after an interval of every 24 hours to determine the time taken for the disappearance of parasites from the blood. Concurrently, the time taken for the disappearance of the clinical symptoms was also recorded and was based on remission of temperature. All the cases were kept under observation for a period of two and a half months to three months.

The following observations have been recorded.

NIVAQUINE.

Nivaquine tablets 0·2 gm. each were administered to 96 patients out of which 24 had *P. falciparum*, 54 *P. vivax* and 18 mixed *P. falciparum* and *P. vivax* infections.

Dosages.—

First day	...	Six tablets in three divided doses after every eight hours.	Adult dose.
Second day	...	Two tablets single dose.	
Third day	...	Two tablets single dose.	

This was followed by two tablets every week for a period of five weeks.

Results.—There was complete clearance of parasites from the peripheral blood in majority of the cases within 24 hours, and in all cases within 96 hours irrespective of the species of malaria parasites involved (Table I).

In the majority of *P. vivax* and the *P. falciparum* cases, the clinical symptoms disappeared within 48 hours and in the rest within 72-96 hours. In the mixed *P. vivax* and *P. falciparum* infections, majority of the cases became free from clinical symptoms within 24 hours, and the rest within 96 hours (Table II).

Twelve *P. vivax*, one *P. falciparum* and five mixed infection cases had relapses within a period from 7 to 14 days. Five *P. vivax* and one *P. falciparum* cases did not respond to this drug and had to be treated ultimately with quinine and paludrine.

TABLE I.

Effect of nivaquine on parasite clearance.

Species of parasites.	PARASITE CLEARANCE IN HOURS.					Total	Remarks.
	24 hours.	48 hours.	72 hours.	96 hours.	No response.		
P. falciparum	14 (58·3)	5 (20·8)	1 (4·2)	3 (12·5)	1 (4·2)	24	One relapsed after 14 days (4·1 per cent).
P. vivax	23 (42·6)	11 (20·3)	13 (24·1)	2 (3·7)	5 (9·3)	54	12 relapsed within 7 to 14 days (22·2 per cent).
Mixed *P. falciparum* and *P. vivax*	6 (33·2)	6 (33·2)	3 (16·6)	3 (16·6)	... ...	18	5 relapsed within 10 days (27·7 per cent).

Figures within brackets indicate percentage.

TABLE II.

Effect of nivaquine on clearance of clinical symptoms.

Species of parasites.	Clearance of clinical symptoms in hours.					Total.	Remarks.
	24 hours.	48 hours.	72 hours.	96 hours.	No response		
P. falciparum	4 (16·6)	15 (62·5)	2 8·7)	2 (8·7)	1 (4·2)	24	
P. vivax	11 (20·4)	32 (59·2)	6 (11·1)	... ...	5 (9·3)	54	
Mixed *P. falciparum* and *P. vivax*	12 (66·6)	1 (22·2)	1 (5·5)	1 (5·5)	...	18	

Figures within brackets indicate percentage.

CAMOQUIN.

Thirty-one patients were put on camoquin out of which seven showed *P. falciparum*, 20 *P. vivax* and four mixed *P. falciparum* and *P. vivax* infections.

Dosages.—All the *P. falciparum*, 13 *P. vivax* and 2 mixed infection cases were given a single dose treatment of three tablets each and the rest of the *P. vivax* and mixed infection cases were given three tablets in three divided doses in a day. The results observed in both these regimes were the same.

Results.—In majority of the *P. falciparum* and *P. vivax* cases, there was complete disappearance of parasites from the peripheral blood within 24 hours and in the rest within 48-72 hours. In the mixed infection cases, this occurred within 48 hours (Table III).

TABLE III.

Effect of camoquin on parasite clearance.

Species of parasites.	Parasite clearance in hours.					Total	Remarks.
	24 hours.	48 hours.	72 hours.	96 hours.	No response		
P. falciparum	4 (57·1)	3 (42·8)	...	...	...	7	
P. vivax	10 (50·0)	8 (40·0)	2 (10·0)	...	...	20	2 relapsed within 16 to 17 days. (10·0)
Mixed *P. falciparum* and *P. vivax*	2 (50·0)	2 (50·0)	...	...	...	4	

Figures within brackets indicate percentage.

There was disappearance of the clinical symptoms in majority of *P. falciparum* and *P. vivax* cases within 48 hours and in the rest within 72 hours, whereas in the mixed infection cases, this happened within 96 hours (Table IV).

Two *P. vivax* cases had relapse within 16 to 17 days.

Some of the patients taking the drug complained of loss of appetite and feeling of uneasiness.

TABLE IV.

Effect of camoquin on clearance of clinical symptoms.

Species of parasites.	Clearance of clinical symptoms in hours.					Total.	Remarks.
	24 hours.	48 hours.	72 hours.	96 hours.	No response.		
P. falciparum ...	2 (28·5)	3 (42·8)	2 (28·5)	...	...	7	
P. vivax ...	7 (35·0)	11 (55·0)	2 (10·0)	...	...	20	
Mixed *P. falciparum* and *P. vivax*	1 (25·0)	1 (25·0)	1 (25·0)	1 (25·0)	...	4	

RESOCHIN.

Altogether 100 patients were put on this drug (tablets, each of 0·15 gm. base) during a period of 18 months. As it was decided to administer this drug by two different methods, *i.e.*, the usual ten tablets in three days and the single dose method, these patients were divided into two groups.

Group 1.—There were 60 patients in this group out of which 30 had *P. falciparum*, 15 *P. vivax* and 15 mixed *P. falciparum* and *P. vivax* infections. Gametocytes were found in 17 *P. falciparum* and three *P. vivax* cases.

Dosages.—

First day ... Six tablets in three divided doses after every eight hours.
Second day ... Two tablets.
Third day ... Two tablets.

Followed by two tablets on same day of the week for a period of five weeks.

Results.—In majority of the cases, there was complete clearance of parasites from the peripheral blood within 24 hours irrespective of the species of parasites involved. In the rest of the cases, the peripheral blood became negative within 96 hours in *P. falciparum* and *P. vivax*, whereas in mixed infections the blood was free of parasites within 48 hours (Table V).

The clinical symptoms also disappeared in majority of the cases within 24 hours, and within 72 hours in the cases of *P. falciparum* and *P. vivax*, and within 48 hours in the case of mixed infections (TableVI).

Gametocytes disappeared within 96 hours in all cases, most of them within 48 hours (Table VII). Few cases complained of pruritis.

TABLE V.

Effect of resochin (divided doses method) on parasite clearance.

Species of parasites.	Parasite clearance in hours.						Remarks.
	24 hours.	48 hours.	72 hours.	96 hours.	No response.	Total.	
P. falciparum ...	16 (53·3)	8 (26·6)	4 (13·3)	2 (6·6)	...	30	
P. vivax ...	8 (53·3)	3 (20·0)	2 (13·3)	2 (13·3)	...	15	*One relapsed on the 14th day. (6·6).
Mixed *P. falciparum* and *P. vivax*	10 (66·6)	5 (33·3)	...	...	...	15	

Figures within brackets indicate percentage.

*The case was put on single dose method and there was complete clearance of parasites from peripheral blood and clinical symptoms within 24 hours and thereafter he had no relapse during the period of observation.

TABLE VI.

Effect of resochin (divided doses method) on clearance of clinical symptoms.

Species of parasites.	Clearance of clinical symptoms in hours.						Remarks.
	24 hours.	48 hours.	72 hours.	96 hours.	No response.	Total.	
P. falciparum ...	18 (60·0)	10 (33·3)	2 (6·6)	...	...	30	
P. vivax ...	9 (60·0)	3 (20·0)	3 (20·0)	...	...	15	
Mixed *P. falciparum* and *P. vivax*	9 (60·0)	6 (40·0)	...	...	...	15	

Figures within brackets indicate percentage.

TABLE VII.

Effect of resochin (divided doses method) on gametocyte clearance.

Species of parasites.	Gametocyte clearance in hours.					Remarks.
	24 hours.	48 hours.	72 hours.	96 hours.	Total.	
P. falciparum ...	4 (23·5)	8 (47·1)	3 (17·6)	2 (11·7)	17	
P. vivax ...	...	2 (66·6)	...	1 (33·3)	3	

Figures within brackets indicate percentage.

Group II.—There were 40 patients in this group out of which 20 had *P. falciparum*, ten *P. vivax* and ten mixed *P. falciparum* and *P. vivax* infections. There were ten *P. falciparum* and five *P. vivax* gametocyte carriers.

Dosages.—Each patient was given a single dose of three to four tablets followed by one tablet on same day of the week for a period of five weeks.

Results.—In *P. falciparum* cases, the clinical symptoms disappeared within 24 hours and the blood became negative within 48 hours.

In *P. vivax* cases, both the clearance of the parasites from the peripheral blood and the disappearance of clinical symptoms occurred within 48 hours.

In mixed infections, the blood became clear of parasites within 24 hours and the clinical symptoms disappeared within 48 hours.

Gametocytes disappeared within 48 hours in both *P. falciparum* and *P. vivax* infections.

TABLE VIII.

Effect of resochin (single dose method) on parasite clearance.

Species of parasites.	Parasite clearance in hours.				Remarks.
	Dosage.	24 hours.	48 hours.	Total.	
P. falciparum ...	3 tablets	5 (33·3)	10 (66·6)	15	
	4 tablets	3 (60·0)	2 (40·0)	5	
P. vivax	3 tablets	4 (50·0)	4 (50·0)	8	
	4 tablets	1 (50·0)	1 (50·0)	2	
Mixed *P. falciparum* and *P. vivax*	3 tablets	7 (100·0)	...	7	
	4 tablets	3 (100·0)	...	3	

Figures within brackets indicate percentage of cases.

TABLE IX.

Effect of resochin (single dose method) on clearance of clinical symptoms.

Species of parasites.	Dosage.	Clearance of clinical symptoms in hours.			Remarks.
		24 hours.	48 hours.	Total.	
P. falciparum ...	3 tablets	15 (100·0)	...	15	
	4 tablets	5 (100·0)	...	5	
P. vivax	3 tablets	6 (75·0)	2 (25·0)	8	
	4 tablets	2 (100·0)	...	2	
Mixed *P. falciparum* and *P. vivax*	3 tablets	7 (100·0)	...	7	
	4 tablets	2 (66·6)	1 (33·3)	3	

Figures within brackets indicate percentage of cases.

TABLE X.

Effect of resochin (single dose method) on gametocyte clearance.

Species of parasites.	Dosage.	Gametocyte clearance in hours.			Remarks.
		24 hours.	48 hours.	Total.	
P. falciparum ...	3 tablets.	4 (50·0)	4 (50·0)	8	
	4 tablets	2 (100·0)	...	2	
P. vivax	3 tablets	2 (66·6)	1 (33·3)	3	
	4 tablets	1 (50·0)	1 (50·0)	2	

Figures within brackets indicate percentage of cases.

DISCUSSION.

All the three drugs were very prompt in their action. Taking into account all the cases, the parasite clearance was attained within 24 hours in 48·4 per cent, within 48 hours in 79·0 per cent and over 90 per cent of the cases within 72 hours.

Taking the parasite clearance within 48 hours, a comparative observation of the three drugs is given in Table XI below :—

TABLE XI.

Comparative observation on the effect of nivaquine, camoquin and resochin on parasite clearance within 48 hours (Per cent).

Species of parasites.	Nivaquine.	Camoquin.	Resochin.
P. falciparum ...	80·0	100·0	80·0
P. vivax	60·0	90·0	73·3
Mixed *P. falciparum* and *P. vivax*	50·0	100·0	100·0

The figures indicate percentage of cases.

From the parasite clearance achieved within 24 hours, it appears that all the three drugs are equally effective against *P. falciparum*; camoquin and resochin a bit superior against *P. vivax*, and against mixed infections resochin appears to act the best.

Relapse rates were 18·7, 6·4 and 1·6 per cent under nivaquine, camoquin and resochin, respectively. The one case of relapse under resochin reacted very favourably to the single dose method and had no further relapse during the period of observation (three months).

There was no response to nivaquine in 6·25 per cent of cases and these had to be treated with other antimalarials. On going through records of the cases, it was found that all the six cases (*P. vivax* 5, *P. falciparum* 1) who did not respond to nivaquin but did so under quinine or paludrine, were children below ten years of age and it is very likely that the drug was vomited out soon after administration.

Some of the patients complained of pruritis, loss of appetite and feeling of uneasiness after administration of resochin and camoquin but these symptoms were not as serious as to warrant discontinuance of the treatment.

As regards gametocyte clearance, no comparison could be made between the three drugs as there were no gametocyte carriers under the regimes of nivaquine and camoquin. Resochin acted very effectively, the gametocyte clearance being achieved in majority of the cases within 48 hours.

The single dose method of resochin gave very encouraging results. The following table shows the results obtained within 24 hours and 48 hours as compared to camoquin.

TABLE XII.

Comparative observation on the effect of resochin (single dose method) and camoquin on parasite clearance. (Per cent).

Species of parasites.	PARASITE CLEARANCE WITHIN 24 HOURS.		PARASITE CLEARANCE WITHIN 48 HOURS.	
	Camoquin.	Resochin.	Camoquin.	Resochin.
P. falciparum ...	57·1	40·0	100·0	100·0
P. vivax ...	50·0	50·0	90·0	100·0
Mixed *P. falciparum* and *P. vivax*	50·0	100·0	100·0	100·0

The figures indicate percentage of cases.

The disappearance of clinical symptoms under single dose resochin was within 48 hours in all cases whereas under camoquin it was within 96 hours.

SUMMARY.

The authors' findings show that all the three drugs have marked effect on the erythrocytic stages of the malaria parasites under review. Unfortunately no *P. malariæ* cases were encountered and so no observation could be made against this species.

The best results were obtained with resochin, next camoquin and then nivaquine.

Resochin single dose method has given the best results compared even to camoquin, and this method is to be recommended.

No observations were made as regards the value of these drugs concerning mass clinical prophylaxis and it would be interesting to study and record the finding on this aspect.

Indian Journal of Malariology, **8**, 3, *September* 1954.

A SIMPLE BIOLOGICAL METHOD OF TESTING THE TOXICITY OF RESIDUAL INSECTICIDES.

BY

M. K. AFRIDI,

M. Z. Y. HUSAIN,

S. H. NAQVI

AND

ABDUL MAJID.

(*Malaria Institute of Pakistan.*)

(August 16, 1954.)

INTRODUCTION.

It is now generally recognised that climatic conditions may modify the effects of a residual insecticide to the extent that an assessment of its toxicity in one place may not be applicable to another. It is therefore essential that local arrangements should exist for assessing the duration of toxicity of such insecticides. Since the need for these tests usually arises in countries which are under-developed and where elaborate mechanical devices are not always available, it is particularly important that the testing method be simple and cheap.

Keeping the above objects in view, experiments were started in the Malaria Institute of Pakistan towards the end of 1948 and were continued through 1951 when an appropriate technique was developed which forms the subject of this paper. In the course of these experiments, much useful data was collected on the relative efficacy of D.D.T. and B.H.C. which have been analysed and is also presented in this communication.

Somewhat similar mechanism of testing residual toxicity of insecticides was described by :

(*i*) Technical Department Establishment Laboratory, Kanpur. (*Tech. Rept.*, 1946).

(*ii*) Simmons *et al.* (1945) Supplement No. 186 to *Pub. Hlth. Rep.* Washington, pp. 3-20).

EDITOR.

I. MATERIAL AND METHOD OF TEST.

(*a*) *Preparation of wall area.*—The surface of the walls both inside a room and in a verandah is marked off, 4 feet above the floor, by a black painted border one inch in width, taking care that the enclosed space is exactly one square foot (Plate IX, Fig. 1).

The surface of the square may be plastered with mud, lime wash, cow-dung or any other material currently used in the local houses. Alternatively, thatch, matting or bamboo and wood surfaces may be fixed to the squares and tested. (Plate IX, Figs. 2 and Plate X, Fig. 1).

(*b*) *Spraying of the prepared square.*—Using a De-Vilbiss sprayer, the prepared square is sprayed with the insecticide measured accurately to give the desired dosage per square foot. To ensure a uniform spread of the insecticide, trial sprayings with plain water are practised on a square set apart for the purpose. In case of a strong wind, a wooden box 14 × 14 inches and 6 inches deep is placed on the square to prevent the drift of the spray (Plate X, Fig. 2).

(*c*) *Exposure chamber.*—This consists of an ordinary glass funnel three to five inches in diameter with its stem cut off. The funnel is held against the sprayed surface by rubber straps (salvaged from discarded cycle tubes) stretched between nails projecting from two wooden strips nailed to the wall on either side of the square. Funnels made from perforated copper, zinc and iron of different sizes were tried to see whether aeration or the size of the chamber affected the results of the tests. This was not, however, found to be the case and a glass funnel was ultimately selected because in it the reaction of mosquitoes can be readily studied and because the mosquitoes are disinclined to rest on its glazed surface.

(*d*) *Introduction of mosquitoes into the exposure chamber.*—A batch of ten laboratory-bred 24-hour-old female mosquitoes is introduced into the funnel by means of a catching tube and the time of introduction noted. A match stick, passed through a small hole in the rubber strap, (Plate IX, Fig. 2), effectively dislodges any mosquito that may attempt to settle on the glass surface, thereby ensuring its continuous contact with the sprayed surface. Actually, most of the mosquitoes preferred to rest on the wall rather than on the slippery glass surface and it is only the odd specimen that had to be so dislodged.

When a pre-arranged proportion of the mosquitoes is knocked out, the time is again noted and the batch transferred to clean " Observation hotels " to determine the mortality counts after 24 hours.

(*e*) *Observation hotels.*—These consist of small glass lantern chimneys placed on an enamel dish containing a piece of wet lint. The upper opening of the chimney is closed by mosquito-proof netting held in position by a rubber band or a tin ring. For food, lint soaked in glucose solution or moistened raisins are placed on the top of the netting.

(*f*) *Precautions.*—(1) Persons engaged on testing work are not allowed to come in contact with insecticides.

(2) Those working in the insectarium are similarly debarred from handling any of the equipment etc., used in the testing section.

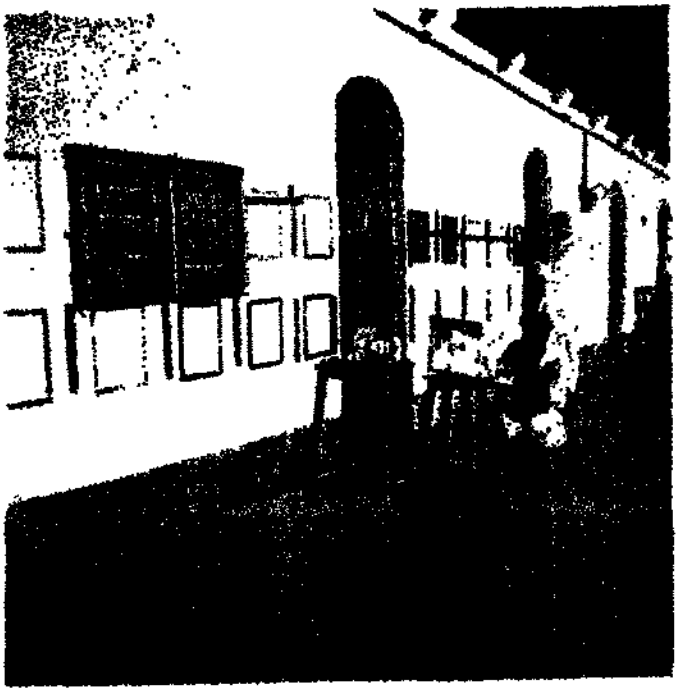


Fig. 1. Verandah.

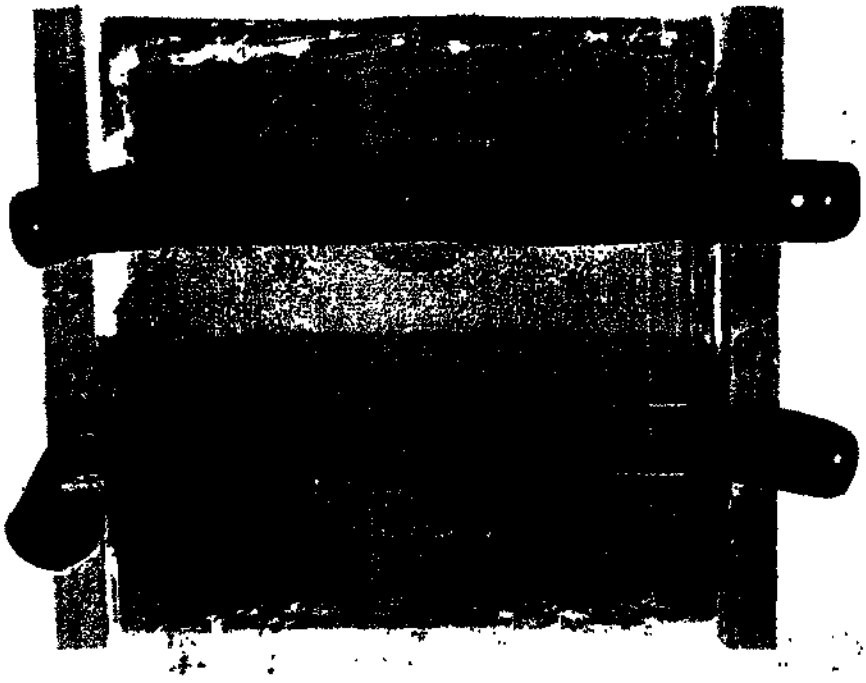


Fig. 2. Mud and wooden and mud combined plastered screens.

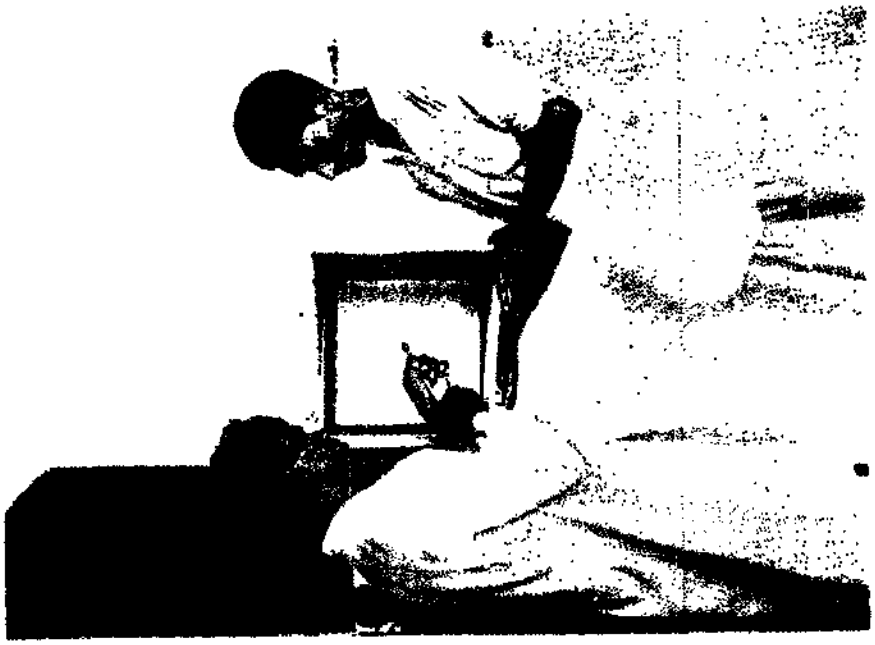


Fig. 2. Wooden bars placed on the square.

Fig. 1. Mat and thatch fixed surfaces

(3) The insectarium itself is located at as safe a distance from the testing section as possible.

(4) All contaminated apparatus are cleaned and washed with kerosine oil, then with soda and finally with soap and water and thereafter stored separately.

(5) With each experiment a control is run on an unsprayed square.

II. ASSESSMENT OF RESULTS.

The obvious advantage of this testing procedure is that the sprayed surface remains exposed to the atmospheric climatic changes as well as to the weathering effect of breeze under natural conditions. Secondly, multiple variables can be introduced in the tests and run simultaneously.

For the proper evaluation of the sprayed surface, however, a standard has to be set. In the earlier tests, mosquitoes were exposed daily and the time taken to secure a knock-down of 50 per cent of each batch was determined. The maximum period of exposure allowed was six hours. The exposed batch was then observed for 24 hours for mortality rates. The adoption of this standard, however, yielded results which fluctuated from day to day. In latter tests, therefore, the standard adopted was to determine the number of weeks during which exposure up to a maximum of two hours gave 100 per cent knock-down and a 100 per cent mortality in 24 hours. Such exposures were made once a week. Minor variations were observed even with this method of assessment but, on the whole, the results obtained were fairly consistent and clear-cut.

Basing analysis on these two standards, the results of observations carried out under the climatic conditions prevalent in Karachi on the efficacy of D.D.T. and B.H.C. are reproduced in the following paragraphs.

(1) On the customary surface of lime plaster on cement, the solutions, emulsions and suspensions of D.D.T. and B.H.C. remained effective against the local species of anophelines for the number of weeks shown in Table I :—

TABLE I.

Effect of solutions, emulsions and suspensions of D.D.T. and B.H.C. on customary surface of lime plaster on cement.

Dose per m^2.	Solution.		Emulsion in water.		Suspension of water-dispersible powder in water.	
	Varandah.	Room.	Varandah.	Room.	Varandah.	Room.
D.D.T. 1 g. ...	8 weeks.	*	5 weeks.	5 weeks	8 to 10 weeks.	14 weeks.
B.H.C. 0·1 g. of gamma isomer ...	1 week.	2 to 3 weeks	1 week	2 weeks	1 week	5 to 7 weeks

*Data incomplete.

The superiority of suspensions to solutions and emulsions on this type of surface is well brought out in Table I. It should, however, be mentioned that the test emulsions were locally prepared with gum and resin and not with a high grade emulsifier such as Triton. This perhaps explains the unusually short residual effect of this formulation.

(2) The effectiveness of the water-dispersible powder of D.D.T. at the rate of 1 g. per m² (100 mg. per square foot) on different types of wall surfaces based on 100 per cent knock-down standard, is shown in Table II.

TABLE II.

Effectiveness of water-dispersible powder of D.D.T. of different types of wall surfaces.

	Matting	Pure cement plaster, split bamboo or wood	Thatching material	Mud plaster, or mud and cow-dung plaster
Room ...	24 weeks	29 to 30 weeks	9 weeks	5 weeks
Varandah ...	11 weeks	11 to 13 weeks	8 weeks	3 to 5 weeks

In the case of B.H.C. applied at a dose of 0·1 g. of gamma isomer per m² (11 mg. per square foot,) the toxic effects lasted less than one week in the varandah whereas inside the room it was uniformly effective for two weeks on all surfaces except mud and cow-dung plaster where its toxicity lasted three to four weeks. B.H.C. was thus apparently more effective on absorbent surfaces than on the non-absorbent ones whereas from Table II it will be seen that D.D.T. had the opposite tendency being more effective on non-absorbent surfaces.

(3) The duration of the residual effect of the varying dosages of water-dispersible powders of D.D.T. and B.H.C. on lime plaster, against local anophelines, is shown in Table III.

TABLE III.

Duration of residual effect of water-dispersible powders of D.D.T. and B.H.C. on lime plaster.

Dose per m²	Room	Varandah
D.D.T. 0·5 g.	7 weeks	4 weeks
1·0 g.	16 ,,	9 ,,
2·0 g.	40 ,,	26 ,,
B.H.C. 0·033 g. of gamma isomer	2 ,,	0 week
0·066 g. of gamma isomer	2 to 3 weeks	1 week
0·100 g. of gamma isomer	5 to 7 ,,	1 to 2 weeks

In this as well as in the two preceding series, although the residual effect of D.D.T. lasted longer than of B.H.C., the knock-down effect of the latter was significantly more rapid.

(4) Compared with anophelines, the culicines proved extremely refractory to the action of D.D.T. Thus, at a dose of 1·0/g. per m² it took more than four hours exposure to secure 50 per cent knock-down and 83 per cent mortality inside the room in the very first week of spraying. These results gave rise to the suspicion that the local culicines might have acquired resistance to D.D.T. following the extensive spraying operations which had been carried out in Karachi in war years. To eliminate this possibility, tests were repeated on culicines bred from pupæ collected from the interior of Sind where no previous D.D.T. sprayings had been carried out. These specimens proved equally resistant.

B.H.C. on the other hand was fairly effective against culicines in that at a dose of 0·1 gm. of gamma isomer per m², 100 per cent knock-down occurred in 35 minutes in the first week and in one and a half hours in the second week with 100 per cent mortality. In the succeeding three weeks the exposure time rose to 4 hours 45 minutes but the mortality continued at 100 per cent. In the sixth week mortality fell down steeply to 30 per cent while in the eighth week it was nil even after 6 hours' exposure.

The quick knock-down effect of B.H.C. and its special lethal action on culicines was taken advantage of by mixing it in equal proportion with D.D.T. for use as a general sanitary insecticide, which gave excellent results in the spraying of refugee colonies in Karachi, during 1949 and 1950.

(5) In an effort to determine how far the short palpi of culicines contributed to their excessive resistance to D.D.T., tests were made on a batch of *A. subpictus* whose palpi had been amputated. This operation did not, however, lead to a significant decrease in the susceptibility of *A. subpictus*, showing that the differential reaction of the local species of anophelines and culicines to D.D.T. was occasioned by some factor other than the difference in the length of the palpi.

SUMMARY.

1. A simple and cheap method of testing the toxicity of residual insecticides is described in which walls exposed to natural weather conditions are utilized. The results of such tests, therefore, closely approximate those expected under actual field conditions.

2. The testing procedure can readily incorporate a variety of variables such as the different types of surfaces, varying dosages and formulations of insecticides, and the different genera and species of the local mosquito fauna.

3. The investigations carried out in Karachi into the relative efficacy of D.D.T. and B.H.C. *inter alia* showed that although the residual effects of D.D.T. lasted much longer than those of B.H.C., the latter had a quicker knock-down effect and was a more potent toxicant to the culicines than D.D.T.

ACKNOWLEDGMENT.

The authors acknowledge with gratitude the active co-operation and assistance of Messrs. M. A. Bokhari, M.Sc., Malaria Officer (Science Graduate), A. Aziz, Senior Laboratory Assistant, and other members of the staff in the conduct of these tests.

OBITUARY

BRIGADIER GENERAL JAMES S. SIMMONS.

THE Journal records with deep regret the death of Brigadier General James Stevens Simmons (U.S. Army—retired), Dean of the Harvard School of Public Health, at the age of 64, at Hartford, Connecticut, after a lifetime devoted to military and civilian health.

General Simmons was born on June 7, 1890, in Newton, North Carolina (U.S.A.), graduated from Davidson College, in 1911, got his doctorate in medecine from the University of Pennsylvania, School of Medicine, in 1915, Ph.D. from the George Washington School of Medicine, in 1934, and doctorate in Public Health, in 1939, from the Harvard School of Public Health. In 1937, Davidson College conferred on him the honorary degree of D.Sc. and later he received similar honorary degree from Duke University, University of Pennsylvania, Marquette College, the University of North Carolina and Harvard University.

General Simmons undertook several important assignments in his army career. During World War II, as Chief of the Preventive Medicine Service, Office of the Sugeon General, he was responsible for preventing sickness and accidents among the U.S. armies constituting nine million officers and men.

His outstanding services won many honours including 'Sedgwick Memorial Medal for Distinguished Service in Public Health' from the American Public Health Association. After 30 years of Army Service, General Simmons retired to become Dean of the Harvard School of Public Health.

His work at the School was also recognised with honours. In 1948 he was awarded the James D. Bruce Memorial Medal for outstanding achievement in preventive medicine at the annual convocation of the American College of Physicians, San Francisco. He delivered the annual Charles V. Chapin Oration at the 141st meeting of the Rhode Island Medical Society in 1952 and received in the same year Chapin Medal, which is awarded by the City of Providence for outstanding contributions in the field of Public Health.

As a consultant in Tropical Medicine and as a member of most important national health associations, General Simmons always worked for an increased public and private awareness of the benefits to be derived from providing more attention to prevention of disease. He always maintained that the objective of Medical Science should be the prevention of sickness in the service of human life. Towards acceptance of this concept, he appeared several times before congressional committees considering Government aid to Health Education and made innumerable speeches and wrote several scientific papers.

Very recently he undertook a round-the-world tour with a colleague from the Harvard School of Public Health and exchanged ideas with health leaders in ten European and Asian Nations in pursuance of a long-visualized plan for establishing what he termed a 'Bridge of Health' between the Harvard School of Public Health and an Asian Institution interested in exchanging students, faculty members and ideas on a long term basis.

He was editor-in-Chief of *A History of Preventive Medicine in World War 2nd.*

J.S.

Indian Journal of Malariology, **8**, 4, December 1954.

A WRITTEN SYMPOSIUM ON *PLASMODIUM BERGHEI* VINCKE AND LIPS, 1948.

Introduction.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B.(Edin.), D.P.H.(Eng.), D.T.M. & H.(London).

Director, Malaria Institute of India, Delhi.

(December 31, 1954.)

THE discovery by Vincke and Lips of a new Plasmodium, namely, *berghei*, in 1948, followed by an announcement that mice and rats were susceptible to it, was most enthusiastically received by all malaria workers. These rodents which are easily bred and handled, are well understood in the laboratory. Experimental malariology commenced with fresh vigour and during a period of six years, there have been nearly a thousand publications on research connected with malaria due to *Plasmodium berghei*, which (research) has proved to be highly useful in our studies in malaria.

The Expert Committee on Malaria of the World Health Organization at its fifth meeting at Istanbul in September, 1953, considered *berghei* malaria as one of the three items of considerable interest in the recent scientific developments, and emphasized on its special value in opening up fresh fields of research in malaria. The Committee considered that the World Health Organization might well promote the coordination of such research activities at the different centres in the world.

The large volume of work on *Plasmodium berghei* has confirmed many of the principles of human malariology already known and has shown that, to a large extent, the natural history of the disease in rodents is similar to that in man.

It would be appropriate to pause and critically take stock of what has already been achieved and to formulate the lines of future work. But there appears to be one difficulty. The centres of research are so widely scattered round the world that research workers can hardly meet in one place and discuss the problems at length. It is for this reason that a written symposium on the subject was contemplated and most of the workers engaged in research on *Plasmodium berghei* were invited to participate. The response to such an invitation was spontaneous

and highly satisfactory. The readiness with which a doyen of malariology, as Professor Sergent, agreed to contribute, is an honour to the symposium. It is by no means complete but a great deal has been achieved towards the fulfilment of our objective.

The original articles written in French, have perforce been translated and published in English. It is realized that justice to the facile expression in the original language can hardly be reflected in any translation. At the same time, the translations were necessary to serve majority of the readers of this Journal. The publication of the French articles in original was also not possible due to the non-availability of punctuated matrix at the press of the Journal. All the same it is hoped that the original articles in this special Symposium Number of the Journal will serve as a collective work of reference for a long time to come.

Documentation by van den Berghe of the discovery of the parasite by Vincke and Lips, is fascinating. One is reminded of many incidents of " mice and men " when he recalls the manner in which the discovery was announced to the world. Natural history of the disease among rodents and *A. dureni* in the Belgian Congo, is ably dealt with by Vincke. The finding that as a result of clearance of the forest galleries, the rodents had been driven away and it became difficult to find natural infection in *A. dureni*, is a profound observation on the epizootiology of the disease.

Transmission of *Plasmodium berghei* by mosquitoes in nature, is dealt with by Vincke. Bray summarizes persevering attempts which have proved partially successful. These reviews bring out forcefully many aspects of the different strains of *Plasmodium berghei* and its host parasite relationship with special reference to gametogenesis.

The current history of mosquito transmission of *Plasmodium berghei* in the laboratory is not without a parallel. A number of attempts to infect mosquitoes with *Plasmodium knowlesi* (Sinton and Mulligan, 1932) from *Macacus mulatta* monkeys (accredited abnormal hosts), were unsuccessful in the past. Mulligan and Knowles (quoted by Russell, 1942) obtained cöcysts in only one specimen of *A. stephensi* out of 400 that were dissected after being fed on an infected monkey. Russell and Mohan (quoted by Russell *et al.*, 1946) found sporozoites in a single specimen of *Aedes reginae* fed on an infected monkey. Further attempts to infect the same species and other mosquitoes failed. Indeed, the failure to transmit *P. knowlesi* infections through mosquitoes in the laboratory became so well " established " that standard text books record ". . . . mosquito-induced infections, not yet possible with *P. knowlesi*, are desirable " (Boyd, 1949). Thus, for seventeen years since the discovery of *P. knowlesi* in 1932, determined efforts to infect mosquitoes with *P. knowlesi* in the laboratory were not successful. All these years, the strain was maintained by blood passage in the laboratories. And yet in 1949 and 1950 fresh attempts to infect *A. stephensi* and *A. annularis* with *P. knowlesi* were successful (Jaswant Singh, Ray and Nair, 1949 : 1950). The actual reasons for the early and consistent failures and later success in infecting mosquitoes with *P. knowlesi* are not yet clear. A concurrent observation, along with the successful mosquito transmission, was that the strain had lost its virulence and was no more producing 100 per cent fatal infections in *Macacus mulatta* monkeys. It would appear permissible to infer that prolonged passage of the strain

through unnatural hosts was in some way responsible for the successful infection of mosquitoes. The high degree of geographical and host localization of *Plasmodium berghei* pointed out by Bray, would also appear to be similar to that of *P. knowlesi* which is confined to Malaya in *irus* monkeys.

Maegraith's approach to the physiological and pathological processes in *Plasmodium berghei* infections, is interesting. His discovery of the effects of milk diet in malaria has opened out an entirely new field of research on food requirements of the malaria parasites. Study of the nutritional requirements is essential in understanding the physiology of the parasite. A complete change in the physiology of a strain of *Plasmodium berghei* resistant to sulphadiazine observed by Jaswant Singh and collaborators, is also of interest. Among other things, it illustrates that the degree of parasitism of Plasmodium is not total and is still capable of adaptation to gross environmental changes, namely, complete lack of PABA.

The discovery of *Plasmodium berghei* has made it possible for the first time to critically appraise the rôle of host nutrition on the course of the disease in a laboratory mammal. Ramakrishnan has reviewed his work at the Malaria Institute of India. His studies on the course of infection in starved and semi-starved hosts have brought out a clear cut explanation of observed facts during the epidemic of malaria during the last widespread famine of 1943 in Bengal. The noticeable effect of poor nutrition of the host was reduced parasitæmia and mild first attacks. But relapses in the under-nourished hosts caused greater mortality than in well-nourished hosts.

The subject of immunology in *Plasmodium berghei* malaria has been reviewed independently by Sergent, Raffæle, Fabiani; and jointly by Galliard and Lapierre. Immunity in malaria, being of the nature of premunition, it will be remembered, was first established by Sergent a few decades ago. In his analysis of the numerous investigations on *berghei* malaria, he sees no cause to change his concept of the mechanism of immunity in malaria. He rightly points out the fallacies in techniques generally adopted to prove radical cure of animals.

Raffæle has raised an interesting point on the rôle that age of the host plays in innate natural immunity to infection. The importance of further study of this problem is stressed by him. Another important emphasis is on the paradox of severe degrees of anæmia in *berghei* infection despite the accredited preference of the parasite to immature erythrocytes. He also points out the greatly modified course of infection in mice on milk diet and their survival for greatly prolonged periods.

Smet and Frankie have shown that *Cricetomys ansorgei* can acquire real immunity to *Plasmodium berghei*.

Fabiani has dealt with experimental investigation of the rôle of endocrines in malaria, a subject which has been little investigated in the past. His concluding sentence, "There is probably no other plasmodial species than *Plasmodium berghei* which provokes so great a diversity of infection patterns in spite of the uniformity of immunological reactions" emphasizes the scope for further study on host factors in malaria.

Roy and Bose have dealt with the specific rôle of estrogen in *Plasmodium berghei* infection.

Galliard and Lapierre have approached the subject of immunity from three different angles, namely, its modification by biological substances, non-specific

substances and antimalarials. Particularly the last is of current interest as the present day availability of antimalarials to the malaria-stricken populations of the world, is unprecedented.

Rodhain has established the specificity of the new parasite *Plasmodium vinckei* by the absence of cross-immunity between it and *Plasmodium berghei*.

Greenberg and Coatney have dealt with particular aspects of immunity to *Plasmodium berghei* infections and have discussed the distinct rôles of mature and immature erythrocytes involved in the infestation.

Corradetti and his co-workers have reviewed their own work on host-parasite relationship of *Plasmodium berghei* infections and point out the direct relationship between progress of infection and host response resulting in increased numbers of immature erythrocytes in the peripheral blood. Their studies on serum proteins show a relative increase of gama-gloubins at the time of parasite crisis. They are of the opinion that a true residual immunity of a high degree occurs in rats after a spontaneous radical cure. Such a true immunity has been unknown heretofore in protozoal infections.

Schneider reviews the chemotherapy of *Plasmodium berghei* infections and points out the usefulness as well as limitations of using the infection for purposes of screening potential antimalarials.

When the symposium was first mooted, it was intended to circulate the manuscripts to different panels for comments. Unavoidable delay in the receipt of contributions have made it necessary to publish the papers in a Special Number without the comments. Copies of the Special Number will now be distributed and any comments received, will be published in a subsequent Number of the *Indian Journal of Malariology*.

It is evident from the contributions to the symposium that hosts, their age, strains of parasites and methods, and terminology employed by different workers, are not according to any set pattern. It would be ideal for research if cooperation between laboratories could be extended under the overall direction of a committee to furnish standard methods and material for work (for instance, by the World Health Organization Expert Committee on Malaria). Such cooperative effort may be further reinforced by the maintenance of a roster of all institutions where work on *Plasmodium berghei* is carried out and by a quick exchange of reprints and reports amongst them. Some of these ideas may take time for implementation and necessitate a certain reorientation in the existing and established methods prevalent in the different laboratories. But the results accruing from such changes may be out of all proportion to the effort involved.

REFERENCES.

Boyd, M. F. (1949)	*Malariology*. W. B. Saunders & Company, Philadelphia and London.
Jaswant Singh, Ray, A. P. and Nair, C. P. (1949)	*Ind. J. Mal.*, **3**, p. 145.
Idem (1950)	*Ibid.*, **4**, p. 317.
Russell, P. F. (1942)	*Final report of the Madras Malaria Investigation Project*. International Health Division of The Rockefeller Foundation.
Russell, P. F., West, L. S. and Manwell, R. D. (1946)	*Practical Malariology*. W. B. Saunders & Company, Philadelphia and London.
Sinton, J. A. and Mulligan, H. W. (1932)	*Rec. Mal. Surv. Ind.*, **3**, p. 257.

Indian Journal of Malariology, **8**, 4, December 1954.

THE HISTORY OF THE DISCOVERY OF *PLASMODIUM BERGHEI*.

BY

LOUIS van den BERGHE.

(Institut pour la Recherche Scientifique en Afrique Centrale.)

(October 1, 1954.)

THE author of this introductory note has personal reasons to relate some historical data of the discovery of *Plasmodium berghei*. The parasite has been dedicated to him as a close friend of Dr. I. Vincke but also because he had the good fortune of following step by step the most classical and, at the same time, perfect example of biological research and discovery.

Dr. I. Vincke, after some fifteen years of service in the Belgian Congo Government in the many capacities of a health officer, a laboratory doctor, and finally the Director of the Service d'Etudes et de Recherches Anti Malariennes (S.E.R.A.M.—a special service of malarial research in the Katanga Province), had developed a remarkable knowledge and understanding of the biology of Anopheles. Much to his credit and as a real foundation to his discovery of *Plasmodium berghei*, he did not restrict his interest and studies solely to the sole Anopheles vectors of human malaria. As a medical man, he wisely thought that the study of any Anopheles might contribute directly or indirectly to our knowledge of malaria and as an all-round naturalist he maintained an equal curiosity towards the observation of all species of Anopheles, no matter how rare or how apparently unimportant. Such was the case of *Anopheles dureni* the adult of which was discovered by Dr. Duren from the region of Mwela in Kwango (Belgian Congo) and named after him in 1938. The larva of *A. dureni* was later found in the same region by Henrard, van Wymeersch and Wanson in 1944. Dr. I. Vincke and F. d'Ursel in 1943 found *A. dureni* adult and larvæ near Elisabethville at the Keyberg experimental farm along the forest gallery of the Kisanga, in the Comite Special du Katanga territory. At the same time and as the second step of discovery, *A. dureni* were discovered infected with a presumed malarial parasite. For three years the infection was repeatedly found in *A. dureni*. In 1946, Vincke brought stained smears of the stomach contents to the laboratory of the author,

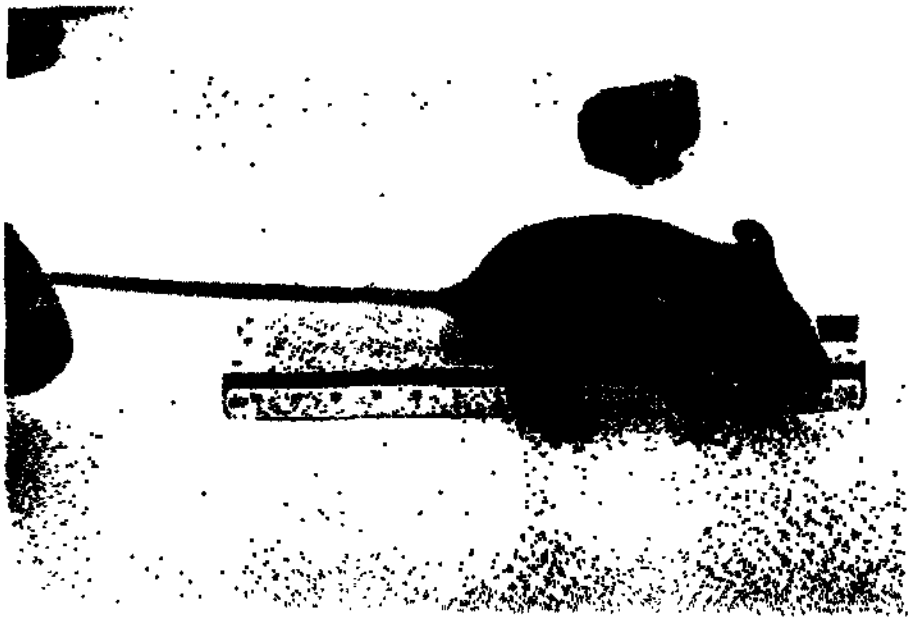
who as Professor of the Institute for Tropical Medicine in Antwerp, Belgium, was conducting at the time extensive hæmatological research. The red blood cells were found to be similar to those of monkeys or rodents. The white blood cells offered no clues. Early in 1947, Vincke was back in the Katanga and prepared agglutinating sera for as complete series of mammals as possible. Only the anti-rodent serum proved difficult to make, and was therefore not considered too reliable. None of the anti-sera precipitated the extract from *A. dureni* stomach contents. However, the anti-rodents serum results were suspicious.

But no conclusion could be made. Fortunately, very soon two *Thamnomys* were found heavily infected. Blood was transmitted to white mice with good positive results. Some slides were sent to the author in Antwerp and also to Rodhain. The parasites belonged unmistakably to the genus *Plasmodium* and were identical with those seen in the stomach contents of *A. dureni*.

The third step of research, namely, the discovery of the intermediate host, as a result was begun on the assumption that a rodent was the probable carrier. For almost a year, hundreds of specimens of the mammalian fauna of the small forest gallery were captured and their blood examined. In January 1948, a captured rodent, *Thamnomys surdaster* (Plate XI), showed a few suspicious forms in the blood. The slides were first seen by M. Lips, Medical Assistant at the S.E.R.A.M. Vincke, on his return from a short field survey, examined the blood smears carefully.

Vincke undertook the transmission of the strain (Strain K. 173) to all the available wild and domestic rodents. He soon made the exceedingly important discovery that the white mouse developed almost 100 per cent lethal infection. Experimental workers in malaria had at their disposal for the first time a *Plasmodium*, highly pathogenic for one of the commonest and the most economical of all laboratory mammals. Not only could the mechanism of acute malaria and death be studied as well as the therapeutic action of drugs but it could be done with a greater accuracy and speed than in birds and monkeys. The different rodents presented various degrees of susceptibility ranging from complete resistance to a 100 per cent mortality, which offer, for the first time, the possibility of experimental study of the immunity in malaria. Although the identity of the parasites from the *Thamnomys* and the one from *A. dureni* was not established until 1950, it was felt that a preliminary announcement should be made. An American scientific expedition had just claimed to have discovered a *Plasmodium* of the elephant shrew in Sudan. Several dozens of *Elephantulus* had been shipped by plane to the United States of America. From the vague, although largely publicised data (some newspapers spoke of the discovery of a malaria parasite in the African elephant instead of the elephant shrew), the parasite could have been the same as the one observed in the Congo rodents. In January 1948, the author had just been appointed the Director of the new Research Institute in Central Africa called I.R.S.A.C., and in May 1948, before joining his post in the Belgian Congo he was invited to attend the Fourth International Congress of Tropical Medicine and Malaria held in Washington. He vainly attempted there to present for Vincke and Lips the description of the parasite which in the meantime they had named *Plasmodium berghei*. The regulations of the Congress could not be waived whereby papers could only be presented by invited members of the Congress. The author, being on the programme with a paper of a general character on the new

Plate XI

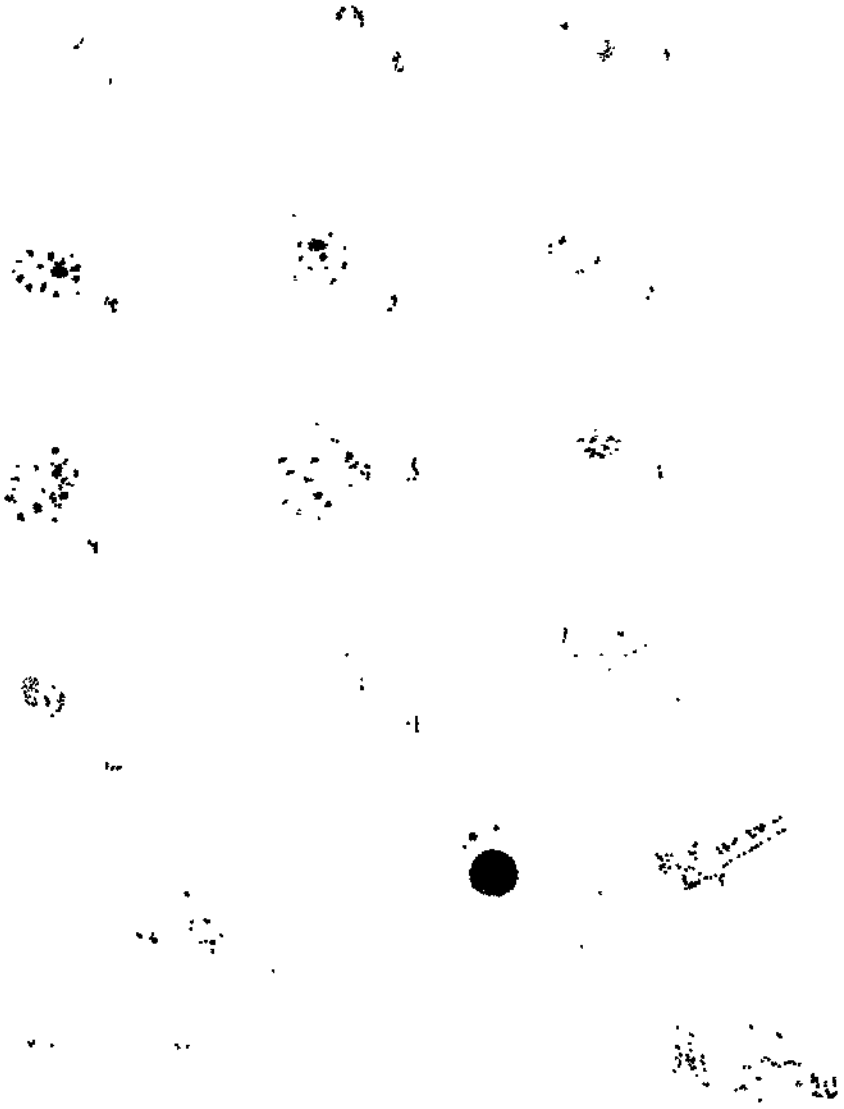


THALNOATA SURDASTER SURDASTER.

This plate was very kindly were made available by the Institut pour la Recherche Scientifique en Afrique Centrale, Rue Montoyer, 42, Brussels.

PLATE XII.

PARASITE OF *PLASMODIUM BERGHEI*



Figs. 1, 2, 3. Annular trophozoites.
4, 5. Rosettes of schizogony.
6. Erythrocyte with multiple infection.
7, 8. Blue substance showing multiple chromatin nuclei; hypertrophied red cells nearly filled by conglomerate of trophozoites.
9. Extracellular parasite.
10, 11, 12. Gametocytes.
13. Erythrocyte parasitized by a half-grown macrogametocyte and two annular trophozoites.
14. Two annular trophozoites in an erythroblast.

This plate has very kindly been made available by the Institut pour la Recherche scientifique en Afrique Centrale, the Manager, 12, Brussels.

medical research in the Tropics, used the discovery by Vincke as an illustration of one type of research, introducing at the same time a foot note for Vincke and Lips with a brief but satisfactory description of *P. berghei*. Coloured slides and microscopic preparations were also presented by the author at one of the public demonstrations while no mention was made at the Congress of the alleged *Plasmodium* parasite of the elephant shrew. In a rather unorthodox way, *Plasmodium berghei* made its appearance in this manner in May 1948, while shortly afterwards in the same year a formal paper by I. Vincke and M. Lips appeared in the *Annales de la Societe Belge de Medicine Tropicale*, illustrated by a coloured plate (Plate XII).

Because of the extraordinary importance of the discovery of *Plasmodium berghei*, the strain was sent by Dr. Vincke almost immediately to the Institut de Medecine Tropicale Prince Leopold of Antwerp where Professor J. Rodhain undertook its study and from where the Director, Professor A. Dubois, sent it to the many interested institutes and laboratories. At the same time the new Belgian Congo I.R.S.A.C. Institute granted a research subsidy to Dr. Vincke and put at his disposal the assistance of N. Leleup and M. Chardome both from the I.R.S.A.C. technical staff.

The research in Congo was mainly directed towards the ethology and ecology of *Anopheles dureni* and of *Thamnomys surdaster* with the result that in the following three years many new infected locations were discovered and other strains made available for research. Very rapidly outstanding work originated in all parts of the world, building fundamental new knowledge in the field of malariology. The I.R.S.A.C. Annual Report published in 1952 a bibliographical list of not less than 129 papers on *Plasmodium berghei*. The ancient saying "*Ex Africa aliquid semper novi*" was confirmed in an outstanding way in the field of medical zoology which appears still so full of promise in Central Africal, by far the richest part of our modern world in animal life.

Indian Journal of Malariology, 8, 4, December 1954.

NATURAL HISTORY OF *PLASMODIUM BERGHEI.**

BY

I. H. VINCKE.

(Director, S.E.R.A.M., Elisabethville, Belgian Congo.)

(October 1, 1954.)

INTRODUCTION.

The Katanga region, where *P. berghei* was discovered in 1948, is situated in the south-east of Belgian Congo south of the 5th south latitude. The western extremity exceeds the 24th meridian east of Greenwich whilst the eastern frontier is in the neighbourhood of 30th meridian east of Greenwich. The southern frontier is constituted by the separating line of the ridges between Lualaba basin on the one side and that of Zambeze on the other. The extreme southern point of Katanga extends nearly up to 13°27' south latitude. The Katanga is in short the southern flank of the Congo basin which is bordered to the south, south-east and to the east by the high plateau regions of Central Africa.

The altitude of the north-west zone of the province varies between 500 and 1,000 meters ; this zone can be called low Katanga. The region, situated to the south, south-east and to the east, is a zone of high plateau, the altitude of which ranges from 1,000 to 1,500 meters. The belts of plateaus such as the Kibara-Biano, the Kundelungu, the Marungu, reach and exceed an altitude of 1,700 metres. Elisabethville is at an altitude of 1,230 meters.

The vast table land called high Katanga extends to low Katanga (Biano, Kibara, Kundelungu). Between the extensions are found the low plains ; the plain of Kamalondo (maximum altitude 560 meters) with its marshes and lakes, the Lufira plain from 800 to 1,000 meters and the Moero-Luapula (950 to 1,100 metres) which extends towards the north valley of Luapula. These low zones, riverain of the Tanganyka and Moero lakes, are comparable to those of low Katanga.

*Thanks are due to Mr. K. Gopalakrishnan, Assistant Director General, Posts and Telegraphs, New Delhi, for translating the original paper written in French into English.—*Editor*.

From the north-west to the south-east, the climate is subject to marked variations. It passes from a sub-equatorial climate with 2 to 3 months of dry season to a Sudanian climate the dry season of which grows longer when we move towards the south-east, reaching seven months at the extreme south-east of the province (Sakania).

The vegetation represents faithfully the climatic variations : to the north-west region the sub-equatorial forest ; to the south-east forest-park which transforms itself to brachystegia wooded savanna on reaching high katanga. Further south-east, on the high plateaus, swept in dry season by the trade winds, the vegetation is constituted by a grassy steppe surrounded by a belt of bushy shrubs.

The night temperatures in the high Katanga can be very low in the dry season and even vary in the neighbourhood of freezing point in the high plateaus. By way of example, the minimum mean temperatures observed at Kongolo (Bas-Kat.), Albertville (Bas-Kat. shore of the Tanganyka Lake), Elisabethville (Haut-Kat.) for the years 1930-1939, are given below :—

	Kongolo	*Albertville*	*Elisabethville*
January	19·2	18·4	13·9
February	19·6	18·4	14·4
March	19·3	18·6	12·6
April	19·0	18·2	9·2
May	17·7	16·4	6·5
June	15·6	13·8	3·8
July	14·8	12·7	3·3
August	16·9	12·8	3·9
September	18·6	14·9	7·0
October	19·0	17·3	10·4
November ...	18·5	18·8	13·6
December	18·8	18·7	14·6

The day temperatures do not show any marked difference in the entire extent of the province.

DESCRIPTION OF THE PARASITE.

The young trophozoite appears as ring form with a relatively big vacuole. The chromatin dot is most often single, but rings, with two chromatin dots generally of the same dimension, are sometimes seen.

When the parasite grows, one can distinguish in the cytoplasm very fine grains of a black pigment. Pigmented trophozoites, and the others the pigment of which is so light that it is visible only in fresh films, are also found. This is above all true in multiparasitized cells. The multiparasitism of the same cell is frequent. Erythrocytes invaded by six parasites and even more, are met with. When the latter grow in hypertrophied cells, the cytoplasms merge and their limits are no more visible, and constitute blue masses which measure 10·6 μ in diameter. Parasites of different ages can develop in the same cell—for example, young trophozoites, schizonts and gametocytes. The schizogony gives rise more often to 8, 10 and 14 merozoites.

In intense infections, it is not rare to find young forms having invaded the blue cytoplasm of the nucleated red cells. The gametocytes, males as well as females, keep often their vacuoles during the course of their growth. A black pigment, most often clearly visible, is dispersed throughout the cytoplasm of the macrogametocyte ; that of the microgametocyte is disposed most often in clumps. The nucleus of the macrogametocyte is typically peripheral and small. Those of microgametocytes are much bigger and not densely packed. The colouration of macrogametocyte is intense blue, whilst that of microgametocyte is paler and violet or greenish. The dissemination of the chromatin gives them a general rosy aspect which is very striking.

Apropos of gametocytes, these should be studied on the strains freshly isolated. After a certain number of passages, it has been noticed that their appearance is modified to a point when they can be distinguished with difficulty from the asexual forms and when the males lose their power of exflagellation.

The enlargement of cells is typical. It is necessary to discuss the interpretation given to the enlargement. One can prove in short that it presents itself in the cells which have been polyparasitized. It manifests itself even when the red cell has underdeveloped or young parasites.

The enlargement of red cells would not be due to the parasite itself but due to the fact that the infection occurs in red cells which have not reached maturity ; the proportion of young red cells increases with anæmia and persists when the parasites disappear after treatment.

Tissue forms have been described in the histocytes of the liver. They appeared 36 hours after the blood inoculation when the parasites have not yet reached the blood. They are found equally in the bone marrow and in the endothelial cells of the capillaries. The tissue schizonts should have a larger number of merozoites than the blood schizonts (up to 30 merozoites).

These tissue forms should remind one of those of *P. elongatum*. However, some authors have not found the tissue forms. Baldi (1952) describes the forms which he thinks could be of tissue origin, on which he did not like to conclude. The fact that they are found in the bone marrow after treatment with quinine or atebrin, would go in favour of their being tissue forms and equally explain the relapses observed when the blood forms disappear after treatment. Against this opinion, it has been remarked that there are always some erythrocytes present in the organs and that at the same time are found blood forms resembling tissue schizonts — forms in which the pigment cannot be distinguished. In other words, the suspension of washed organs do not appear to be infective when the blood of the same animal is not infective.

Arguments of an experimental nature go equally against the tissue origin of the parasite found in the organs after infection of blood — likewise it has been discovered that there are no prepatent periods and that when massive injections by peritoneal route are made, it is found that the blood is infective ten minutes after the inoculation. When rats are inoculated by intraperitoneal or intracardiac route with massive quantities of parasitized cells, the cells of the donor are found in the blood of the tail from 15 minutes to one hour after the inoculation. In very short time, the parasites multiply in the red cells of the recipient. This at

least proves that there are no tissue barriers which oppose the immediate passage of the parasites in the general circulation or their multiplication.

The experiments made on *Cricetomys ansorgi* do not certainly enable us to prove, till the present time, the existence of any exœrythrocytic phase after infection of the blood. It is known that *Cricetomys* present periods of latency, often prolonged, and it has been demonstrated that during such period the blood remained always infective. It was the same in two cases after injection of sporozoites; two *Cricetomys* have likewise been injected with nivaquine, one was completely and definitely cured and the other presented an inapparent parasitæmia during which subinoculations have, however, been positive and followed later by severe relapses.

It is, however, evident that a prepatent (pre-erythrocytic) period must exist. It has been demonstrated that after injection of sporozoites to young rats, the latter were not infected before two to three days. However, the pre-erythrocytic forms have not been recognized in the animals injected with numerous sporozoites (up to hundred positive salivary glands in a white rat 15 days old).

The sporozoites have not been sufficiently described but they resemble closely those of parasites of man. Oöcysts in Anopheles fed on infected animals have been described as presenting fine pigment, and in clear diffused light appear as a double line lightly curved. It does not appear that the descriptions correspond to what is commonly met with in *A. dureni*. In any case, the question would merit further study.

THE INVERTEBRATE HOST.

The vector of *P. berghei* is incontestably *A. dureni* Edwards. This Anopheles was described for the first time in 1938 and found in the district of Kwango—region of Mwela (Belgian Congo) in the environs of 6° south latitude and 30° east longitude — (altitude 900-1,000 meters). According to Duren (1944), the number of human malaria cases in the region is low or nil, and the indigenous adults contract the disease probably in very much lower altitudes (about 600 meters). The majority of anophelines captured in the houses were *A. dureni*.

The larvæ as described by Henrard *et al.* (1944) live in sandy rivers in which water flows slowly and shaded by dense vegetation.

A rapid personal survey undertaken in May, 1951, permits us to state precisely the following points :

We have not in this period found *A. dureni* in the houses but they bite man near the river itself. The only larval resting place found was a space cleared of trees and covered with grassy vegetation ; we have not found the Anopheles (fed ones) on the trees. In Kwango, no dissection of *A. dureni* has been carried out to our knowledge to this day.

In Katanga, the habits of *A. dureni* present a totally different pattern. The habitat is always constituted by forest galleries and it appears certainly that there was a relation between the density of the forest and the number of Anopheles; it is thus that a gallery (Kisanga) having been partially cleared, the Anopheles concentrated themselves in a space of some 100 meters. The adults are found easily

in day-light on the trunks of living trees, particularly old ones, which are rough or moss-grown.

It is difficult to give an idea of the density of Anopheles. Some attempts have been made by taking as basis the number of trees. But the indigenous insect collectors have made marks on the most frequented trees—which marks vitiate the results. For the years 1944-45, 10,371 Anopheles were found on 10,848 trees. A trained collector can easily find 200 Anopheles in one morning when the season is favourable.

A very large proportion of males, likewise are found on the tree trunks (about one-third of the collection). Among the females, a large number is in an advanced state of ovarian development and digestion. Here are the values for ovarian stages expressed as percentage (according to Christophers, 1911) :—

I	..	...	3
II	...	...	22
III	...	...	22
IV	...	...	22
V	...	...	31

The *A. dureni* of the Katanga collected, rest on the trees in order to complete their gonotrophic cycle unlike *A. funestus* and *A. gambiæ* which rest in the houses in which they feed. It is hardly necessary to dilate on the adult habitat. Researches were made in 1944-45 round about one of our observation stations. The results were negative for :

(1) The collections were made in the houses of an indigenous camp situated at some hundreds of meters from the larval habitat.

(2) In the neighbouring cattle-sheds.

(3) Collections made in places where the grass was mowed very short.

(4) The traps of Magoon—in 1944, seven *A. dureni* were found among 1,303 anopheline mosquitoes (of which one was found in the trap with human bait). The *A. dureni* of Katanga does not bite man. Different catching stations have been established during the year 1945 in various places. Collections were made day and night including ones on the river. No *A. dureni* was captured. Some Anopheles were captured on man on the first day of our researches, but it was never repeated. There is every reason to think that it was a question of error.

During the same year, no *A. dureni* was captured in Magoon traps with human bait. Other anophelines, sometimes even in large numbers, were found : *A. concolor, A. berghei, A. implexus.*

The adults appear to feed exclusively on rodents In short, the contents of the stomach of a large number among them were submitted to the precipitin test for the following anti-sera :—

Sheep
Ox
Horse

Cat
Dog
Antelope
Man
Monkey

As these sera are rarely specific, it can be admitted that the negative results would thus cover the majority of mammals except the rodents and perhaps the insectivores. In other words, on direct examination, nucleated red cells are not found in the blood.

In Katanga, the larval habitats are constituted typically by the shady banks of the streams of the forest gallery. Generally the current is rapid and larvæ are easily obtained by a scoop scraped over the roots of trees projecting in the water.

From the point of view of adults, they disappear during the dry season, but larvæ are found always in some abundance.

The *A. dureni*, in the Katanga, appears to be essentially a forest dwelling species, hygrophile and shade loving. It spends all its life in the forest gallery where it is enclosed. Howsoever specialized and delicate, they find sometimes in their stations very favourable condition in certain galleries where they can be very abundant. These conditions differ from those of Kwango. To these differences must be added other factors of morphological order, superficial they may be. The adults of Katanga present less of scales on the eighth tergite and the larva presents typically distinct pigmentation — to such an extent that it is easy to recognize it with the naked eye *in situ*.

Will this suffice to create a new species ? We will not dare to affirm it and it could be particularly interesting to apply the cytogenetic methods recently described by Fraizzi (1953). If it were a question of one and the same species, we will come across an interesting case of geographical variation.

GEOGRAPHICAL DISTRIBUTION.

This has been studied only in the Katanga. Map I shows that *A. dureni* is present through all the high Katanga. It is found there at various altitudes from 2,000 to about 1,700 meters. *A. dureni*, Katanga type, has been likewise found in the Tanganyka territory in the environs of Abercorn. It is again a question of grassy plateau at 1,500 meters altitude obstructed by important forest galleries (Plate XIII).

THE PARASITE RESERVOIR.

Up to the present moment, we have found that the reservoir of parasites are the three rodents frequently found in the forest galleries. They are *Thamnomys surdaster*, *Praomys jacksoni* and *Leggada belle*.

Thamnomys surdaster.—This has often been alluded as an arboreal rat. In fact nothing is less certain, and a more intense study is required. It is found in

MAP 1.

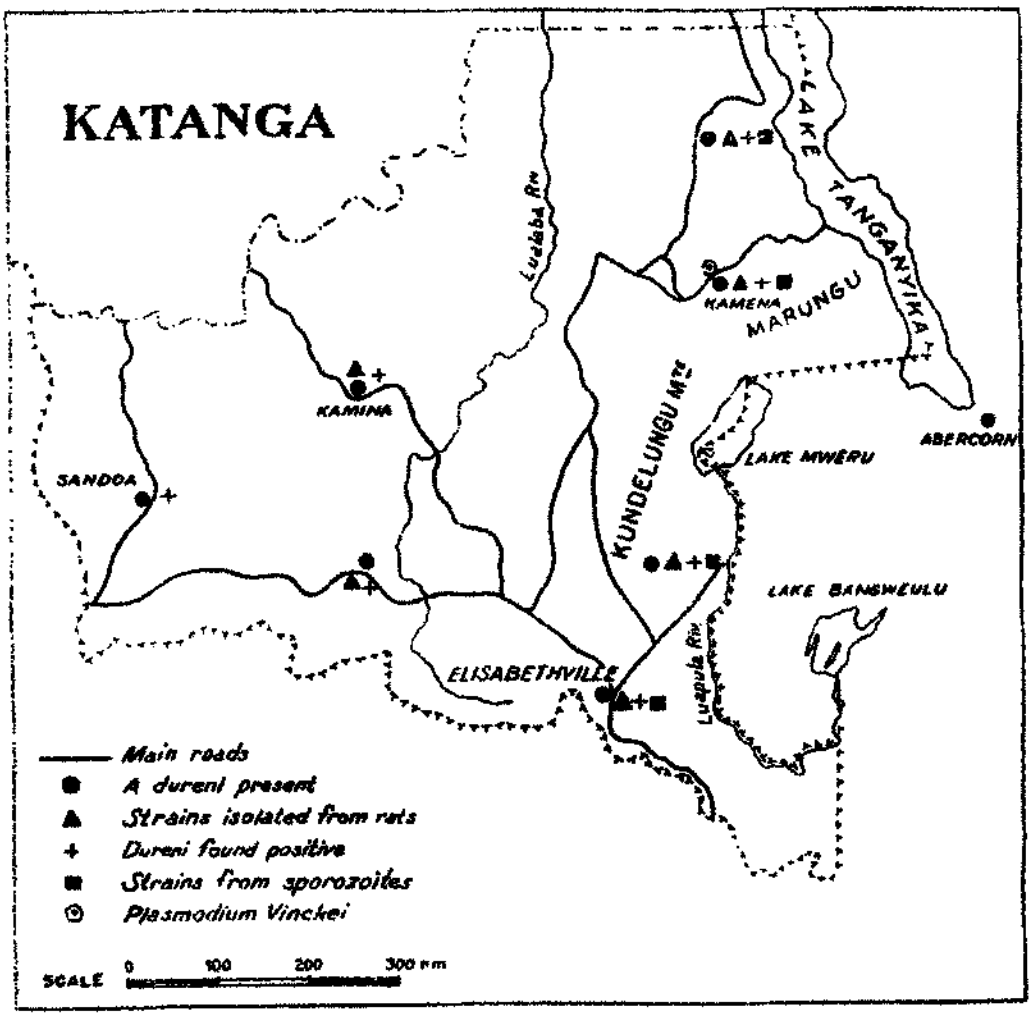
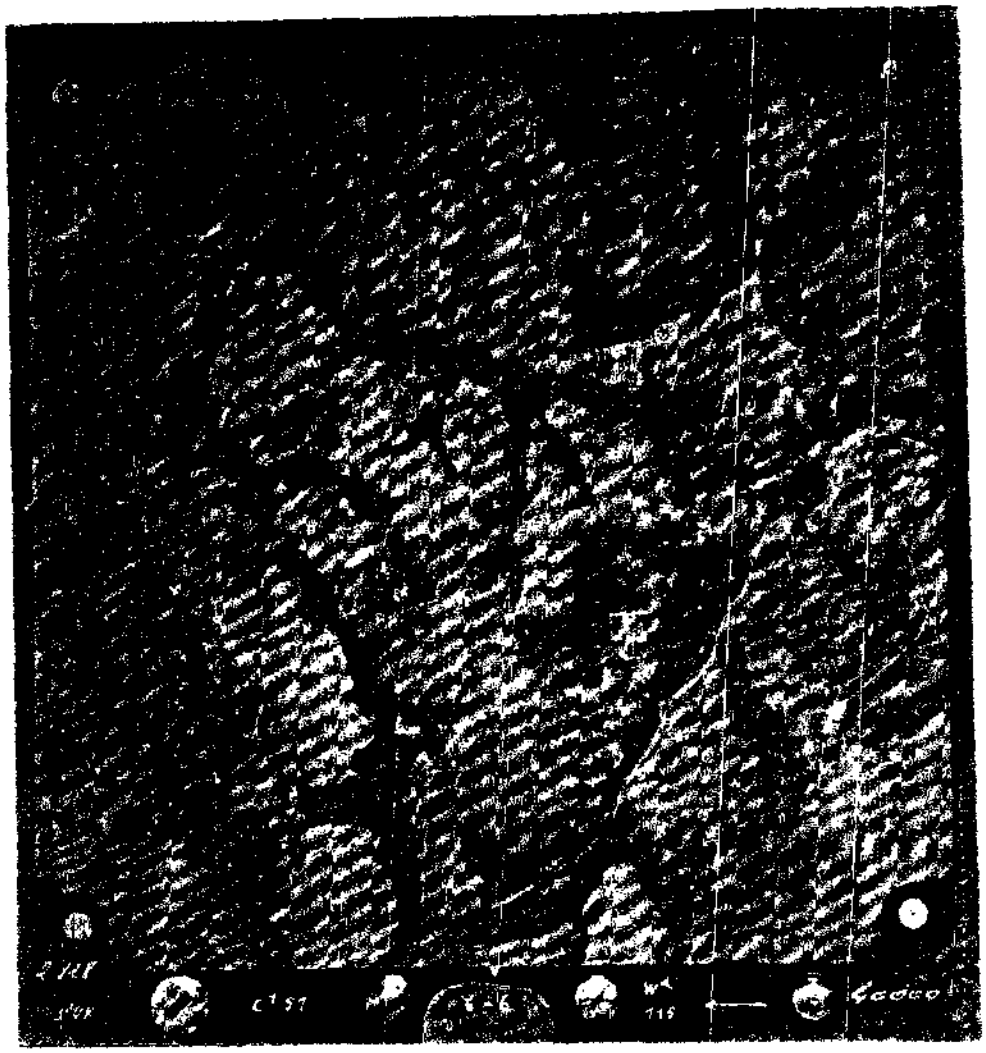

KATANGA
Lualaba Riv
LAKE TANGANYIKA
KAMENA
MARUNGU
KAMINA
SANDOA
KUNDELUNGU Mts
LAKE MWERU
ABERCORN
LAKE BANGWEULU
Luapula Riv
ELISABETHVILLE
Main roads
A dureni present
Strains isolated from rats
Dureni found positive
Strains from sporozoites
Plasmodium Vinckei
SCALE 0 100 200 300 m

PLATE XIII



Cross plateau at 1,5 a.u. meters altitude obstructed by important [illegible]

the tree holes situated 2·5 metres above the ground. But it cannot be described as a squirrel. It moves on the ground very frequently.

Praomys Jacksoni.—Is found in nests at the foot of the trees and bushes between the roots and also in certain thickets. It moves about essentially on the ground and unlike *Thamnomys* withdraws easily from forest covering. *Thamnomys* and *Praomys* are frugivorous.

Leggada belle.—It can live in the same conditions as the other rodents such as *Praomys* but equally in the habitats of the indigenous domestic mice (*Mus musculus*) to which group the *Leggada* must belong. It is omnivorous and can invade wild habitats such as forest galleries in certain cases.

The forest galleries contain other *Muridæ*, all susceptible, such as *Saccolomus campestris*, *Mastomys coucha*, *Praomys jacksoni*, *Æthomys* sp., *Pelomys frater*, *Lophyromys aquilus*, *Soricides* (*Crocidura turba*), some *Sciuridæ* and rarely small *Carnassiers*.

Some forest galleries are inhabited by various antilopes and monkeys (*Cynocephales*).

EPI OR ENZOOTOLOGY ASPECT.

Epi or enzootology aspect is interesting in the study of *P. berghei* malaria :

1. Generally only aged animals are captured : however, a good proportion among them have not attained sexual maturity, but of the least among these have been found infected unlike in the case of human malaria in which it is common to find a high rate of infection in the younger age group when the infection rate of the community is 25 to 60 per cent or more, particularly when the sporozoite index is very high which will be described later.

But this is contrary to every thing and it is one of the principal reasons for which five years have elapsed between the discovery of sprozoites in *A. dureni* and *P. berghei* (1943-48).

Among the positive animals captured in Nature, the parasites are never abundant, are often extracellular and consequently difficult to recognize with certainty. The positive number is very low. From 1947 to 1949, the blood of 358 rats, the majority of which were *Thamnomys* and *Praomys*, was subjected to a very careful examination. In two *Thamnomys* only, the presence of some blood parasites could be observed and the first strain of *P. berghei* was thus isolated.

Later in 1952, seven strains were obtained from 61 *Thamnomys* and five from 99 *Praomys*, from the blood of the captured animals, systematically inoculated into mice and white rats.

Howsoever fruitful these results are, in the positive animals a very feeble infection is encountered. These strains have been obtained in dry season as well as in rainy season.

2. *A. dureni* is probably the only vector of *P. berghei*.

A forest gallery represents in short the natural insectarium where the Anopheles has the greatest chances of longevity, a condition essential for a good vector.

But we find only three other dendrophile species :

A. implexus feeds on man but perhaps not exclusively. It has been found with sporozoites only once out of 739 examined.

A. concolor appeared to feed exclusively on antelopes. Its sporozoite index is very low. The inoculation of positive salivary glands in rats and mice have never given any results and the sporozoites are morphologically different from those of *P. berghei*.

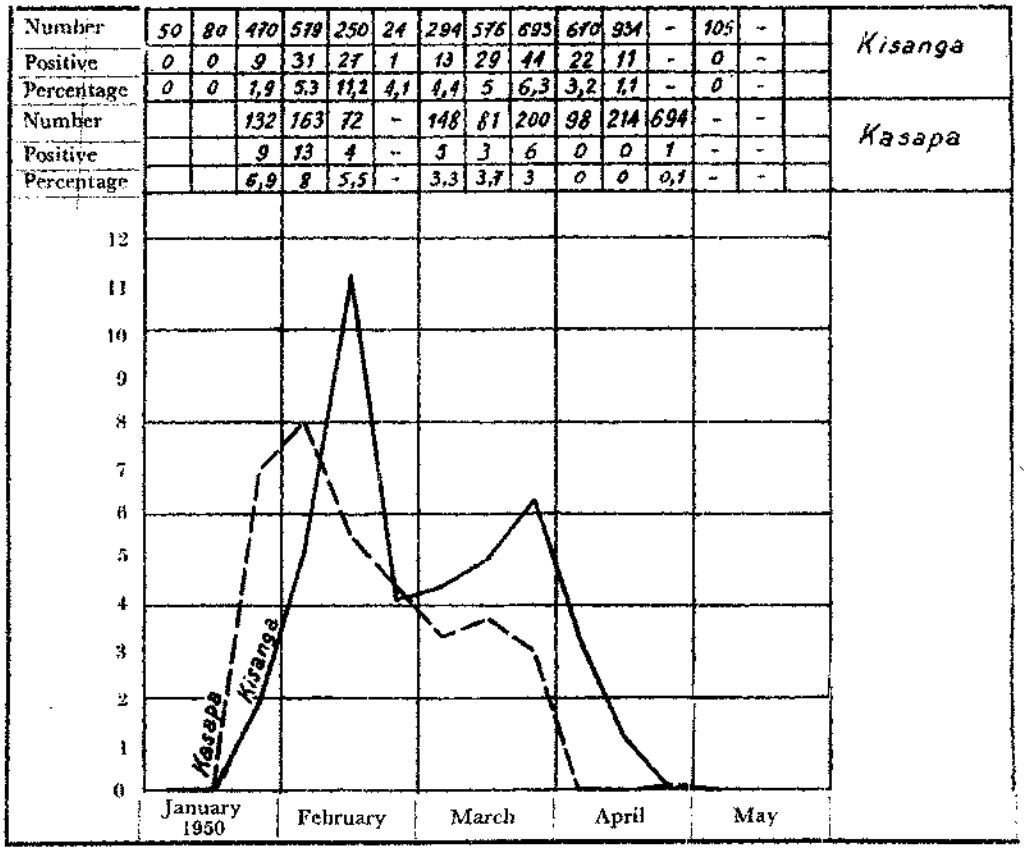
Then remains *A. berghei* which appeared to us very fragile and dissection of which has always been negative — here, however, more investigations are desirable.

6,380 specimens belonging to 15 other anopheline species have given four salivary infections and four gut infections. These anopheline came from different stations of capture from the environs of Elisabethville.

3. Geographical isolation of species in the various stations of capture is remarkable.

The different regions are separated by large valleys and also exhibit a different appearance. Also it could be relied upon to find for three different murine species of animals, several species of *Plasmodia* (in man there are only four species). But

GRAPH 1.



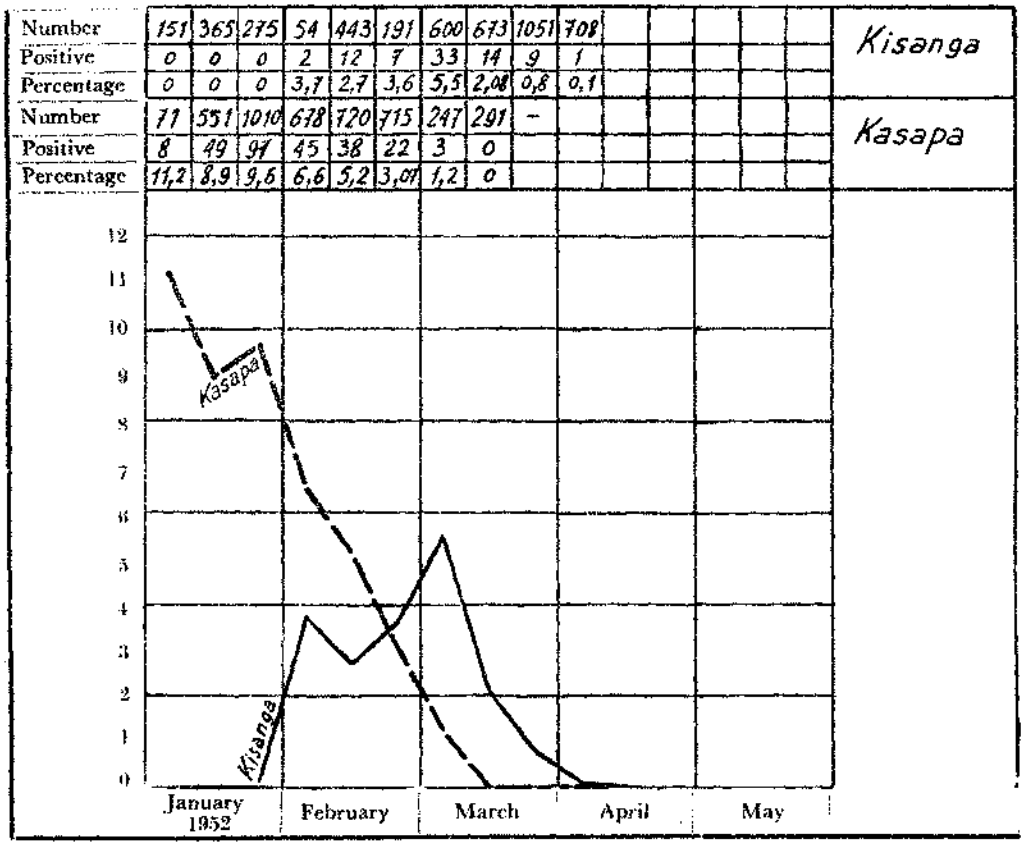
Sporozoite index of *A. dureni*.

till the end of the season of 1953, *P. berghei* has been isolated from 12 rodent strains of seven different stations, of which two are in Elisabethville.

From the sporozoites, 367 strains have to this day been isolated coming from four regions very far apart from each other. At least 297 of them have been obtained in individual mosquitoes. The possibility of a very frequent mixture of several species, less one supressing the other, can thus be excluded. Only in a single case a new parasite has been obtained (*P. vincke*, Rodhain).

4. We have said previously that the density of the Anopheles could vary from region to region. On this subject, one fact has struck us : from our station which had the largest number of observations in Kundelungu, several strains of parasite have been isolated from the blood of *Muridæ*. *A. dureni* have been found there rarely since 1949, to the point that we have suspected for some time *A. concolor* (very abundant in this region) to be the vector. Finally, after patient researches out of 40 *A. dureni* found during the entire season (1943-44) only one was found positive which gave us a strain of *P. berghei*.

GRAPH 2.

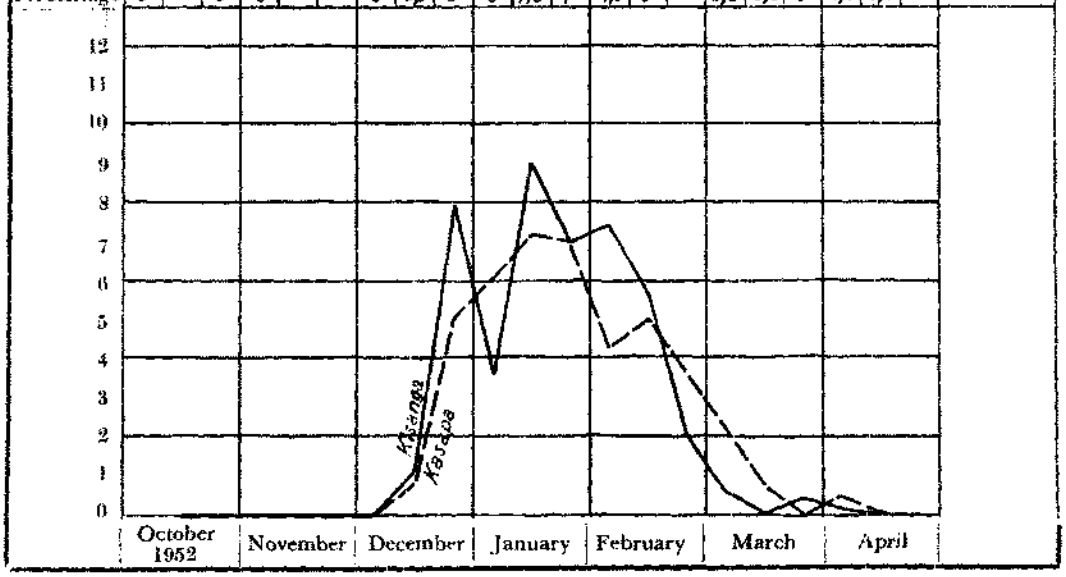


Sporozoite index of *A. dureni*.

5. Sufficiently complete data have been gathered on the subject of potential vector *A. dureni* from 1943 ; two stations were observed in the environs of Elisabethville, stations situated at a distance of 20 kilometers from each other : Kisanga and Kasapa. The cycle of malaria is practically interrupted in the mosquito from May to November as evidenced by the least negative results of 1,593 dissections practised during this period from 1950 up to May 1954. From December, the sporozoite index rises rapidly to reach generally its maximum in January or February and fall again soon thereafter. We have reproduced in Graphs 1, 2, 3 and 4, the four successive seasons for the two stations of capture—Kisanga and Kasapa.

GRAPH 3.

Number	-	105	259	-	-	-	-	606	805	308	1396	1024	442	525	1011	509	382	728	591	343	426	Kisanga
Positive	-	0	0	-	-	-	-	7	64	11	126	74	33	30	21	3	0	3	1	0	0	
Percentage	-	0	0	-	-	-	-	1,1	7,9	3,6	9	7	7,4	5,7	2	0,6	0	0,4	0,16	0	0	
Number	75	-	43	32	-	-	61	135	835	1670	941	1176	1034	1088	-	705	814	272	431	818	-	Kasapa
Positive	0	-	0	0	-	-	0	1	43	104	68	81	45	53	-	16	7	0	2	1	-	
Percentage	0	-	0	0	-	-	0	0,8	5	6	7,2	7	4,3	5	-	2,2	0,8	0	0,5	0,1	-	



Sporozoite index of *A. dureni.*

The data are not always complete. But the curves are always of the same pattern and show the following striking facts :

1. The duration of the season is always the same and the index exceeds three per cent only during a period of 60 days.

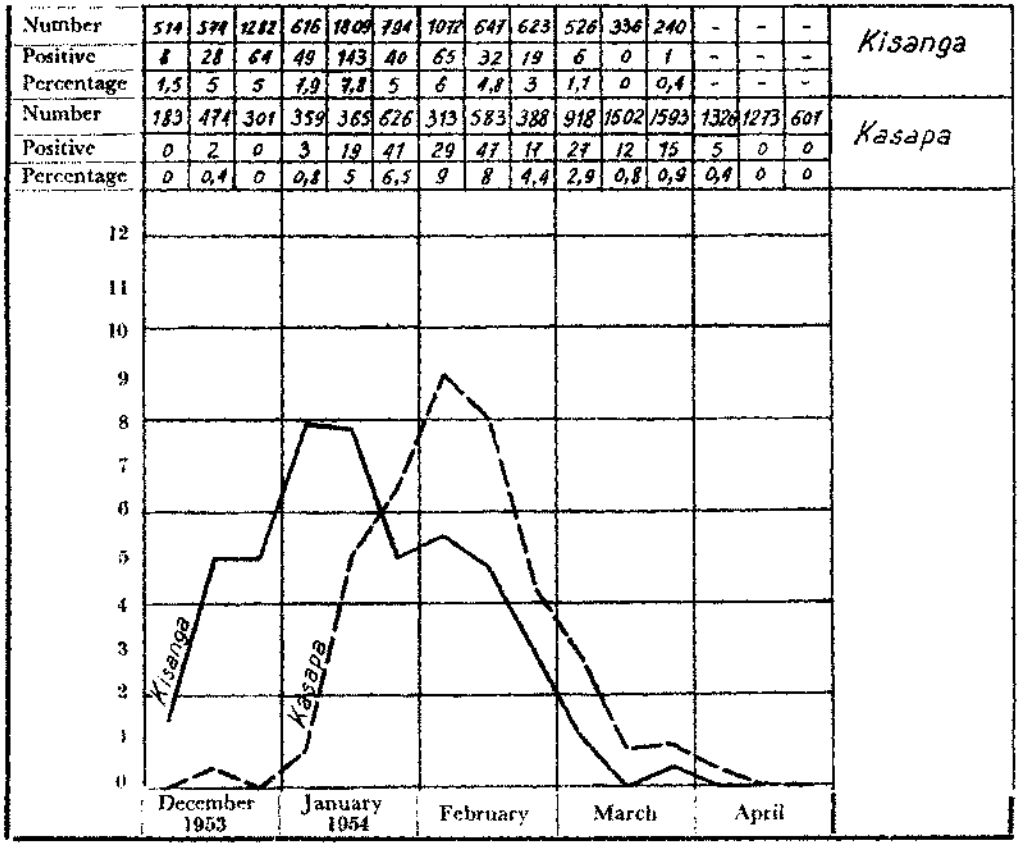
2. It can appear very soon in one station or the other, but the index falls down then also rapidly.

3. The sporozoite index falls down much sooner than the meteorological conditions would permit us to expect it. All this occurs as if an abrupt, brief and sudden epizootic breaks in each year, leaving behind it a small number of infected

animals. Even among individuals, not having still attained full maturity, we have never found an intense infection. It could be objected that a sick animal does not feel well and consequently will not be tempted by any bait—this would be contrary to clinical facts, for a *Thamnomys*, even a white mouse strongly infected with protozoa, will show apparent signs of lack of appetite only in the late stages.

This epizootic would occur explosively only among all young animals and it could equally be inferred from it that only the very young animals could be capable of infecting the invertabrate vectors. Perhaps this is the reason for which it has been so difficult up to the present to infect mosquitoes in the laboratory.

GRAPH 4.



Sporozoite index of *A. dureni*.

6. In the course of our observations, a very curious fact has been proved : gallery of Kisanga has been observed for more than ten years since 1943. Up to the end of May 1946, sporozoites were regularly found. The researches have been interrupted during the 1946-47 season and recommenced at the end of 1947. During the two seasons which followed, we have been able to see only one *A. dureni*

infected. This was out of 2,789 dissections. We then introduced at the end of 1949, in the gallery, a large number of rats inoculated with *P. berghei*, and on January 27, 1950, the sporozoites were found again.

What explanation is to be given to this phenomenon. The gallery has been partially destroyed in 1946 but the Anopheles remained nevertheless abundant. Soon we captured a certain number of rodents, in every manner the biological environment had been modified and this demonstrates once more how easily a biological equilibrium can be disturbed.

The most curious of the entire findings, and we admit to have passed through periods of extreme perplexity, is that *P. berghei* was discovered among the *Thamnomys* in the same Kisanga in January 1948, when the cycle of transmission was already interrupted among the mosquito. And it is through extraordinary luck that we have been able to capture one of the last positive rats.

REFERENCES.

BALDI, A. (1950) ... ... *Riv. di Malariologia*, **29**, p. 349.
Idem (1952) ... ... *Ibid.*, **31**, p. 41.
CHRISTOPHERS, S. R. (1911) ... ... *Transactions of the Committee for the study of malaria in India. Government of India Press, Calcutta.*
DRAPER, C. and WALL, W. (1951) ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **45**, p. 4.
DUREN, A. (1944) ... ... *Review App. Ent.*, **32**, p. 88.
EVANS, A. M. (1938) ... ... *Mosquitoes of the Ethiopian Region.*
FRIEZI, G. (1953) ... ... *Bull. World Hlth. Org.*, **9**, p. 335.
GALLIARD, H. and LAPIERRE, J. (1950) ... *Bull. Soc. Path. Expt.*, **43**, p. 410.
GARNHAM, P. C. C. (1951) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **45**, p. 2.
HENRARD, C., VAN WYMERSCH, H. and WANSON, M. (1944) ... ... *East African Med. J.*, **21**, p. 266.
RODHAIN, J. (1952) ... ... *Ann. Soc. Belge Med. Trop.*, **32**, p. 275.
Idem (1953) ... ... *Ann. Inst. Pasteur*, **84**, p. 572.
SERVICES D'ETUDES ET DE RECHERCHES ANTI MALARIENNES, ELISABETHVILLE (1954) ... *Unpublished reports.*
VAN DEN BERGHE, L., VINCKE, I. H. and CHARDOME, M. (1950) ... ... *Ann. Soc. Belge. Med. Trop.*, **30**, p. 79.
VAN DEN PLAS, A. (1947) ... ... *La temperature au Congo Belge Min. Col.* Dir. de l'Agriculture, Brussels.
VINCKE, I. H., PEETERS, E. and FRANCKIE, Gh. (1953-54) ... ... *Bull. Inst. Roy. Colonial Belge*, **24**, p. 1364.
VINCKE, I. H. (1946) ... ... *Ann. Soc. Belge Med. Trop.*, **26**, p. 161.
Idem (1946) ... ... *Ibid.*, **26**, p. 385.
Idem (1950) ... ... *Compte rendu Congres Scient.*, Elisabethville.
VINCKE, I. H. and LELEUM, N. (1949) ... *Revue Zool. Bot. Afric.*, **12**, Nos. 3 and 4.
VINCKE, I. H. and LIPS, M. (1948) ... *Ann. Soc. Belge Med. Trop.*, **28**, p. 97.

Indian Journal of Malariology, **8**, 4, December 1954.

EXPERIMENTAL TRANSMISSION OF *PLASMODIUM BERGHEI.**

BY

I. H. VINCKE.

(Director, S.E.R.A.M., Elizabetheville, Belgian Congo.)

(October 1, 1954.)

THIS section comprises :—

1. The attempts to infect mosquitoes from infected animals.
2. The cyclic transmission to various *Muridæ*. Observations on the subject of transmission in nature, appear in the section on Natural History.

I. ATTEMPTS TO INFECT MOSQUITOES IN THE LABORATORY.

Since the discovery of *Plasmodium berghei*, one of the problems which the research workers attempted to resolve was the transmission of the plasmodium to various species of mosquitoes. Besides the scientific importance of the problem, the practical importance attached to the study of the tissue phases had attracted the attention of a good number of laboratories.

The natural invertebrate host, *A. dureni*, was the first mosquito used for these investigations. Unfortunately, after some years of research, it has become necessary to abandon it ; the insect is too delicate to be raised in colonies and moreover, even if one succeeds in feeding these Anopheles captured in nature, they do not survive sufficiently long for developing oöcysts. Numerous authors have, therefore, turned towards the mosquitoes more easily handled, and according to our knowledge, the following, at the very least, is the enumeration of the species studied from this point of view :—

Ædes aegypti	*Culex bitæneorhynchus*
A. albimanus	*A. concolor*
A. annularis	*A. coustani* var. *ziemanni*
A. aztecus	*A. fluviatilis*

*Thanks are due to Mr. K. Gopalakrishnan, Assistant Director General, Posts and Telegraphs, Delhi, for translating the original paper written in French into English.—*Editor*.

A. funestus	*A. pulcherrimus*
A. gambiæ	*A. quadrimaculatus*
A. hyrcanus	*A. splendidus*
A. jamesi	*A. stephensi* (type and var. *mysorensis*)
A. maculipennis var. *atroparvus*	*A. subpictus*

Of all these attempts, only some have proved successful.

Yoeli and Wall (1951 : 1952*a* : 1952*b*) found that *A. stephensi*, *A. gambiæ*, *A. maculipennis* var. *atroparvus* and *A. quadrimaculatus* were infected easily when they were fed on hamsters presenting gametocytes. The oöcysts were regularly present and developed up to maturity, but the arrival of sporozoites in the salivary glands was very irregular (in certain cases 62 per cent out of 35 Anopheles showed sporozoites).

These authors succeeded in the cyclic transmission either by inoculating positive salivary glands or by making infected mosquitoes bite. The animals used were young white rats, four weeks old.

Rodhain and Vincke (1952) point out that by making *A. maculipennis* var. *atroparvus* bite splenectomised rats showing numerous gametocytes (males showing exflagellation), the results are negative (39 Anopheles dissected).

The author fed mosquitoes on infected *Thamnomys* and for about a hundred of *atroparvus* dissected at varying intervals of three days to one month after the infective feed, two showed some oöcysts after an interval of three to four days ; two after 18 days and three after a month. Thus oöcysts were very rarely observed which did not develop further.

At present the researches are being continued at the Institute of Tropical Medicine at Anver (Rodhain, Wanson and Vincke with the technical collaboration of Berteaux). Very young hamsters are used and some oöcysts are obtained regularly. In some cases, sporozoites develop but they are always too few and up to the present moment, we have not succeeded in infecting susceptible animals. The small number of sporozoites could be the reason but it is not impossible that these sporozoites may be degenerate. It is to be noted that in the experiment of Yoeli and Wall (1952*a* : 1952*b*), the Anopheles were kept at a temperature of 26 to 27°F., and in this at 23 to 24°F.

Ramakrishnan *et al.* (1953) tried to infect a large number of species of Anopheles. Two strains of parasites were used one of which had undergone 145 passages and the other 45 passages. Mice, albino rats, wild rats, squirrels and hamsters served the infecting meal.

All these led to the production of sporozoites in only one *A. stephensi*. The authors recognized, it is true, that in spite of the presence of gametocytes, they did not obtain exflagellations and concluded that the gametocytes were functionally deficient.

Perez-Reyes (1953) made some attempts with three species of Anopheles, namely, *A. quadrimaculatus*, *A. aztecus* and *A. albimanus*. *A. aztecus* was raised at 24° C. and 70 per cent humidity, the other Anopheles in the same humidity but at 28° C. The strain employed had already sustained weekly passages for one year.

The choice of the animal rested on the hamster, on which the mosquitoes were fed every evening from 7·00 to 9·00 hours during a period of seven days.

The mosquitoes were dissected between the 5th and 14th day after the infective feed. The results were better this time and high percentages of success were achieved. The *A. aztecus* appeared to be more susceptible than *A. quadrimaculatus* while the *A. albimanus* was not susceptible. It was necessary to feed the mosquitoes on these animals during the ascending period of infection, i.e., from the fifth to seventh day after inoculation. In *A. aztecus*, the oöcysts reached maturity towards the 11th day and the sporozoites were capable of infecting the white rat and the hamster.

How can the differences in results and the slowness of success achieved be explained ? It is astonishing at first sight that amongst so many species of mosquitoes tried, so few of the experimental vectors have been found, while in the other cases only one species of plasmodium can be transmitted by a sufficiently large number of mosquitoes. The parasites of man furnish the best example. There is, therefore, ground for believing that the obstacles to be overcome are found among mammals rather than among the mosquitoes.

These difficulties can be of very different nature :

(1) The animal species is important probably, since up to the present time only the hamster has given some results. *Thamnomys* and *Praomys*, the natural hosts, should be equally capable of serving these experiments.

(2) The age of the animals is of great importance since the observations in nature inform us that the plasmodium index of the captured animals is low and the parasites always rare. It is perhaps a question of only very young animals being infected since the period of positive sporozoite index does not exceed a few weeks. Unfortunately very young animals have not been captured up to the present moment and it is there that the lacuna has to be studied . Perez-Reyes (*loc. cit.*) does not inform, on the other hand, the age of the animals used.

(3) The state of advancement of the disease among the experimental animals at the time of infecting feed is specified in the experiments of Perez-Reyes (*loc. cit.*) who obtained the best results among the inoculated animals from the fifth to seventh day.

(4) *The number of previous passages.*—The strains used have been submitted to sufficiently numerous passages—in the experiment of Perez-Reyes (*loc. cit.*) is about 52 passages and his success astonishes us since, in general, after the 15th passage of *P. berghei*, it becomes extremely difficult to obtain exflagellation of male gametocytes. It is true that the last author indicates to have used the Mukata strain. There were several strains named after the name of indigenous sanitary guards who have assisted us. It remains to be known if the attempts of Perez-Reyes (*loc. cit.*) will be repeated regularly.

The question appears to us, therefore, far from being completely resolved and this leads us to discuss the question of maturation of gametocytes.

It has been observed that gametocytes become enfeebled gradually as the noncyclic passages increased and nothing permits us to say that even a single passage of this type would not compromise the results of attempts to infect mosquitoes. The production of gametocytes takes place besides, at indeterminate intervals of three to four days during the ascending period of infection (Rama-

krishnan *et al.*, 1953). We have observed the same thing, but have been struck by the fact that the phenomenon was irregular. This was the case even among some animals (white rats, white mice, *Cricetomys*, etc.) inoculated directly with sporozoites.

We have, therefore, chosen very young white rats 15 to 25 days old, as well as some *Cricetomys* of undetermined age to inoculate directly with sporozoites. *Anopheles maculipennis* var. *atroparvus* in large numbers were fed on these animals during the ascending period of the infection. The results were negative.

At the same time, what significance is to be attached to the presence in abundance of exflaggalation of male gametocytes ? Is the latter a necessary and sufficient condition for formation of zygotes and of subsequent growth? And how to know if the female gametocytes are or not functionally deficient ? Finally even the production of zygotes do not appear to indicate necessarily that the rats which are used, fulfil all the required conditions.

Foy and Kondi (1952) have demonstrated that the gametocytes of *P. falciparum* of a patient treated with daraprim do not develop up to the sporozoite stage in the mosquito fed on his blood.

McKennas and Erede (1947) and Shute and Maryon (1948) have proved that proguanil affects the female gametocyte in the sense that the oöcysts do not reach maturity. This finding agreed moreover with the experiments of Vincke (1952) which have established that the administration of daraprim to the indigenous people leads to the negativity of sporozoite index in *A. funestus*.

For *P. berghei*, it is therefore perfectly possible that the regular formation of oöcysts proved by Yoeli and Wall (1952*a* : 1952*b*) followed by formation of sporozoites, is explained in the same manner. And one is led to wonder if there cannot exist deficiency of certain necessary factors in mammals under certain circumstances for the production of gametocytes for their maturation and for the formation of oöcysts and also for the ultimate maturation of these oöcysts.

In this order of ideas, Thurston (1950*a* : 1950*b*) had established that in some adequate conditions, the antimalarial action of proguanil and of pyrimethamine could be neutralized (antogonised) by PABA. It is this reason for which, in the course of some of our present attempts, we have fed, as a preliminary, to *Cricetomys* some of PABA and also folic acid—unfortunately no positive results were obtained.

The question of infecting mosquitoes and that of creation of conditions favourable or unfavourable to the development of cyclical infection of *P. berghei* is certainly not still achieved. More profound researches appear to us indispensable.

II. TRANSMISSION TO MAMMALS.

(*a*) *Non-cyclic transmission.*—We shall state here only briefly observations of a general nature. The non-cyclic transmission is in general easy. Some drops of blood of the tail are drawn into a watch glass containing an anticoagualent (citrate and/or heparin) and inoculated to a susceptible animal.

1. All routes of injection appeared good. But the peritoneal route is the most convenient for the best results. The intradermal and intrapulmonory routes are dependable. The subcutaneous is practicable only after preliminary scarification.

2. Failure to infect certain specimens of a susceptible animal species, does not necessarily signify a refractory state. Often a second or a third inoculation or more ends in success.

3. The best results are obtained when the donor is selected at the commencement of the infection (prepatent periods are shorter and consistent).

(*b*) *Cyclic transmission.*—This has been obtained in rare cases when the mosquito had been infected in the laboratory, thus demonstrating that the sporozoites had really reached maturity. Experiments with greater success have been made from the salivary glands of *A. dureni* captured in nature. These *Anopheles* came from several stations at Katanga. The mosquitoes are dissected in groups of 10 to 20, and the glands examined by a team of workers. The sporozoites are infected either gradually as they are found or together at the end of dissection of the batch which never exceeds two hours. We have employed successfully physiological heparinised saline, the plasma of heparinised rats and of the red cell extract of rats. Finally our choice stopped with a one per cent or two per cent bovalbumin fraction V in physiological saline. It is unwise to use pencillin or centrifuge lots of salivary glands. Results have been obtained for the following animals : white rats, white mice, *Thamnomys surdaster*, *Cricetomys ansorgei* and *Praomys jacksoni*. The inoculation route has been intraperitoneal.

There is certainly a relation between the number of glands utilized and the results obtained as seen in the following sample experiments :—

When the white rat is used : one to five glands obtained in 46 dissections gave rise to 21 positive; and five glands and more obtained in 21 dissections to 20 positive infections. The rats were not strictly of the same age, but in general from six weeks to two months old. Since the beginning of the experiments dating from 1950, we have been struck by the fact that it was particularly difficult to obtain successful infections in white mice with certain strains of infection in the salivary glands. However, it remained a doubt since the experiments were not numerous. For reasons of non-availability, we have not been able to utilize either animals of the same age or a uniform number of glands.

During the 1953-54 session, we have been able to inoculate a certain number of animals soon after the examination of dissection of a single positive mosquito. It goes without saying that even in this case, the number of sporozoites can still be very variable, but even if a method of enumeration can be accurate, the delay in operations will be still more risky.

Be it, as it may, the following table gives a resume of the results obtained :—

Age groups.	10 to 30 days.			30 to 60 days.			Adults or indeterminate.		
	Total.	Positive.	Percentage.	Total.	Positive.	Percentage.	Total.	Positive.	Percentage.
Mice ...	80	14	17·5	5	0	...	86	8	9·3
White rats	124	93	75·0	74	35	47·3	10	8	...

In addition to the above, for twelve white rats of indeterminate age we registered five successes, which makes the total to 22 positive over 171, that is, 12·8 per cent for the mice and 141/220 for the rats, i.e., 64 per cent.

The age of the animals appeared to exercise a certain influence equally :

(*a*) *Young Cricetomys of indeterminate age.*—Twenty-one *Cricetomys* have been inoculated, of which only four became positive ; the number of glands inoculated at one time varied from 1 to 14, with a total of 35, and an average of 8·7.

(*b*) *Adult* Cricetomys *without determination of exact age.*—Here we obtained one success out of nine animals. The only positive animal received eight glands and the eight others 51 glands in all, thus giving an average of 6·3 glands each. All the inoculations have been made by the intraperitoneal route soon after the examination of the positive glands. Among these animals, five young and one adult have been utilized twice. Here also it is usual that smaller numbers of *Cricetomys* contract malaria after inoculation of sporozoites than with inoculation of blood.

A cyclic transmission has permitted to demonstrate that a pre-erythrocytic phase exists in the white rat. In six cases, the blood was drawn every day from the day of inoculation of sporozoites and inoculated to young white mice. A rat gave infective blood at the end of 48 hours and five at the end of 72 hours. Two rats were sacrificed at the end of 72 hours and mice inoculated with their blood co ntracted the infection, whilst others sacrificed at the end of 48 hours, gave negative results on subinoculation of blood. In the last four cases, the inoculations were massive. The pre-erythrocytic phase would thus be of three days' duration in general.

Apparently the evolution of the disease is the same after inoculation of sporozoites as after inoculation of blood.

REFERENCES.

Foy, H. and Kondi, A. (1952) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 370.
Hawking, F. (1954) ... ... *Brit. Med. J.*, **i**, p. 425.
McKennas, M. I. and Erede, Q. N. (1947) *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, p. 365.
Perez-Reyes, R. (1953) ... ... *J. Parasit.*, **39**, p. 603.
Ramakrishnan, S. P., Satya Prakash, Krishnaswami, A. K. and Mohan, B. N. (1953) ... ... *Ind. J. Mal.*, **7**, p. 67.
Roddain, J. and Vincke, I. H. (1951) ... *Ann. Soc. Belge Med. Trop.*, **31**, p. 297.
Idem (1952) ... ... *Ibid.*, **32**, p. 165.
Shute, P. G. and Maryon, M. (1948) ... *Parasit.*, **38**, p. 264.
Thurston, J. P. (1950*a*) ... ... *Brit. J. Pharmacol.*, **5**, p. 409.
Idem (1950*b*) ... ... *Lancet*, **2**, p. 438.
Vincke, I. H. (1952) ... ... *Ann. Soc. Belge Med. Trop.*, **32**, p. 91.
Vincke, I. H. and Lips, M. (1950) ... *Ibid.*, **30**, p. 1005.
Vincke, I. H. and Peeters, E. (1953) ... *Ibid.*, **33**, p. 87.
Yoeli, M. and Wall, J. (1951) ... *Nature*, **168**, p. 1078.
Idem (1952*a*) ... ... *Ibid.*, **169**, p. 881.
Idem (1952*b*) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 374.

Indian Journal of Malariology, **8**, 4, December 1954.

THE MOSQUITO TRANSMISSION OF *PLASMODIUM BERGHEI*.

BY

R. S. BRAY, Ph.D.

(*Lecturer in Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.* 1.)

(October 8, 1954.)

It is a revealing commentary on the experimental cyclical transmission of *P. berghei* that the most useful contribution that I can make to this symposium is to record two and a half years of failure to establish the parasite satisfactorily in mosquitoes. The experimental details which follow represent the whole of the work on this subject carried out in the London School of Hygiene and Tropical Medicine over the last few years. Transmission experiments have proceeded intermittently in both the Ross Institute and the Department of Parasitology since the success obtained by Yoeli and Wall (1951 : 1952) in this School in 1951. I am indebted to both Professor P. C. C. Garnham and Miss W. J. Wall for permission to include their results in this contribution.

Our chief concern has been to obtain sufficient sporozoites to proceed with an investigation into the nature of the pre-erythrocytic stages of *P. berghei*. Such an enquiry demands at least one hundred sets of salivary glands heavily infected with sporozoites. We have attained one set of salivary glands heavily infected with sporozoites. This is the true measure of our failure and will give some idea of our frustration.

MATERIALS AND METHODS.

The strains of *P. berghei* used in our investigation have been the S.P. 28 S. Keiberg, " K " (so named in the Ross Institute), " R. S. V. P." (so named in this Department), S.P. 228 Kisanga, S.P. 229 Kisanga, S. P. 235 Kisanga, S.P. 236 Kisanga, S.P. 260 Kisanga, S.P. 261 Kisanga, S.P. 263 Kisanga, S.P. 270 Kisanga, S.P. 271 Kasapa, S.P. 171, S. P. 18, " IV ", " V ". None of these strains was used at less than two removes from the original sporozoite infection. The mosquitoes used in our investigations have been *Anopheles*

maculipennis var. *atroparvus*, *A. quadrimaculatus*, *A. stephensi*, *A. gambiæ*, a species thought at one time to be *A. aztecus* but which is more probably either *A. maculipennis* or a hybrid of *A. aztecus* and *A. maculipennis*, and *Ædes ægypti*.

The vertebrate hosts used in our investigations have been the white mouse, the white, brindled, and hooded rat, the golden hamster and the rabbit.

The following has been our routine mode of infecting mosquitoes. The strain is tested for microgamete formation. The host animal is anæsthetized by ether, nembutal or hexabarbitone and strapped out on a board. The abdomen is shaved and the animal introduced into the cage containing mosquitoes. Subsequently the fed mosquitoes are transferred either to a new cage or individually into tubes depending on the species. The mosquitoes are maintained on blood, sugar or fruit.

The day of choice for feeding is normally the fifth and sixth days after inoculation of one or more millions of parasites. Little account is taken of the number, absolute or relative, of gametocytes in the peripheral blood.

Only one major variation of this technique has been used. On several occasions, p-aminobenzoic acid was inoculated into or fed to the vertebrate and/or fed to the invertebrate host in amounts never exceeding 500 mg./kg. body weight or a 0.1 per cent solution. When partial success was obtained, attempts at transmission were made by inoculating suspensions of guts and/or glands or by biting.

In all, some 200 vertebrate hosts and some 5,000 mosquitoes have been used in these experiments which are set out in the accompanying table.

RESULTS.

One mosquito (*A. quadrimaculatus*) was found to have a heavy infection in the salivary glands. All batches of Anopheles infected with the S.P. 28 S. Keiberg strain from hamsters showed a few (> 20) sporozoites in the glands of some of the mosquitoes. The R.S.V.P. strain was established once as a scanty infection in the glands of *A. stephensi*.

Oöcysts were seen frequently particularly with the S.P. 28 S. Keiberg, S.P. 171, S.P. 18, IV and V strains. Mature oöcysts containing sporozoites were usual with these strains. All other strains with the exception of S.P. 236 and S.P. 270 were established in Anopheles at one time or another.

The best results were obtained when *A. stephensi* or *A. quadrimaculatus* were fed on hamsters five or six days after infection with 5 to 50×10^6 parasites. *A. maculipennis* and *Ædes ægypti* were shown to be unsuitable hosts for *P. berghei*. The mouse and the rabbit proved to be of little or no use as hosts in this specialized capacity. The only strains used within five removes from the sporozoite infection S.P. 228, 235, 236, 260, 263, 270) proved to be the least successful in these experiments. Maintenance of the mosquitoes on blood, sugar or fruit resulted in no significant differences in the state or extent of infection. No useful results were obtained by the PABA supplements. It can be said in general that while the extent of infection in mosquitoes was frequently most satisfactory, the state of infection was equally frequently poor. Oöcysts were often small and retarded. Degeneration and lack of synchronous development were usual. The percentage

Table I.

Results with the different strains of P. berghei.

Strain of *P. berghei*.	Vertebrate host.	Invertebrate host.	Feeding time in days after infection.	Special techniques.	Oöcysts, (days after feeding in parentheses).	Sporozoites in oöcysts.	Sporozoites in glands.	Comments.
S.P. 28 S. Keiberg	Rat	*A. maculipennis*	5, 6	Rat inoculated with heparin.	(5, 7–9) +	+	—	Crushed guts inoculated. No infection.
	Rat	*A. stephensi*	5–9	—	(6, 8) +	+	—	—
	Rat	*A. quadrimaculatus*	5–8	—	(3, 5, 7) +	+	—	—
	Mouse	*A. maculipennis*	4, 5	—	(5) +	—	—	Oöcysts small and undeveloped.
	Hamster	*A. maculipennis*	3–10	—	(3–12) +	+	Very scanty	Heavy gut infection. Crushed guts inoculated. No infection. Black spores of Ross present.
	Hamster	*A. stephensi*	3–10, 15	—	(3–12) +	+	Very scanty	,, ,, ,,
	Hamster	*A. quadrimaculatus*	4–8	—	(3–15) +	+	+(one case)	,, ,, ,,
	Hamster	*A. gambiæ*	4–7	—	(4–8) +	+	Very scanty	,, ,, ,,
	Hamster	*Ædes egypti*	6, 22	—	(3–8) +	—	—	All oöcysts small.
"K" A. Kasapa strain	Rat	A. *maculipennis*	5–8		(5–1) +	—	—	Strain considered unsatisfactory.
	Rat	*A. stephensi*	5–9	—	(3–6) +	—	—	,, ,, ,,
	Rat	*A. quadrimaculatus*	5–7	—	(3–5) +	—	—	,, ,, ,,
	Hamster	*A. maculipennis*	4–10	—	(3–6) +	—	—	,, ,, ,,
	Hamster	*A. stephensi*	4–10	—	(3–7) +	—	—	,, ,, ,,
	Hamster	*A. quadrimaculatus*	4–10	—	(3–6) +	—	—	,, ,, ,,
	Hamster	*A. gambiæ*	5–7	—	—	—	—	,, ,, ,,

TABLE I (*Continued*).

Strain of *P. berghei*.	Vertebrate host.	Invertebrate host.	Feeding time in days after infection.	Special techniques.	Oöcysts, (days after feeding in parentheses).	Sporozoites in oöcysts.	Sporozoites in glands.	Comments.
"R.S.V.P."	Mouse	*A. maculipennis*	5, 6	—	—	—	—	—
	Mouse	*A. stephensi*	5, 6	—	—	—	—	—
	Rabbit	*A. maculipennis*	2 mins., 2	—	—	—	—	—
	Rat	*A. maculipennis*	4–6	—	(3–5) +	—	—	—
	Rat	*A. quadrimaculatus*	5–8	—	(5, 8) +	+	—	Oöcysts small. Crushed guts inoculated. No infection.
	Hamster	*A. stephensi*	4–14	—	(1–1) +	+	Very scanty	Crushed guts and glands inoculated. No infection. Black spores of Ross present.
	Hamster	*A. quadrimaculatus*	4–7	—	(6, 8) +	—	—	—
	Hamster	*A. gambiæ*	4–10	—	(5–11) +	+	—	Black spores of Ross present.
S.P. 263, 228, 235, 280	Hamster	*A. stephensi*	5, 6	PABA added to mosquito diet.	(7) +	—	—	One oöcyst only. All strains used simultaneously in one animal.
	Hamster	*A. quadrimaculatus*	5, 6	,,	(7) +	—	—	,, ,, ,,
	Rat	*A. stephensi*	5, 6	—	—	—	—	—
S.P. 228	Rat	*A. maculipennis*	5, 6	PABA inoculated into rats and fed to mosquitoes	—	—	—	—
	Rat	*A. quadrimaculatus*	4–6	—	—	—	—	—
	Rat	*A. aztecus* (?)	4–6	—	—	—	—	—

TABLE I (*Concluded*).

Strain of *P. berghei*.	Vertebrate host.	Invertebrate host.	Feeding time in days after infection.	Special techniques.	Oöcysts, (days after feeding in parentheses).	Sporozoites in oöcysts.	Sporozoites in glands.	Comments.
S.P. 236 and 270	Rat	*A. stephensi*	5, 6	PABA inoculated into rats	—	—	—	
S.P. 171	Hamster	*A. stephensi*	6, 7	—	(8) +	+	—	Very heavy gut infections.
S.P. 18	Hamster	*A. stephensi*	3, 5–7	—	(7, 8) +	+	—	" " "
IV	Hamster	*A. stephensi*	4, 5, 10	—	(7, 8, 15) +	+	—	" Oöcysts small and retarded at 15 days.
V	Hamster	*A. stephensi*	3	—	(3, 4–7) +	+	—	"

of oöcysts showing the degenerative changes leading up to the formation of black spores of Ross as described by Garnham (1955) was disproportionately high. Many mature oöcysts were seen not to have burst, and the contained sporozoites were undergoing degeneration. At no time has it been possible to transmit the infection from mosquitoes to a new vertebrate host since the success obtained by Yoeli and Wall (1952) in these laboratories.

DISCUSSION.

The problem of the mosquito transmission of *P. berghei* or indeed any *Plasmodium*, presents many considerations, any one of which may be decisive in the final issue. It is useful to enumerate these aspects of the problem of *P. berghei* transmission. Under the general heading of the parasite, the following must be taken into account :—

1. The strain or line of parasite.
2. The state of the gametocytes.
3. The state and extent of the general infection in the vertebrate host.
4. The state and behaviour of the parasite in the invertebrate host.
5. The nutritional requirements of the sporogonic stages of the parasite.

Under the general heading of hosts one must consider :—

1. The choice of vertebrate host.
2. The choice of invertebrate host.
3. The reactions, passive or active, of the invertebrate host to the presence of the parasite.

It is obvious from a study of the accompanying table (Table I) that the strain of *P. berghei* used is important. It is not obvious however, what the ideal criteria are. It can usually be assumed that the fewer the number of blood passages through laboratory animals the better chance the *Plasmodium* has of establishment in an alien mosquito. Vincke, Peeters and Frankie (1953) go so far as to state that it is of importance to use only sporozoite-induced infections of *P. berghei*, a condition unfortunately impossible in laboratories remote from the gallery forests of the Katanga. Yet is this the case? In our series the least successful strains were the most freshly isolated as sporozoite-induced infections from wild caught *A. dureni*. Equally the strain used in the most successful attempts at cyclical transmission to date, those of Perez-Reyes (1953), had been passaged a number of times through rats. If, however, the admonition of Dr. Vincke and his colleagues is well-founded it gives proof of what an unamenable parasite *P. berghei* is. As far as I have been able to discover, there exists only one strain of *P. gallinaceum* in the laboratories of the world. All lines stem from the strain isolated by the late Professor Brumpt in 1935. Despite this, and despite the innumerable transfers, the haphazard modes of transmission and the lengthy series of blood passages, *P. gallinaceum* remains as easily transmitted cyclically by a wide variety of anopheline and culicine mosquitoes as it was on the day it was isolated.

The whole question of the strains of *P. berghei* requires more careful examination than it has received in the past. In the first place it is dubious if it is correct to refer to the numerous infections of *P. berghei* maintained in various laboratories as " strains ". The confined geographical and biological distribution of *P. berghei* makes it likely that many of the lines isolated derive from one parent strain. For instance, five infections in five rats resulting from the bites of five *A. dureni* trapped at the same time and in the same place will not as a rule represent five genetically differing strains. They will represent five homogenetic lines. There is even the distinct possibility that all lines of this parasite derive from the one parent strain. On the other hand, it is true to say that *P. berghei* is a relatively unstable organism. A strain of *P. berghei* maintained in one laboratory behaves differently from another strain maintained under only slightly differing circumstances in another laboratory. Two lines in the same laboratory though derived from one infection but later passed in different host species, will display variations in virulence, rate of multiplication and production of gametocytes when subsequently compared in the same host species. Such variations were well illustrated in a recent correspondence in the *Transactions of the Royal Society of Tropical Medicine and Hygiene* (Galliard, 1954 ; Hawking, 1954*a* ; Maegraith, 1954). There is ample evidence for this instability in the behaviour of the gametocytes which will be discussed later. Although some of this behaviour may be due to the host (Greenburg, Nadel and Coatney, 1954), many variations are undeniably parasite phenomena.

This situation leads the biologist to a consideration of the phylogenetics of *P. berghei*. This parasite fulfils many of the characteristics of a " rare species " as discussed by Huxley (1942). It is geographically and biologically highly localized and can have little opportunity for progressive adaptive change and no great evolutionary plasticity. Under such conditions the parasite will be expected to indulge in a series of random and frequently regressive or at least useless changes in genetic character (the so-called Sewall-Wright effect) which is likely to lead it to become even more reliant on a specific environment. If this view is accepted, it is not surprising that *P. berghei* should grow successfully only in *A. dureni* and the completely unrelated *A. aztecus*. At the same time, it would not be unreasonable to assume that the genetic character would be more labile than that of a parasite more in accord with a wider range of environmental stresses but only in the sense of possessing neutral genes which under different conditions of increased growth will produce chance characteristics. It must be remembered though, that such characteristics would be unlikely to prove biologically useful to the parasite, useful as they may be to the malariologist. The parasite then would be expected to react quickly to alien hosts and diets. Further the parasite could be expected to throw up random variations of itself, the " variations which seem to us in our ignorance to arise spontaneously " (Darwin, 1859), which if isolated will develop into sub-species, or, among the protozoa, even into species. Such a phenomenon probably has occurred in this case and given us *P. vinckei*.

In conclusion it may be said that such a closed vector-host system as prevails in the case of *P. berghei* will prove difficult of access from a laboratory point of view. Attempts to break into this system will frequently provide inconclusive and confusing data. Previous experience will prove as often useless as not, and chance will play the major rôle.

This depressing conclusion reinforces my growing belief that useful as *P. berghei* may appear as a tool in the hands of the malariologist, the biochemist and the physiologist, it is to the biologist a *cul-de-sac* and as such should not be accorded a falsely important position among the malaria parasites. This view may also be taken as a warning that much information collected about this parasite will prove misleading if applied generally in mammalian malaria.

It now becomes necessary to study this parasite from the point of view of gametocytogenesis. This has been done with considerable thoroughness by Refaat (1954) and provides an example of a neutral gene character becoming prominent owing to an increased rate of growth in a new homogenous environment. A brief resumé of Refaat's findings can be made as follows :—

1. The absolute numbers of gametocytes reached a peak between 14 and 16 days after intraperitoneal inoculation of one million parasites into three months old rats.

2. Gametocytogenesis failed completely in the S.P. 28 S. Keiberg strain after thirteen serial blood passages through newly weaned rats and in the R.S.V.P. strain after seven serial blood passages through white mice. Microgamete formation proceeded normally up to the disappearance of the microgametocytes. These results were shown to hold true on several occasions.

3. Gametocytogenesis remained normal for two years when the R.S.V.P. strain was blood passaged alternately through rats and mice. It also remained normal after four months of latency in adult rats.

4. The power of gametocytogenesis, once lost, could not be regained by
   (*a*) passage of the line through refractory hosts, whole or splenectomised,
   (*b*) allowing the line to remain latent in normal hosts,
   (*c*) various alterations in the hosts' diet, including additions of *p*-aminobenzoic acid, pteroylglutamic acid or Vitamin B complex,
   (*d*) subsequent alternate passages through rats and mice.

5. A gametocyteless line has the power of causing a normal line to lose the faculty of gametocytogenesis after only one week, when the two lines are present in the same host. This was shown not to be a simple overgrowth phenomenon but a definite loss of gametocytogenesis on the part of the normal line. (These two lines were derived from two different so-called strains of *P. berghei* originally isolated three years apart). A gametocyteless strain of *P. vinckei* did not suppress gametocytogenesis in a normal strain of *P. berghei*. The effect could only be obtained by using the living parasite. I have had the opportunity of confirming the results listed 1, 2 and 3 above with the S.P. 172, S P. 208 and S.P. 235 strains. Similar results have been noted by Vincke *et al* (1953). On the other hand, apparently not all strains behave similarly. The S.P. 27 S. Keiberg strain retained its power of gametocytogenesis after numerous blood transfers through hamsters. The Mukata strain used by Perez-Reyes (1953) infected *A. aztecus* despite an unspecified number of blood transfers through rats.

Two somewhat surprising conclusions are to be drawn from this work. Firstly, gametocytogenesis in *P. berghei* is of a labile character, while the loss of the power of gametocytogenesis is an apparently fixed character. Secondly, the factor

causing the loss of gametocytogenesis is transferable. While the former conclusion merely reinforces my belief that *P. berghei* is genetically unstable, the second conclusion is an exciting one. The only analogy seems to be the ability of the desoxyribonucleic acid from a drug-fast strain of bacteria to confer drug-resistance upon a normal strain when it is introduced into the substrate of the normal strain. In the case under consideration we have the transfer of a quality apparently more permanent than drug-resistance under much more complex and fleeting circumstances. It would seem that the newly dominant gene causing the loss of gametocytogenesis in one line can cause the similar but still neutral gene in the other line to become quickly dominant. Since there can be no hybridization of the two lines it is difficult to understand how this influence is brought to bear.

An interesting speculation which is suggested by this instability in the sexual behaviour of a *Plasmodium* is the support it may give to one or other of the two conflicting theories concerning the phylogenetics of the genus. Does such an instability tell against the theory of insect host origin of plasmodia and support the theory that the plasmodia represent the last stage of an evolutionary shift within the Coccidiida and away from the sexually stable Eimeriidæ ?

While on the subject of the gametocytes of *P. berghei* some comment should be made concerning their morphology. A healthy gametocyte of *P. berghei* always displays easily visible pigment scattered over its entire cytoplasm. If pigment is not easily visible then the parasite is not a gametocyte. If the pigment is clumped and yet the parasite is uninucleate and fills the host cell then it is a degenerate gametocyte. Sometimes in mice a large asexual trophozoite will be seen in a host red cell with punctate basophilia and this can be misleading. Ramakrishnan, Satya Prakash, Krishnaswami and Mohan (1953) posed the question of what constitutes a mature gametocyte. They answer the question by stating that the generally accepted criterion is a scattering of the pigment throughout the cytoplasm of the parasite (Gambrell, 1937). Under no circumstances should this definition which was intended for avian malaria parasites be accepted with reference to mammalian malaria parasites. The only possible criterion which can be accepted for the mammalian malaria parasites is that the macrogametocyte should entirely fill the host red cell and that the microgametocyte should all but fill the host red cell. I am conscious that many illustrators show what purport to be mature gametocytes, particularly of *P. malariæ* and *P. ovale* only one half or three quarters filling their host cells. This is completely misguided. I have seen many gametocytes of *P. ovale* with well scattered pigment incompletely filling the red cell. These gametocytes were immature and unable to infect Anopheles. This remains true of *P. berghei* and no other criteria are acceptable. Ramakrishnan *et al.* (1953) have rendered a most useful account of their experiences and their discussion is most valuable. Their experiences with the gametocytes of *P. berghei*, however, require some comment. It should be said at the outset that it is doubtful if their Strain I was at the time producing gametocytes. The mode of maintaining the strain was such as to cause the disappearance of gametocytes. The illustrations show several forms which I should not have diagnosed as gametocytes of *P. berghei*. Some gametocytes were said to be in polychromatophilic erythrocytes, a circumstance which I, in agreement with Corradetti and Verolini (1951), do not believe occurs. It is also significant, I believe, that these authors noted that after ten serial

passages through rats, Strain II took on the appearances of Strain I, but after one passage through a hamster, Strain II regained its normal appearance. This roughly accords with the experiences of Refaat (1954).

The next consideration is the state of the infection within the vertebrate host. Numerous experiments have shown that the fifth and sixth days after inoculation of more than one million parasites are the ideal times for infecting mosquitoes. This has been confirmed fully by the results of Perez-Reyes (1953). This forces the conclusion that the number of gametocytes present in the peripheral blood has little bearing on the problem as they do not reach a maximum until the fourteenth day. These experiences are similar to those of Lumsden and Bertram (1940) who found that the gametocytes of *P. gallinaceum* are most infective to *Ædes ægypti* prior to the time of maximum gametocyte production. It appears that the same mechanism may be at work with *P. knowlesi* infections in rhesus monkeys. Recent research with *P. falciparum* (Young, Hardman, Burgess, Frohne and Sabrosky, 1948 ; Muirhed-Thompson, 1954) has shown that sheer numbers of gametocytes are not the deciding factor in the infection of mosquitoes. It seems probable that the decisive factor is the amount of nutrient material, chiefly hæmoglobin that is available to the growing gametocyte. In heavy infections with such active parasites as *P. berghei*, *P. gallinaceum* and *P. knowlesi*, there is a great loss of hæmoglobin on the part of the host and a tendency especially in *P. berghei* to invade immature red cells having a poor supply of hæmoglobin. Below a certain critical level of nutriment the gametocyte will not be capable of gamete formation and subsequent fertilisation. In particular, the formation of the microgamete is often incomplete in heavy infections. I have seen violent microgamete formation taking place in heavy *P. berghei* infections ten days after inoculation when a high proportion of the resultant free microgametes, on staining, could be seen to lack nuclei. It would be useful to know the percentage of microgametes lacking nuclei on successive days of the vertebrate infection.

The behaviour of *P. berghei* in the invertebrate host presents the most interesting problem of all. Repeatedly we have been successful in obtaining oökinetes and young oöcysts in large numbers. Frequently we have obtained nearly mature and fully mature oöcysts on the seventh and eighth days after feeding. Yet on only one occasion have we seen numerous sporozoites in the glands of a mosquito. Somewhere sporogony is halted. We know that it is often retarded and halted at the young oöcyst stage. We know the formation of the black spores of Ross in the nearly mature oöcysts is abnormally frequent. But what of the mature oöcysts filled with sporozoites ? These have been seen on the ninth and tenth days showing a thickening of the cyst wall and degenerative changes in the sporozoites. Despite all this we have been left with the conviction that sporozoite bearing oöcysts do burst and release sporozoites into the hæmocœlome but none or very few of the sporozoites reach the salivary glands. *P. berghei* is not alone in displaying this last phenomenon. It has been noted in *P. schwetzi* infections by Rodhain and Lassman (1940), in *P. gonderi* infections by Garnham (1953) and in *P. knowlesi* infections by Hawking (1954*b*). One or both of two mechanisms may be at work here. Is it that the body fluids of unnatural invertebrate hosts kill the naked sporozoites and in some cases even retard the oöcysts (Garnham, 1953) ? Or is it that the chemotropism which draws the sporozoites to the salivary glands

is not suitable for *P. berghei* in these unnatural vectors ? Either theory is attractive and both are difficult to prove or disprove. It would be useful to repeat the experiments of Weathersby (1952) using *P. berghei* by inoculating gametocytes into the thorax of mosquitoes.

One method of attack upon this problem was to assume that the laboratory mosquitoes lacked some metabolites essential to the sporogonic development of *P. berghei*. The obvious metabolite was *p*-aminobenzoic acid which we know to be essential to *P. berghei* (Thurston, 1954 ; Hawking, 1953 : 1954*c*) and also essential to sporogonic development of *Plasmodium* (Terzian, Stahler and Ward, 1952). However, the addition of this substance in varying quantities has not brought any improvement in the sporogonic development of *P. berghei*. Fulton (1954) has recently shown that methionine is also essential for the growth of *P. berghei* and this substance might well be tested in this connection.

Vincke *et al.* (1953) echo the opinion of many other workers in considering the choice of vertebrate host to be of great importance. This opinion has arisen due to the repeated failures experienced by various workers using a variety of animals and the success of Yoeli and Wall (1951 : 1952) using the hamster. While the hamster may be the animal of choice, a number of facts militate against too great an importance being placed upon such a choice. Firstly, since the success of Yoeli and Wall (1952), failure to transmit has been as complete with hamsters as with rats. Secondly, although Yoeli and Wall (1952) reported failure with rats, I was able at that time to obtain similar results in a few cases using rats. Lastly, no real test has been made of the relative virtues of hamsters and rats, owing to a reluctance to transfer from the already proven hamster.

I am doubtful if the hamster has any intrinsic value which makes it a superior host to the rat in studies of this type. I also entertain the suspicion that the best vertebrate host for these purposes should be *Thamnomys surdaster* as being the animal least likely to select regressive genetic characters not apparent under the natural conditions of transmission. It is obvious that the mouse should be avoided as it is a host of uncertain capacity for the sporozoites of *P. berghei* and because it allows a rate of development which may be inimical to both gametocytogenesis and gametogenesis. None of our experiences gives any indication of the relative value of age or sex of the vertebrate hosts.

As to the choice of invertebrate hosts there is little that can be said which is not immediately obvious. The laboratory mosquito of choice is *A. aztecus* if it can be obtained. *A. stephensi*, *A. gambiæ* and *A. quadrimaculatus* have been shown to be of limited value. The outstanding problem in this field is the cage-colonization of *A. dureni* which one feels should be possible but which is obviously hedged about with the greatest difficulties.

The behaviour of the mosquito in relation to the parasite has been discussed above to some degree. What makes a mosquito susceptible or insusceptible to plasmodial invasion remains a question beyond our present knowledge. Considerable advances have been made in recent years in rendering insusceptible vertebrate hosts more liable to parasite invasion. The use of cortisone, A.C.T.H., certain dietary deficiencies and splenectomy are well known. More complex are

the techniques involving the introduction of susceptible host extracts into refractory hosts. Such techniques have allowed McGhee (1951) to establish *P. lophuræ* in infant mice, and Desowitz and Watson (1953) to establish *Trypanosoma vivax* in rats. Is it too wild a surmise to suggest that some enterprising malariologist should inoculate extracts of *A. dureni* into laboratory bred Anopheles or hybridise *A. dureni* with a laboratory bred Anopheles to produce a cage-colony of susceptible Anopheles ?

CONCLUSIONS.

Details of various attempts to transmit *P. berghei* through mosquitoes are given.

Some attempt is made to analyse these and other results with special reference to the genetic characteristics of *P. berghei*.

REFERENCES.

Corradetti, A. and Verolini, F. (1951) ...	*Riv. Parassit.*, **12**, p. 69.
Darwin, C. (1859)	"*The origin of species*". 1st Ed. J. Murray, London.
Desowitz, R. S. and Watson, H. J. C. (1953)	*Ann. Trop. Med. Parasit.*, **47**, p. 62.
Fulton, J. D. (1954)	*Lancet*, **i**, Jan. 16, p. 162.
Galliard, H. (1954)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, p. 274.
Gambrell, W. E. (1937)	*Amer. J. Trop. Med.*, **17**, p. 689.
Garnham, P. C. C. (1954)	*Lectures on the scientific basis of medicine*, Vol. II, p. 323. University of London. The Athlone Press, London.
Idem (1955)	*Mechanisms of microbial pathogenicity*. Fifth symposium of the Society for General Microbiology, p. 191. University Press, Cambridge.
Greenberg, J., Nadel, E. M. and Coatney, G. R. (1954)	*J. inf. Dis.*, **95**, p. 114.
Hawking, F. (1953)	*Brit. Med. J.*, **i**, p. 1201.
Idem (1954*a*)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, p. 274.
Idem (1954*b*)	Personal communication.
Idem (1954*c*)	*Brit. Med. J.*, **i**, p. 425.
Huxley, J. (1942)	"*Evolution. The Modern Synthesis*". George Allen and Unwin Ltd., London.
Lumsden, W. H. R. and Bertram, D. S. (1940)	*Ann. Trop. Med. Parasit.*, **34**, p. 135.
McGhee, R. B. (1951)	*J. inf. Dis.*, **88**, p. 86.
Maegraith, B. G. (1954)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, p. 275.
Muirhead-Thompson, R. C. (1954) ...	*Idem*, **48**, p. 208.
Perez Reyes, R. (1953)	*J. Parasit.*, **39**, p. 603.
Ramakrishnan, S. P., Satya Prakash, Krishnaswami, A. K. and Mohan, B. N. (1953)	*Ind. J. Malariology*, **7**, p. 67.
Refaat, M. A. (1954)	"*Studies on gametocytogenesis in* Plasmodium". Doctorate Thesis. University of London.
Rodhain, J. and Lassman, P. (1949) ...	*Ann. Soc. Belge Med. Trop.*, **29**, p. 179.
Terzian, L. A., Stahler, N. and Ward, P. A. (1952)	*J. Inf. Dis.*, **90**, p. 116.
Thurston, J. P. (1954)	*Parasitology*, **44**, p. 99.
Vincke, I., Peeters, E. and Frankie, Gh. (1953)	*Inst. Roy. Col. Belge, Bull. Seances*, **24**, p. 1364.
Weathersby, A. B. (1952)	*J. Inf. Dis.*, **91**, p. 108.
Yoeli, M. and Wall, W. J. (1951) ...	*Nature*, **168**, p. 1078.
Idem (1952)	*Ibid.*, **169**, p. 881.
Young, M. D., Hardman, N. F., Burgess, R. W., Frohne, W. C. and Sabrosky, C. W. (1948)	*Amer. J. Trop. Med.*, **28**, p. 393.

Indian Journal of Malariology, **8**, 4, December 1954.

PLASMODIUM BERGHEI AND CHEMOTHERAPY*.

BY

J. SCHNEIDER.

(*Paris.*)

(September 21, 1954.)

SINCE 1949, when I first obtained a strain of *P. berghei*, I tried to find out if this new plasmodial strain could possibly be used for the study of new antimalarial drugs. From 1937 onwards, we used for our investigations first *P. relictum præcox* of the canary, then *P. gallinaceum* and also *Hæmoproteus paddæ*. The discovery of *P. berghei*, a parasite capable of infecting a laboratory mammal, seemed to be of primary importance as it would enable to study activity and at the same time toxicity of drugs.

In fact, up to the present time, the investigation of the chemotherapeutic index of drugs (to which the pharmacologist seems to attach perhaps too great an importance) was carried out under unreliable conditions. Since they thought that the study of toxicity in birds was unreliable, one compared the activity of the drug against an avian plasmodium and assessed its toxicity on mammals such as mice, rabbits, etc. Until recently the only mammals on which the study of the activity and toxicity of drugs could be carried out at the same time, were monkeys, animals the scarcity of which was a limiting factor for systematic investigation. Since the discovery of *P. berghei*, we were justified, therefore, in hoping that the results of laboratory investigation on this parasite might be applicable to man more directly than heretofore.

During our preliminary work, we intended to test the susceptibility of this plasmodium to know antimalarials. In our first paper (Schneider *et al.*, 1949) we have reported in our findings that *P. berghei* was susceptible to the action of quinine, mepacrine (quinacrine) and chloroquine (nivaquine). On the basis of these results, we attempted to establish a more precise laboratory test. To do that, it was necessary to induce in the experimental animals a disease of standardized severity. Until now, all attempts to transmit the parasite through the Anopheles were either a failure or presented extreme difficulties. We were, therefore, compelled to use a direct inoculation of infected blood from one animal to another healthy one. We soon found that this method gave us satisfactory results and that the inoculated animal behaved as if there had been no exoerythrocytic form of the parasite and the normal course of the infection was not altered in the later host. We were able to show (Schneider and Schneider, 1950) the absence of 'incubation' period since the blood of the receptor was immediately infective after the intraperitoneal inoculation.

*The editor is grateful to Dr. L. J. Bruce Chwatt, Malaria Service, Medical Department, Nigeria, for kindly translating the original paper from French to English.—*Editor.*

After Vincke, we found that, of all the usual laboratory rodents, the mouse was by far the most susceptible to *P. berghei* and it is on this animal that we standardized our test and carried out the investigations.

The test aims at producing a considerable parasitæmia within a standard time of less than 24 hours and at the production of a disease of an increasing severity and constant mortality. These results could be obtained with equal ease by intraperitoneal inoculation as by intravenous inoculation.

To obtain a standard infection, we use, as mentioned in our paper (Schneider and Montezin, 1950), an intraperitoneal inoculation of one-tenth millilitre of blood parasitized by *P. berghei*. The constancy of the results is assured by using a pooled and citrated (sodium citrate two per cent) blood of several mice which have more than ten per cent of parasitized red blood cells. The use of pooled blood makes it easy to inoculate under standard conditions a large number of mice and increases the chances of obtaining standardized infection.

The course of the infection is rapid and the parasitæmia increased regularly during the first five days. After that the parasitæmia increased less constantly and is always followed by the death of the animal within the following periods based on a study of 150 mice :—

42 per cent die during the first five days;
23 per cent die between five and ten days;
20 per cent die between 10 and 20 days;
15 per cent die after 20 days.

These results are of adequate constancy and allow to carry out systematic studies of antimalarial drugs. After three years of work, we have summarized in a paper (Schneider *et al.*, 1952) our results and compared *P. berghei* from this point of view with *P. relictum* and *P. gallinaceum*. We were able to assess the activity of investigated drugs on the basis of our " test of cure " (" *test du traitement curatif* ") which consists in administering the drug under investigation during five days after the inoculation. We believe that the minimum effective dose of the drug is that which is followed by a disappearance of parasites from the peripheral blood for at least three days after the end of the treatment. Our results were always interpreted with reference to control mice inoculated on the same day with the same pooled blood as used for experimental mice.

On the basis of this test, we were able to work out the minimal effective dose against *P. berghei* for the following drugs :—

Plasmochin
Rhodoquine
Chloroquine
Pentaquin
Isopentaquin
Primaquine
Mepacrine
Proguanil
Metachloridine
Pyrimethamine.

The results of our investigation are shown in Tables I and II.

TABLE I.

Comparative activity of various antimalarials on P. Berghei *in mice (test of cure).*

Investigated drug	Minimum active dose mg./kg.		Toxicity to mouse LD_{50} mg./kg.		Chemotherapeutic index C/T.(C=1)	
	Subcutaneous	Oral	Subcutaneous	Oral	Subcutaneous	Oral
Quinine (sulphate) ...	125	200	400	625	1/3,6	1/3, 1
Plasmochin (hydrochloride)	> 15	inactive	15	30	≧ 1	*Nil*
Rhodoquine (hydrochloride)	> 10	inactive	20	20	≧ 1	*Nil*
Chloroquine (base)* ...	2,5	2,5	150	400	1/60	1/160
Pentaquin (monophosphate)	20	20	100	150	1/5	1/7,5
Isopentaquin (oxalate)	10	20	20	20	1/2	1
Primaquine (phosphate)	5	5	100	100	1/20	1/20
Mepacrine (hydrochloride)	5	5	300	800	1/60	1/160
Proguanil (acetate) ...	12,5	12,5	17,5	≧ 17,5	≦1	≦1
Metachloridine ...	25	15	1000	1250	1/40	1/80
Pyrimethamine (hydrochloride) ...	0,25	0,25	20	25	1/80	1/100

* Expressed as the base, the sulphate having been used.

TABLE II.

Chemotherapeutic index (C/T — C=1) *compared in* P. Berghei, P. Relictum *and* P. Gallinaceum.

(Subcutaneous administration)

	Quinine	Plasmochin	Rhodoquine	Chloroquine	Pentaquin	Isopentaquin	Primaquine	Mepacrine	Proguanil	Metachloridine	Pyrimethamine
P. berghei ... Mice (*a*) Test of cure	1/3,6	≧ 1	≧ 1	1/60	1/5	1/2	1/20	1/60	1	1/40	1/80
P. relictum Canaries (*b*) Early treatment	1/8	1/120	1/40	1/30	1/200	1/100	1/200	1/24	1	1/4	1/80
P. gallinaceum Chicks (*c*)	1/50	1/100	1/40	1/150	1/1000	1/200	1/400	1/30	1/12,5 (*d*)	1/2000 (*d*)	1/800

(*a*) Curative dosage for five days. (*b*) Preventive treatment for six days. (*c*) Preventive treatment for eight days. (*d*) Oral administration. C=Min. effective referred as 1. T=LD_{50}.

Our data have shown considerable differences between the activity of some drugs on *P. berghei* and on the two avian plasmodia, *P. relictum* and *P. gallinaceum.* Several other authors have also studied the activity of antimalarials on *P. berghei* (Goodwin, 1949 ; Thurston, 1950 ; Hill, 1950 ; Black, 1951) and some of them have shown that drugs which are poor antimalarials in human infections, have a very high activity against *P. berghei.*

We found that proguanil which is an excellent schizonticide in cases of susceptible strains of malaria in man, shows a relatively poor activity on *P. berghei* since its minimal effective dose is less than half the LD_{50}.

On the other hand, we found that pyrimethamine (Daraprim, Malocide) shows an extremely high activity against *P. berghei.* It is a drug the chemotherapeutic index of which is by far the highest, while in human malaria its therapeutic value is less than that of chloroquine.

Both chloroquine and mepacrine have their chemotherapeutic indices close to one another against *P. berghei.* Chloroquine in man has much higher activity than that of mepacrine. Its chemotherapeutic index for *P. relictum* is higher than that of mepacrine while in *P. gallinaceum* its chemotherapeutic index is five times as high as that of mepacrine.

Finally, one must remember that drugs classified as gametocide may have a considerable therapeutic value on account of their preventive action on relapses of *P. vivax.* Plasmochin, Rhodoquine, Pentaquin and Isopentaquin, all have chemotherapeutic indices less than one. Only primaquine has a relatively high chemotherapeutic index although below that of *P. relictum* and *P. gallinaceum.* Thus had *P. berghei* been the only parasite used in the laboratory, neither proguanil nor Plasmochin, Rhodoquine, Pentaquin and Isopentaquin would have been selected for their value in human malaria.

After three years of work on the use of *P. berghei* for evaluation of antimalarials, we think that this parasite may be used in the laboratory for testing antimalarial drugs. Its particular advantages are the regularity of the course of induced infection and the simplicity in interpreting the results of tests. A word of warning is, however, necessary against the use of this plasmodium alone for testing antimalarials. We consider that strains of *P. relictum* and *P. gallinaceum* must be maintained in a chemotherapeutic laboratory and must be used also for systematic testing of drugs. We also believe that the Roehl's test on *Hæmoproteus paddæ* is needed for completion of any systematic drug testing. Only the comparative assessment of results obtained by means of the latter tests will enable one to obtain the best information with regard to activity of antimalarials and pave the way for the most successful stage of clinical experimentation.

SUMMARY.

Since 1949, we have investigated the possibilities of employing *P. berghei* infections in mice for routine tests on antimalarials. Our preliminary investigations showed that several antimalarials were active in *P. berghei* infections. We then proceeded to determine the minimum active dosage (D.M.A.) for all the known antimalarials against *P. berghei* infection in mice.

It was found that the chemotherapeutic coefficient ($\frac{C}{T}$) which is the ratio between the D.M.A. and the lethal dose for 50 per cent mortality (L.D. 50), was in many cases very different for many drugs against *P. berghei* from the coefficients previously determined by us for the same drugs against *P. relictum præcox* in canary and *P. gallinaceum* in chicks.

P. berghei is highly susceptible to some antimalarials such as chloroquine, mepacrine and pyrimethamine. On the other hand, its susceptibility to Plasmochin, Isopentaquin and Proguanil is low. It is obvious that had *P. berghei* been the only parasite used for screening the latter groups of drugs, not one of them would have been considered useful in human malaria.

Although we consider that *P. berghei* is convenient and useful for laboratory investigations to assess the activity of new drugs, we believe that this parasite should not be the only one used for such investigations.

The main superiority of *P. berghei* in the screening of antimalarials is that both activity and toxicity of drugs can be studied simultaneously while with avian plasmodia, toxicity must be studied in mammals.

We consider that prior to clinical trials of new antimalarials proved effective in *P. berghei*, they should be tested against other plasmodia in the laboratory, specially in *P. relictum præcox* and *P. gallinaceum* infections.

The comparison of results of activity of drugs against all the three of the above species will enable one to assess the potency of drugs more accurately and to avoid failures in clinical trials.

REFERENCES.

Black, R. H. (1951) ... ... *Ann. Trop. Med. Parasit.*, **45**, p. 127.
Goodwin, L. G. (1949) ... ... *Nature*, **164**, p. 1133.
Hill, J. (1950) ... ... *Ann. Trop. Med. Parasit.*, **44**, p. 291.
Schneider, J., Decourt, Ph. and Montezin, G. (1949) ... ... *Bull. Soc. Path. Exot.*, **42**, p. 449.
Schneider, J. and Montezin, G. (1950) ... *Ibid.*, **43**, p. 144.
Schneider, J., Montezin, G. and Mille Bihéu, O. (1952) ... ... *Ibid.*, **45**, p. 776.
Schneider, J. and Schneider, C. R. (1950) *Ibid.*, **43**, p. 324.
Thurston, J. P. (1950) ... ... *Brit. J. Pharmaco. Chemo.*, **5**, p. 409.

Indian Journal of Malariology, **8**, 4, December 1954.

SOME PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES IN *PLASMODIUM BERGHEI* INFECTIONS IN WHITE RATS.

BY

B. G. MAEGRAITH.

(*Liverpool School of Tropical Medicine, Liverpool-3.*)

(October 18, 1954.)

THE COURSE OF INFECTION IN ADULT RATS.

In this laboratory we use a standard intraperitoneal blood inoculum containing one million parasitized cells. Parasites which have been passaged by intraperitoneal inoculation once weekly produce a severe infection from which most animals recover. The parasites first appear in the peripheral blood three to four days after inoculation, and steadily increase in numbers up to the 7th to 12th day. The infection rate then falls rapidly, parasites disappearing from the peripheral blood within a few days. If parasites are subinoculated frequently and passaged at a stage in which the parasitæmia is increasing rapidly in the donor animal, much higher infection rates tend to develop and more animals die, usually in hæmoglobinuria.

The infection rates recorded by various workers in *P. berghei* infections in adult rats differ considerably. Galliard (1954), for instance, states that not more than five per cent infection rate is reached in his adults, though much higher rates occur with the same strain in young animals. Hawking (1954) describes a parasitæmia of 5 to 20 per cent in the ordinary laboratory infection. This is in conformity with our own experience but we have observed much higher rates in some animals, particularly after rapid passaging of the strain as described above.

The course of an infection which develops in a host depends on many factors, which include the environment under which the animal is kept. This is clearly seen in the inhibitory effects of milk or starvation on the parasite (Maegraith *et al.*, 1952; Ramakrishnan, 1953).

It has been claimed that the results of experiments with organisms such as *P. berghei* cannot be interpreted unless the exact age and weight of the animals concerned is stated. It is our belief that, although such factors are no doubt of considerable importance, they must not necessarily be regarded as rigid inherent

qualities, which may determine the success or otherwise of invasion by a given parasites in a given host (Maegraith, 1954*a*). It is becoming increasingly clear that extraneous circumstances, many of which may be fortuitous, such as the diet of the host and the presence or absence therein of essential substances, may govern the result of parasite-host contact. This seems to be true not only of the acceptance of the parasite by the host, but also *vice versa* (Maegraith, 1954*b*). For instance, increasing anorexia and failure to take food, as the disease state progresses, may considerably influence the progress and outcome of an infection in a given animal. It may be for reasons of this sort, for example, that infections in rats fed on milk diet *plus* para-aminobenzoic acid are sometimes more severe and more commonly fatal than those in controls fed on a normal diet.

The increase of parasitæmia to a peak and the subsequent rapid disappearance of the parasites closely parallels the course of infections in self-limited forms of simian malaria. The evidence so far available suggests that the same processes are at work and that the development of a crisis followed by recovery can be related to the appearance of acquired resistance (Thurston, 1953).

CHANGES IN THE HOST.

Anæmia.—As the infection progresses, the numbers of circulating red cells fall considerably, until the crisis is reached, after which they are gradually restored to normal. Counts as low as one to two million cells per c.mm. may be met in severe infections.

The anæmia which develops in a given case is usually roughly proportional to the degree of red cell infection and the fall in blood hæmoglobin concentration keeps roughly parallel. This is not always the case, however, and at present we have no certain information as to whether infected cells only are destroyed at sporulation or whether, as in other forms of mammalian malaria, uninfected cells are also lysed. On the whole, the severity of the anæmia attained and the heavy deposits of hæmosiderin found in the spleen indicate that there is some lysis of unparasitized erythrocytes.

Uninvaded red cells.—In infections in adult rats, reticulocytes commonly increase in numbers in the peripheral blood before parasites appear. The subsequent development of reticulocytosis is discussed below. A few nucleated red cells also appear as the infection develops. During the patent infective and early stages of the post-critical recovery phase, uninfected erythrocytes may show little change in appearance except in very heavy infections, particularly in young rats, in which there may be considerable anisocytosis and some poikilocytosis. The reticulocytes and other young red cells exhibit some polychromatophilic staining reactions.

Invaded cells.—The parasites show a pronounced predilection for young red cells. Most invaded cells are polychromatophilic reticulocytes.

These cells are larger in diameter than the mature erythrocytes. Most contain reticulum which can usually be demonstrated in infected cells during the early stages of the parasite. Invaded reticulocytes are present in the earliest stages of the blood infection, when a few mature erythrocytes may occasionally become

infected. At the height of parasitæmia almost all the invaded cells are reticulocytes; the few normoblasts present may also be invaded. After the crisis, parasites are still found most commonly in reticulocytes, but an occasional mature cell may be also infected. At this stage, when the infection rate is rapidly falling, more than half the circulating red cells may be reticulocytes. During the recurrences, reticulocytes are again much the most commonly invaded cells.

Invasion of the host cell, whether mature or reticulocyte, is commonly multiple.

Reticulocyte production.—Reticulocytes, as mentioned above, appear early in the disease and increase in numbers with the rise of the parasitæmia. Subsequent to the crisis the numbers continue to rise for several days after the parasites have cleared from the blood and then reduce slowly. Immune rats frequently develop a persistent reticulocytosis after infection.

The rise in reticulocyte numbers which occurs during the parasitæmia in *P. berghei* infections is unusual in mammalian malaria. In *vivax* malaria, for instance, although the parasite has a somewhat similar predilection for reticulocytes, the numbers of immature cells do not appreciably increase during the overt infection. Significant reticulocytosis develops only after spontaneous recovery or treatment has ended the parasitæmia, in spite of the fact that reticulocyte production in the bone marrow continues during the parsitæmia. It is therefore believed that in *vivax* infections some kind of inhibition is exerted on the flushing of young cells from the marrow so long as parasites are present in the peripheral blood (Maegraith, 1948). This is clearly not the case in *berghei* malaria, in which reticulocytes are apparently being produced in large numbers as the infection progresses. For example, in an infected adult white rat we observed 16 per cent red cell infection at the crisis, the vast majority of which were reticulocytes. Five days later, when only one per cent of red cells were infected (again almost all of which were reticulocytes), 67 per cent of the total red cells present in the peripheral circulation were reticulocytes.

The differential invasion of reticulocytes sometimes appears to influence the progress of an infection. Thus the development of invasion may be explosive in animals with an already existing reticulocytosis, such as occurs in phenylhydrazine poisoning or in immune animals after splenectomy. Again, the parasite count in non-immune rats after very heavy infective inocula reaches two to three per cent (roughly the percentage of reticulocytes in normal blood) in the first few days and subsequently falls for a day or two before rising again as the reticulocyte numbers begin to rise (Fabiani *et al.*, 1952*a* : 1952*b*).

The significance of the invasion of reticulocytes is not understood. It is possibly concerned with the nutritional requirements of the parasite. The importance of the reticulocyte invasion in the assessment of the result of studies on the respiration of parasites in whole blood is referred to below.

The contents of red cells.—Red cells from rats infected with *P. berghei* have been found to have a slightly higher than normal content of glucose. On the other hand, the serum glucose and total blood glucose fall considerably when the parasitæmia is high (Mercado, 1952). This suggests some disturbance in the interchange of sugar between the cell and its plasma environment. No work appears to have

been published concerning the cell/plasma proportions of other substances, such as potassium and sodium, which are considerably affected in other forms of mammalian malaria.

Microincineration of the parasitized cells has, however, revealed the interesting fact that the content of calcium in the body of the parasite increases as it becomes more fully developed. Since it appears that calcium is not normally present in any quantity in red cells, it may be inferred that the parasitized red cell removes this ion from the plasma (Kruszynski, 1952).

Evidence of uptake of phosphorus by the invaded cell has also been obtained. Whitfield (1953) studied the nucleic acids in solid residues of the blood of mice infected with *P. berghei* (25 per cent parasitæmia) and showed that they were present in considerable excess over the amounts in normal blood. Nucleic acid from parasites freed from their host cells had an absorption spectrum similar to nucleic acid of yeast and had a somewhat similar purine and pyrimidine content. Recent work by the same author has demonstrated the uptake of radioactive phosphorus from the plasma in increasing amounts during the development of the asexual cycle. The lipid fraction of the parasites exposed to phosphorus contained half the total isotope present. Smaller amounts were incorporated in the nucleotides.

Fragility.—Mercado and Coatney (1951) have suggested that the commonly observed increase in the number of extracellular parasites seen in the infection towards the climax, might indicate some increase in fragility in the parasitized red cells. No measurements of saline fragility seems, however, to have been reported; no obvious difference in the reaction of normal and infected blood to shaking in saline at 37°C. has been noted in our laboratories.

Hæmoglobin pigments.—The infected cell becomes paler as the parasites develop within it. This is in keeping with Black's observations (1947) in *falciparum* malaria, and may be interpreted as indicating the disappearance of hæmoglobin from the cell. It is presumed that the hæmoglobin is metabolized and converted in part into hæmozoin, which appears relatively late in the parasite as pale small yellowish brown granules or dust.

Inert pigments, other than hæmozoin, have not been detected in the blood during the infection. No intracellular methæmoglobin has been detected.

Hæmoglobin is found in the plasma in severe infections with high degrees of anæmia, but no other pigments have been detected. Free hæmatin or hæmatin-albumin are not produced.

Organ function.—The fall of blood sugar, evident especially in the serum, which Mercado (*loc. cit.*) had demonstrated in the late stages of severe cases is similar to that recorded by Fulton (1939) in *P. knowlesi* infections and Marvin and Rigdon (1945) in the terminal stages of *P. gallinaceum* infections in ducks. Recent studies by Mercado and von Brand (1954) have revealed that there is a concomitant fall in liver glycogen, together with a more slowly developing fall in carcase glycogen. This fall in glycogen could not be explained by the semi-starvation which occurred during the infection and the authors have concluded that, in view of the fact that infected animals fed sugar deposited less liver glycogen than controls, a true disturbance of liver function has occurred. The same possibilities

as to origin of this dysfunction exist in this as in other protozoal infections (Maegraith, 1951).

Nothing appears to have been done regarding possible changes in the renal function of rats infected with *berghei*, which might be expected to be reflected in the plasma. Since animals which die frequently show hæmoglobinuria, a full-scale investigation of the effects of the infection on kidney function would be rewarding. The intrinsic difficulties of studying renal function in rats probably accounts for there being so little information on this subject. The pigments derived from hæmoglobin which appear in the urine are oxyhæmoglobin and methæmoglobin. Where the methæmoglobin is produced is uncertain, as in other forms of hæmoglobinuria. In those animals which suffer from hæmoglobinuria, hæmoglobin can usually be detected in the plasma, but methæmoglobin cannot. It is probable therefore that the latter is formed either in the kidney or in the urine collected in the ureters and bladder. Recent experiments in our laboratories in dogs in hæmoglobinuria have indicated that methæmoglobin is in fact formed in the kidney.

The carriage of oxygen by infected blood.—The oxygen uptake of whole rat blood, volume for volume, is greatly increased when the red cells are parasitized with *berghei* and also in the post-critical recovery, when parasites are few or absent and reticulocytes numerous. The oxygen uptake of parasitized blood is almost invariably linear with time with or without added glucose. Respiratory curves drawn when parasites and nucleated cells have been inhibited by cyanide, are normal in appearance. In *berghei* infection, therefore, the *in vitro* dissociation and association of oxyhæmoglobin is apparently normal. The potential of blood oxygen carriage is thus a function of the available intracellular hæmoglobin so that, except in extremes of anæmia, which are unusual, the carriage of oxygen from lungs to tissues must be thus considered normal in *berghei* infections (Jones *et al.*, 1951).

In this respect the infection behaves like other forms of mammalian malaria.

THE METABOLISM OF *P. BERGHEI*.

The metabolic pathways and nutritional requirements of the parasite are still little understood. Studies of the effects of variation in host diet and of starvation on the development of the parasites (Ramakrishnan, 1953) have so far revealed little more than was already indicated by the reaction of the orgarism to antibiotics and antimalarial drugs and their antagonists.

The inhibition of the development of the asexual cycle of *P. berghei* in rats on a milk diet first described by us (Maegraith *et al.*, 1952) has been confirmed by a number of workers, though not by all. It is presumed that the action of para-aminobenzoic acid in these experiments is similar to that demonstrated by chemotherapeutic experiments. The indications are that the amino acid is required for folic acid metabolism. Thurston (1953) has suggested that *P. berghei* can utilize para-aminobenzoic acid to synthesize folic acid, unlike *P. gallinaceum*, which requires preformed folic acid. This suggestion is particularly interesting in view of the recent demonstration of the exacerbation, rather than suppression, of *P. gallinaceum* infections in birds given a milk diet (Ramakrishnan *et al.*, 1953).

The effect has also been observed in *P. knowlesi* and *P. cynomolgi* infections in monkeys and in *P. vivax* infections in man. Hawking (1953) and others have demonstrated the re-establishment of the cycle when para-aminobenzoic acid is added to the diet. We have confirmed this, but find with Bray and Refaat (1953) that the restoration of the cycle, when the amino acid is given, is incomplete. In our experience other substances, including methionine, also partly restore the development of the parasite. The restorative action of para-aminobenzoic acid in the milk-fed infected animal is in keeping with the known sensitivity of the parasite to sulphonamides and the limitation of this response by the amino acid. It also links up with the action of the amino acid in *P. berghei* infections which have been suppressed by starvation, a suppression which, incidentally, may also be overcome by the exhibition of methionine.

The study of the metabolic processes of the parasite by ringing the changes in the host diet is important in so far as the parasite during such experiments is present in the host in its natural red cell environment. The use of biological products such as milk has obvious disadvantages which could be overcome by developing synthetic diets of known constitution, in which various test substances could be added or excluded at will. Fulton (1954) has pointed this out recently and recorded some of his observations on *P. berghei* infections in rats on synthetic diets. He has in this way confirmed the partial activity of para-aminobenzoic acid, and other substances, including methionine.

It seems to me that there is a considerable future in experiments of this kind which should help in the understanding of the fundamental metabolic processes of the parasite developing undisturbed in its natural environment. So far, little has been achieved that had not already been demonstrated by experiments with chemotherapeutic agents and their antagonists, but we are only at the beginning.

The study of the respiration of parasitized red cells with the object of determining the respiration of the parasite, is open to criticism in any plasmodial infection, since it has to be assumed that the respiration of the host cell is unchanged by the invasion and is the same as that of the uninfected erythrocyte. In *berghei* infections the proposition is made much more difficult because the host cell is the reticulocyte, which itself respires freely, as may be seen in terms of oxygen uptake in Table I.

TABLE I.

O_2 uptake of parasitized rat blood related to parasitæmia.

Day of infection	R.B.Cs. $\times 10^6$ per c.mm.	Percentage of R.B.Cs. parasitized.	Parasites $\times 10^6$ per c.mm.	O_2 uptake ml. per cent.
6	5·3	2·5	0·13	3·0
7	3·6	2·9	0·11	4·7
8	1·9	16·0	0·3	8·4
13	3·5	1·0*	0·04	17·8

* 67 per cent of the red blood-corpuscles were reticulocytes. (Jones *et al.*, 1951).

Our own experiments have shown that the examination of whole blood or washed parasitized cells is thus valueless so far as determination of parasitic metabolic activity is concerned. We have, however, studied the reticulocyte itself and obtained some interesting information regarding its metabolic pathways which may be of some significance so far as the parasite is concerned, and possibly help to explain the predilection of the organism for this particular stage of the erythrocyte. In these experiments reticulocytes obtained from rats treated with phenylhydrazine and from rats in the post-critical stage of *P. berghei* infections, behaved identically. The pattern of carbohydrate metabolism indicated that they oxidized pyruvate *via* the Krebs cycle. The source of energy could not be determined in the absence of information concerning the permeability of the reticulocytes to glucose which itself had no apparent effect on the oxygen uptake. Krebs has suggested that the energy might come from glucose present within the reticulocyte envelope. In view of the fact that other plasmodia when separated from their host cells also oxidize pyruvate *via* the Krebs cycle (Moulder, 1948), it can be concluded that the parasites metabolize carbohydrate at any rate in the same way as reticulocytes, though not mature red cells. It appears to us therefore to be impossible with present techniques to study the carbohydrate metabolism of *P. berghei* in its natural environment. Investigations of its metabolism must be made after the parasite has been forcibly removed from its host cell and thus from its natural environment. Whether observations made under such conditions can be related to the reality of the respiring parasite within the reticulocyte in the host is open to considerable doubt (Jones *et al.*, 1953).

The oxygen uptake of parasitized blood in *P. berghei* infections is, however, interesting from another point of view. Taken as a whole, it presumably represents the uptake of both parasite and host cell, and thus should give some indication of the oxygen requirements of the blood *per se* in relation to other tissues during the infection. Even in heavy infections in anæmic rats, we have found that the oxygen used by the infected blood (about 0·7 ml. per hour) was comparatively small and represented roughly only 1/20th that required by the liver tissue of the same animal. This suggests that qualitatively the oxygen used by the blood was too little to affect the host directly. To discover what the quantitative importance of this usage may be in the infected animal is at present beyond the range of our technical methods. The indications are, however, that the demands of the parasitized blood are unlikely to be of such dimensions as to provide direct competition between host and parasite, particularly in view of the apparently normal carriage and discharge of oxygen by parasitized blood (Jones *et al.*, 1951).

One particularly interesting feature of the metabolism of *P. berghei* is the production of hæmozoin, which appears late and rather sparsely in the parasite, but which is often distributed extensively in the tissues.

Fulton and Rimington (1953) have shown that this pigment contains hæmatin. Its precise composition has not, however, been satisfactorily determined. In view of its insolubility it is possible that the contained hæmatin may be combined with some larger molecule. It has been observed that more hæmozoin is produced by occasional parasites which have invaded mature cells than by those in reticulocytes. This may be explained partly in terms of the hæmoglobin content of the two types of red cells, but may also be the result of slightly different metabolic

processes. The breakdown of hæmoglobin by the parasite has not yet been studied but there is evidence that the protein fraction is utilized and may be a source of methionine, which is also probably obtained from the plasma outside the infected cell. The fate of hæmozoin after its escape at sporulation has not yet been determined. It is known, of course, that it is picked up by the reticulo-endothelial tissues or lies free in tissue spaces, but whether the iron contained in the pigment is available for the reconstitution of hæmoglobin is unknown. Preliminary experiments in our laboratories suggest that it is not available, and that in individual animals living on an iron-deficient diet or possessing a minimal iron reserve, infection with *berghei* may remove sufficient iron from effective circulation to interfere with the full reconstitution of hæmoglobin after recovery from the infection. This work, which is still in progress, emphasizes the importance of studying iron metabolism in malaria in general, especially in areas in which *falciparum* malaria is endemic, since in many of these the available mineral may be sparse and the indigenous population may well be living on the verge of deficiency.

CHANGES IN TISSUES.

An account of the pathological effects of *P. berghei* infection in white rats is being prepared in our laboratories. The work is still incomplete, but the results in a few instances may be of interest to readers of this symposium.

The spleen.—The spleen in the rat enlarges rapidly and becomes pigmented early in the infection. It may eventually become enormous. In the early stages, there is massive accumulation of blood cells in the pulp and sinusoids and deposition of pigment is evident mostly in the intercellular spaces. As the infection progresses, the blood cells held in the tissues increase in number and parasitized and unparasitized cells alike are eventually taken up by the macrophages, which come to contain large amounts of pigment. Some of this pigment is hæmozoin, but the great bulk of it gives the Prussian Blue reaction and is probably hæmosiderin. It thus appears that large quantities of iron, presumably available for the reconstitution of hæmoglobin, are held in the splenic tissues during the infection. After the crisis the spleen rapidly diminishes in size and pigment content.

As the crisis approaches, the phagocytosis of cells and pigment appears to increase and the numbers of lymphocytic cells decrease. The picture thus closely resembles that described in self-limited simian malaria (Taliaferro and Cannon, 1936). In fatal cases, signs of cellular degeneration and necrosis may appear in the pulp tissue. These have also been observed in the mouse (Rodhain, 1951). The relation of this phagocytosis to the appearance of acquired immunity has not yet been determined.

The adrenals.—In view of the wide interest in the functional significance of adrenal hormones in malaria (Overman, 1951; Fabiani and Izzo, 1952), it is notable that in our experience changes in adrenal tissue seem to be exceptional in *P. berghei* infections. The commonest change is congestion of the vessels of the medulla and the inner layers of the cortex. Parasites are often present in large numbers in the smaller blood vessels and deposits of hæmozoin are common. Occasionally, small focal areas of degeneration may be seen in the cortex. As in tissue elsewhere in *P. berghei* malaria, the tendency of invaded cells to lie along the vascular endothelium is less marked than in other plasmodial infections.

PLATE XIV

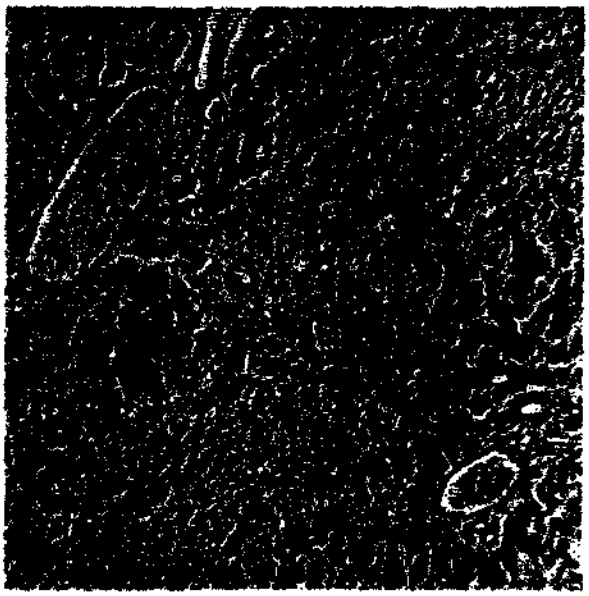


Fig. 1. Liver in fatal *P. berghei* infection in the adult rat. (H and E; medium power).
Note the centrilobular distribution of the degenerative and necrotic tissue, and the relatively normal cells near the portal tracts. The central and midzonal sinusoids contain Kupffer cells loaded with haemozoin. The Kupffer cells near the periphery contain much less pigment.

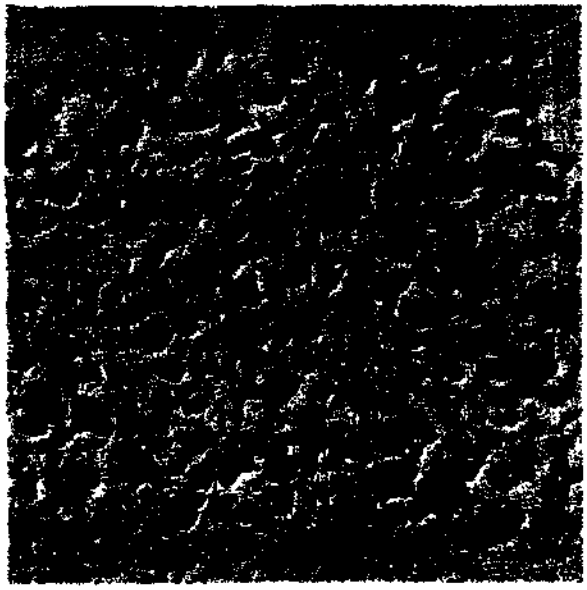


Fig. 2. Liver in fatal *P. berghei* infection in the adult rat. (H and E; high power). The same liver as in Figure 1 above.
Apparently normal cells around a portal tract. Little pigment in Kupffer cells.
The centrilobular region. Note cellular changes and pigment-containing swollen Kupffer cells.

PLATE XV

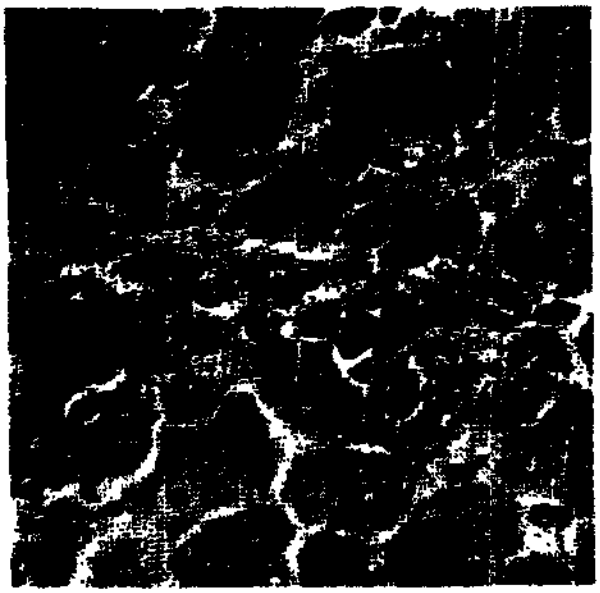


Fig. 1. Liver in fatal *P. berghei* infection in the adult rat. (H and E: high power). The same liver as in Plate XIV, Fig. 1.

The centrilobular region. Note cellular changes and pigment containing swollen Kupffer cells.

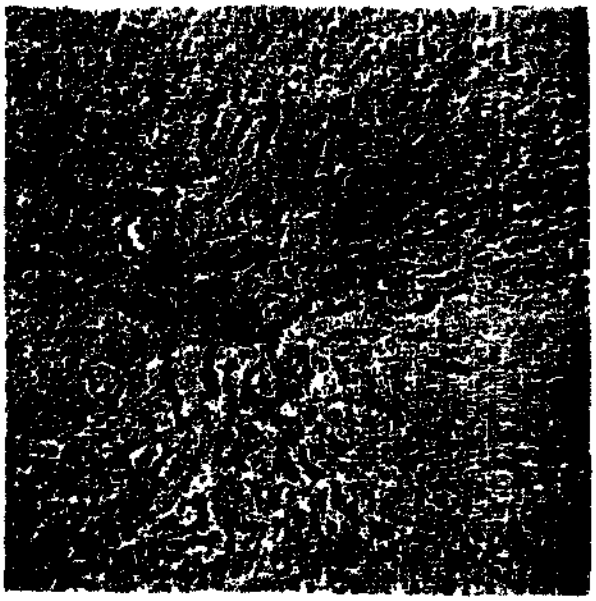


Fig. 2. Liver in acute *P. berghei* infection in adult albino rat (sacrificed early in infection). (H and E; medium power). Intense centrilobular congestion including sinusoids and central vein.

The liver.—Foci of leucocytes and areas of necrosis have been described in the liver in mice infected with *P. berghei* (Levaditi and Vaisman, 1950) ; in cotton rats Rodhain (1951) has described pigmentation of the liver and necrotic lesions.

We have studied liver changes at various stages of the infection in white rats. In animals killed at the height of infection or killed by the infection, the lesions are very similar to those described in other forms of malaria (Plate XIV, Fig. 1).

Changes in the polygonal cells vary from fatty degeneration to frank necrosis. The cells near the periphery of the lobule are usually little affected. Those in the mid-zone and central regions show extensive fatty change and necrosis (Plate XIV, Fig. 2 and Plate XV, Fig. 1).

The Kuppfer cells in the peripheral region of the lobules are often little affected. In some animals, especially those in which deposition of pigment is extensive, the peripherally placed cells may be somewhat swollen and filled with pigment. The Kuppfer cells in the mid-zone particularly are commonly swollen and choked with pigment, often more so than the cells nearer the central vein. This distribution of pigment is interesting in view of the relation of its intake by the Kuppfer cells to the prevailing interlobular circulation and differs from the peripheral distribution sometimes described in other malarial infections, especially after repeated infection. In contrast to the pigment found in the spleen, most of that held in the Kuppfer cells does not appear to contain free iron. Occasionally bile pigment is found in the centrally placed hepatic cells. The cells do not contain any other pigment, even hæmosiderin. Hæmosiderin may thus be apparently sparse in the liver although plentiful in the spleen of the same animal. Edington (1952) has reported similar absence of hæmosiderin in the livers in *P. falciparum* infections in the Gold Coast.

In occasional specimens, some swelling of the liver cells is suggested by their close-set appearance. This has been observed in all regions of the lobule. In most, however, there is no evidence of swelling. The sinusoids are open and filled with blood cells, many of which may be parasitized. In early lesions the sinusoids in the central region are often grossly congested as is the central vein (Plate XV, Fig. 2).

The liver lesion in its fully developed form thus closely resembles that seen in other plasmodial and protozoal infections. The lesion may ultimately involve the central and midzonal lesions but it appears to be essentially centrizonal. Its pathogenicity is discussed elsewhere (Maegraith, 1951).

SUMMARY.

Some aspects of the development of blood transmitted *P. berghei* infections in white rats are described. The predilection of *P. berghei* for reticulocytes is stressed, particularly in relation to measurement of the metabolic activity of both host cell and parasite. Information regarding the latter acquired indirectly by various techniques indicates that the metabolic pathways are similar to those of other mammalian plasmodia and to those of the reticulocyte. The composition of the hæmozoin produced by the parasite is discussed, especially in relation to the iron reserve of the infected host. The evidence suggests that the iron contained in the pigment is not readily available for resynthesis of hæmoglobin. Certain features

of organ function are discussed and lesions developed in severe and fatal infections in the spleen, adrenals and liver are briefly described.

The evidence presented here concerns the development of *P. berghei* infection transmitted by blood inoculation. It is assumed that the erythrocytic phase is the pathogenic form of the parasite, and that the clinical course of invasion is thus probably independent of the method of infection. It is advisable nevertheless to recognize that this is an assumption and that in some respects evidence based solely on artificial blood infections may need to be interpreted with caution in so far as the vector-borne disease is concerned.

REFERENCES.

Black, R. H. (1947) ... ... *Ann. Trop. Med. Parasit.*, **41**, p. 215.
Bray, R. S. and Refaat, M. A. (1953) ... *Brit. Med. J.*, **ii**, p. 1047.
Edington, G. M. (1954) ... ... *Malnutrition in African mothers, infants, and young children. Report of Second Inter-African (C.C.T.A.) Conference on Nutrition (Colonial Office), Gambia*, 1952. (H. M. Stationery Office), p. 115.

Fabiani, G., Clausse, J. and Fulchiron, G. (1952*a*) ... ... *C. R. Soc. Biol.*, **235**, p. 274.
Fabiani, G., Fulchiron, G. and Clausse, J. (1952*b*) ... ... *Ibid.*, **146**, p. 1584.
Fabiani, G. and Izzo, M. A. (1952) ... *Ibid.*, **146**, p. 1155.
Fulton, J. D. (1939) ... ... *Ann. Trop. Med. Parasit.*, **33**, p. 217.
Idem (1954) ... ... *Lancet*, **i**, p. 162.
Fulton, J. D. and Rimington, C. (1953) ... *J. Gen. Microbiol.*, **8**, p. 157.
Galliard, H. (1954) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, p. 274.
Hawking, F. (1953) ... ... *Brit. Med. J.*, **i**, p. 1201.
Idem (1954) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, p. 274.
Jones, E. S., Maegraith, B. G. and Sculthorpe, H. H. (1951) ... ... *Ann. Trop. Med. Parasit.*, **45**, p. 244.
Jones, E. S., Maegraith, B. G. and Gibson, Q. H. (1953) ... ... *Ibid.*, **47**, p. 431.
Kruszynski, J. (1952) ... ... *Ibid.*, **46**, p. 117.
Levaditi, C. and Vaisman, A. (1950) ... *Bull. Soc. Path. Exot.*, **43**, p. 693.
Mackerras, M. J. (1953) ... ... *Aust. J. Sci.*, **16**, p. 24.
Maegraith, B. G. (1948) ... ... *Pathological Processes in Malaria and Blackwater Fever.* Blackwell Scientific Publications, Oxford.
Idem (1951) ... ... *Brit. Med. Bull.*, **8**, p 28.
Idem (1954*a*) ... ... *Proc. Roy. Soc. Med.*, **47**, p. 626.
Idem (1954*b*) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, p. 275.
Maegraith, B. G., Andrews, W. H. H. and Gall, D. (1947) ... ... *Lancet*, **253**, p. 781.
Maegraith, B. G., Deegan, T. and Jones, E. S. (1952) ... ... *Brit. Med. J.*, **2**, p. 1382.
Marvin, N. H. and Rigdon, R. H. (1945) ... *Amer. J. Hyg.*, **42**, p. 174.
Mercado, T. I. (1952) ... ... *Amer. J. Trop. Med. Hyg.*, **1**, p. 932.
Mercado, T. I. and Coatney, G. R. (1951) *J. Parasit.*, **37**, p. 479.
Mercado, T. I. and von Brand, T. (1954) ... *Exptl. Parasit.*, **3**, p. 259.
Moulder, J. W. (1948) ... ... *Ann. Rev. Microbiol.*, **2**, p. 101.
Overman, R. R. (1951) ... ... *Physiol. Rev.*, **31**, p. 285.
Ramakrishnan, S. P. (1953) ... ... *Ind. J. Malariol.*, **7**, p. 53.
Ramakrishnan, S. P., Prakash, S., Krishnaswami, A. K. and Channan Singh (1953) *Ibid.*, **7**, p. 61.
Robhain, J. (1951) ... ... *Ann. Soc. Belge. Med Trop.*, **31**, p. 289.
Idem (1953) ... ... *Ibid.*, **33**, p. 245.
Schneider, J. and Montezin, G. (1953) ... *Bull. Soc. Path. Exot.*, **46**, p. 947.
Taliaferro, W. H. and Cannon, R. P. (1936) *J. Infect. Dis.*, **59**, p. 72.
Thurston, J. P. (1953) ... ... *Exptl. Parasit.*, **2**, p. 311.
Whitfield, P. R. (1953) ... ... *Aust. J. Biol. Sci.*, **6**, p. 234. and 591.

Indian Journal of Malariology, **8**, 4, December 1954.

ON SOME PROBLEMS ARISING FROM THE OBSERVATION OF THE INFECTION WITH *PLASMODIUM BERGHEI* IN MICE AND RATS.

BY

G. RAFFAELE.

(*Istituto di Malariologia "E. Marchiafava", Roma.*)

(September 9, 1954.)

THE interest aroused by the discovery of *Plasmodium berghei* (Vincke and Lips, 1948), a parasite of several rodents, is stated by the vast literature grown in a few years on the subject.

The availability of a malaria parasite of mammals as common as the mouse and the rat awakened the hope that, it might be possible to study the various problems connected with malarial infection, which are difficult to follow in man and too expensive in monkeys, owing to the cost of each single specimen. Bird malaria was considered by many researchers to differ too much from that of mammals, especially so after Shortt and Garnham (1948) affirmed that the exoerythrocytic phase of mammals develops in the hepatic parenchima cells, whereas that of birds develops in the cells of the reticuloendothelial system. Although the writer of this note (Raffaele, 1952) made some objections to the interpretation the British workers gave of their findings, his objections remained up to now without an answer ; the opinion of the British workers was generally accepted and bird malaria was put on a different category than the malaria of mammals. In consequence, *P. berghei* was considered as the choice parasite for the study of some problems concerned with malarial infections in mammals, especially owing to the low cost of the animals susceptible to the infection.

It will be suitable, for shortness sake, to omit the usual chronological list of all the works done on infection with *P. berghei* and to limit our task to call the attention upon the results obtained by us and by other researchers.

I. BEHAVIOUR OF *P. BERGHEI* IN MOUSE AND RAT.

All workers on the subject have ascertained:

(*a*) that the white mouse (*Mus musculus* var. *albus*) is extremely susceptible to the infection, from which it dies in a few days. The common mouse (*Mus musculus*) shows the same susceptibility;

(*b*) the white rat (*Mus norvegicus* var. *albus*) is equally susceptible to the infection, which causes a high mortality in young rats, but heals spontaneously in adult rats, giving way to a complete and lasting immunity against reinfection;

(*c*) both in mice and rats, the parasite develops in erythrocytes and gives a high bone-marrow reaction, to which corresponds the immission in the circulating blood of young erythrocytes (macrocytes, reticulocytes) and sometimes, especially with high parasitæmia, of normoblasts.

To explain this different susceptibility towards infection in young and adult rats various opinions were advanced, in which the important factor of age was perhaps not always held in sufficient account. The experience gained in the *Istituto di Malariologia* on 130 adult rats, of more than five months of age, is that the infection with *P. berghei* never caused death.

The different behaviour of the infection, according to the age of the rat, is one of the most characteristic features of *P. berghei* and finds no correspondence in any other type of malarial infection in man or animal, at least in none that was experimentally studied.

It is not easy to establish the intervening factor, which with the growth of the animal modifies the course of infection. Often the infection gives rise in adult rats to a severe parasitæmia, which, having reached a high peak, subsides quickly and in a few days the parasite disappears from the common blood films.

In young rats the infection acts in the same way as in mice and mortality, at least in rats aged one to two months, is 100 per cent. Splenectomy makes the infection in adult rats as severe and lethal as in young rats and in mice.

Fabiani *et al.* (1952*a*) give a variable rate of mortality in rats infected with two different strains of *P. berghei*, respectively 47 per cent and 19 per cent, but they do not mention the age of the rats. It is possible that the different races of white rats, kept in different laboratories, react in a different way. Galliard and Lapierre (1950) observed that the rate of mortality decreases with the increase of the animals weight, that is with their growing up. This was observed also by Fabiani *et al.* (1952*b*) who state that the Wistar race of rats is less resistant than other races to infection, thus confirming the opinion that the different results are due to the different races of white rats used.

Raffaele and Baldi (1950) with six new-born rats, from different mothers, inoculated with *P. berghei*, had a 100 per cent mortality 10 to 19 days after inoculation. Out of four rats, inoculated during the second month of age, two recovered spontaneously and one had a lethal infection; the fourth died from chance causes. Out of five rats born from one delivery, and inoculated one after the other at different ages, the following results were obtained: 10, 20, 30 and 45 days after birth, death ; 60 and 80 days after birth, inoculation fails; 130 days after birth infection is followed by spontaneous recovery. It seems that between the second and third month the rat acquires the power to control infection. It is therefore, possible to find already in the second month of life some tolerance to infection, which becomes the norm in the adult rat. This has been demonstrated by the recent extensive work of Zuckerman and Yoeli (1954).

The different behaviour towards infection of adult and young rats is perhaps worth of a careful study. It is possible that in adult rats the immunity reaction is quicker and more effective than in young rats, but it would be interesting to see if the phenomenon is correlated only with the immunity reaction or if there is some other factor.

The quick setting in of anæmia, together with a strong medullary reaction, is one of the most characteristic features of the infection with *P. berghei*. It seems obvious that anæmia is the result of the destruction of erythrocytes by the parasite; but the doubt arises that the destruction of the red blood cells might be connected with other factors, considering that there are other kinds of malarial infections, which, although giving way to high parasitæmia, do not cause such a quick fall in the number of red cells.

The researches led by Mercado and Coatney (1953) on the infection with *P. berghei* in a meadow mouse (*Microtus pennsyl vanicus*) with a mortality of about 33 per cent, show a swift decrease of red cells, both in mice which had an infection followed by death and in mice which recovered. The number of parasites in the first three to four days was remarkable, but not higher than that observed in other malarial infections. Even in human infections with *Plasmodium falciparum*, where a high parasitæmia is often reached, there is not always such a quick and dramatic fall in the number of red cells. Maegraith (1948) reported of *P. falciparum* infections which were not followed by severe anæmia. In 20 cases observed by Fairley and Bromfield (1933), the number of red cells differed from 1·8 to 5·2 millions per c.mm. It might be that the severe anæmia that sometimes affects the patients of acute malaria is to be ascribed not only to the destruction of red cells by the parasite, but also to processes of hæmolysis or to a still unknown process of destruction of erythrocytes.

In the infection with *P. berghei* in rats and mice, after the prepatent period, there is such a sudden fall in the number of erythrocytes that it seems unlikely to ascribe it only to the destructive action of the parasite. First of all, as observed by Galliard (1949), Baldi (1950), Ramakrishnan and Prakash (1950) and Corradetti and Verolini (1951), there seems to be no doubt that most of the parasites develop into the reticulocytes. Fabiani *et al.* (1952*b*) observed that parasitæmia increases at the same rate as reticulocytes in the blood. The problem is why the adult normal red blood cells, scantly invaded by the parasites, disappear so quickly, giving way in all cases to a rapid and severe anæmia. It would be interesting to investigate which factor brings about the destruction of the adult normal erythrocytes, the number per c.mm. of which in rats and mice is, as we know, nearly double as in other mammals or in man.

II. DURATION OF INFECTION.

In the animals kept in the laboratory of our Institute, the average length of infection was more or less as observed by other workers. In 93 mice the infection lasted in the average 13·5 days from the day of inoculation (Baldi, 1952). The prepatent period lasted in the average 5·5 days, with a minimum of four and a maximum of ten days. The average duration of patency was of eight days.

Out of 75 inoculated rats, 13, less than three months old, died. In lethal infections, the prepatent period was in the average of 18 days, with a minimum of 12 and a maximum of 22 days. The young rat seems to oppose a greater resistance to infection than the mouse.

In 62 adult rats, the duration of patency was variable; some had only a slight and short parasitæmia of about two to three days, followed by a complete recovery. In heavier infections with a higher parasitæmia (up to 20 per cent of infected erythrocytes) the average duration of patency was of 15 days.

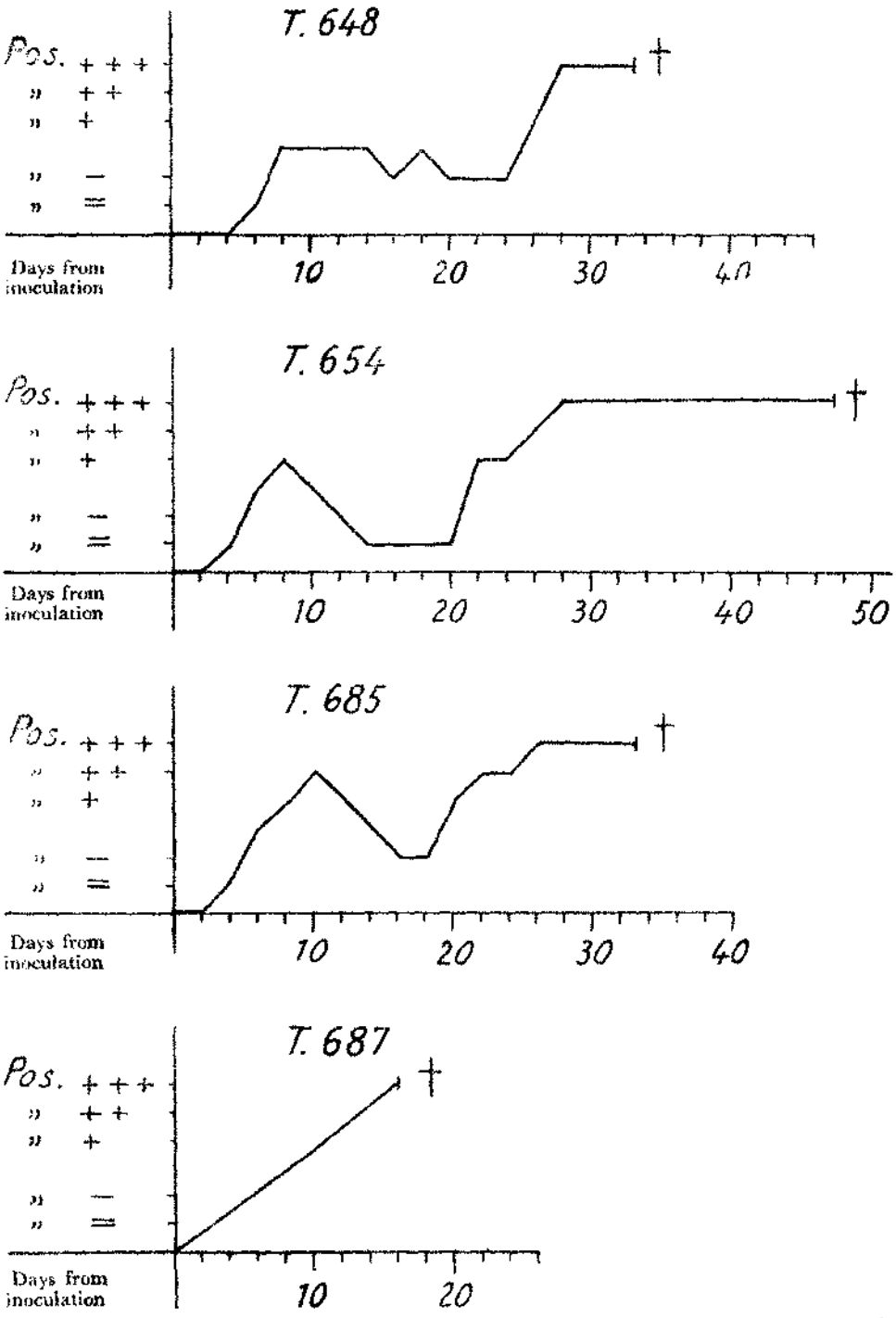
As Fabiani *et al.* (1952*b*) observed, the fall of parasitæmia in rats which recover is very quick and often a high parasitæmia disappears completely in two to three days. The same authors observed that during the fall of parasitæmia, although the blood contains many young erythrocytes (polychromatophilic macrocytes and reticulocytes), the few surviving parasites are often seen in erythrocytes of the same diameter as in those of normal rat. It would, therefore, seem that in the adult rat the tendency of the parasite to invade young erythrocytes disappears by the setting in of immunity, so that only those parasites remain which invade adult, normal erythrocytes. It looks as if the persistence of infection, which can be noted only after subinoculation or splenectomy, is due to the few parasites developing in normal erythrocytes.

Fabiani *et al.* (1951) observed that in splenectomized rats after the disappearance of the parasites from the blood films, there is often a violent relapse with fatal ending; but in 13 rats, splenectomized after the end of the primary infection, there was no relapse This possibly means that in those rats parasites disappeared from the blood completely. Unfortunately the authors do not say how long after the disappearance of parasites from the blood films, splenectomy was performed and it is therefore impossible to calculate how long it takes for infected rats to recover. In our laboratory a rat splenectomized 77 days after disappearance of parasites from the blood films had no relapse. Corradetti's opinion (1950) is that in the surviving rat, the infection subsides within a period from 28 to 30 days.

III. IMMUNITY.

The mechanism of immunity towards *P. berghei* develops slowly, as is shown by the fact that infected albino mice all die, even when infection lasts beyond the ordinary limits of time, which are generally short. Out of 93 inoculated mice, 20 had infections lasting more than three weeks: during this time immunity did not reach such a degree as to avoid death. Thriteen mice, treated with synthetic antimalarial drugs, recovered ; but reinoculated two to four months after recovery they all died (Baldi, 1952). On the contrary mice kept on milk diet, following the technique of Maegraith *et al.* (1952), and having a rather long infection (Raffaele and Carrescia, 1954), achieved a remarkable degree of immunity. Out of 22 mice kept on milk diet, ten survived ; in those the infection lasted from 47 to 74 days and the mice recovered. Of those animals, four were put again on normal diet and reinoculated (Chart 1). The infection lasted a long time with a considerable swaying of parasitæmia. In two of the reinoculated mice, an initial increase in the number of parasites was followed by a strong decrease, which lasted a few days and was followed by a new increase. The four reinoculated mice died of

CHART 1.

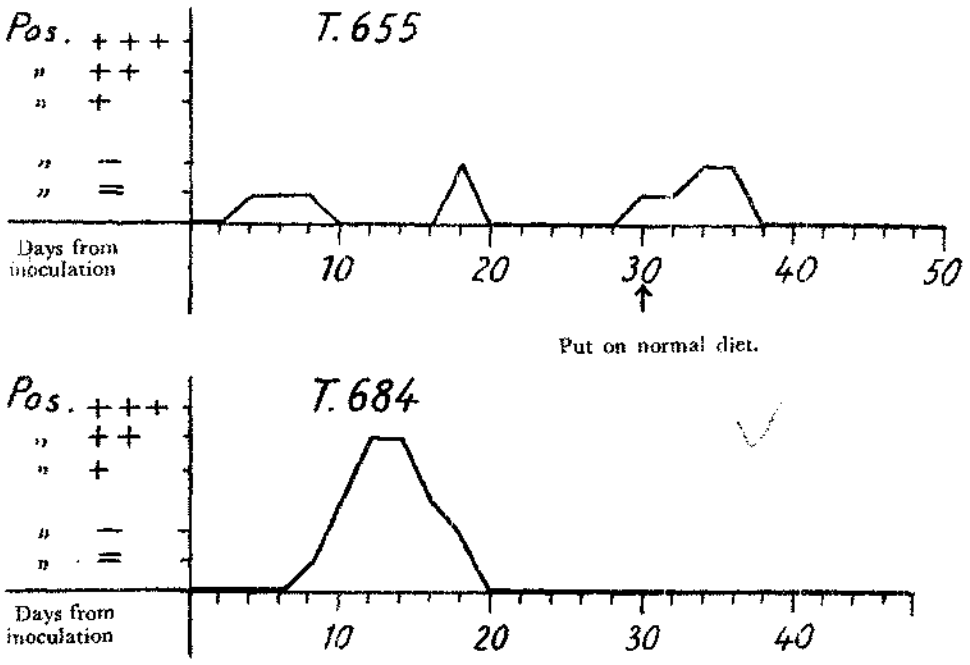


Mice, on milk diet and recovered from the first infection, put on normal diet and re-inoculated.

the infection, which lasted respectively 48, 34, 33 and 16 days, with an average of 32·7 days, whereas in the controls the average duration (calculated from the day of inoculation) lasted 14·6 days. This seems to demonstrate that mice, which owing to milk diet have a prolonged infection followed by spontaneous recovery, develop a certain degree of immunity sufficient to oppose a considerable resistance to parasitic invasion due to reinoculation.

The acquired immunity of the infected mice kept on milk diet and recovered was sufficient to protect them from a second inoculation, if this was performed after they were put again on milk diet (Chart 2). In one of the mice, a scanty parasitic invasion lasted a few days and was followed by short periods of light parasitæmia; those periods were not modified by the return to a normal diet until spontaneous recovery set in. In the second mouse kept at milk diet and reinoculated a speedy parasitic invasion was observed followed by a rapid fall of parasitæmia, until recovery set in. In mice kept on milk diet a noticeable degree of immunity is established, able to protect them from reinfections.

CHART 2.



Mice, on milk diet and recovered from the first infection, maintained on milk diet and re-inoculated.

In adult rats, whatever the duration of the first infection and the attained degree of parasitæmia, immunity is complete. In rats recovered from infection and with negative blood at the microscopic examination as well as at subinoculation tests, we were unable to obtain a clear parasitæmia even by heavy inoculation of parasites in the peritoneum. To three rats we administered from the day of inoculation 100 mg. of PABA daily during seven days, and for other four days each

second day, to observe if an excess of para-aminobenzoic acid could favour in some way the parasitic invasion, but we had no positive results and the blood remained constantly negative. Subinoculations, with blood taken from rats recovered after two months, have always had negative results (Baldi, 1952). We have here immunity and not premunition as Sergent *et al.* (1924) understands it. Immunity appears chiefly as a resistance; tolerance is of a moderate degree and does not last long. Only in mice kept at milk diet a noticeable tolerance is observed.

IV. EFFECT OF MILK DIET.

As already observed by Maegraith *et al.* (1952), milk diet modifies deeply the course of *P. berghei* infection in mice, changing a rapid and always lethal infection into one much longer and less severe, which often recovers. Hawking (1953) established that this was due to the lack of PABA in milk diet and that the addition of PABA to milk neutralizes its effects. The observations of Maegraith *et al.* (1952) and Hawking (1953) awakened a wide interest. Before them Geiman and Mackee (1948) had observed the favourable effects of fasting on the control of *Plasmodium knowlesi* infections in monkeys.

In our Institute, some experiences were done on the effects of milk diet (Raffaele and Carrescia, 1954). The efficacy of such a diet was particularly clear when begun a few days before inoculation; if it was initiated the same day it was less effective. It might be that the mice did not bear the simultaneous and weakening effect of milk diet and infection; sometimes they died even from a low parasitæmia. It seemed preferable to accustom first the animals to milk diet and to inoculate them afterwards. Out of 11 mice kpet on milk diet 2, 4 and 14 days before inoculation, only one died from an infection which lasted 38 days. The other ten mice all recovered after infections lasting from 40 to 74 days.

The course of infection in a group of eight mice, which were put on milk diet 45 days before inoculation, was difficult to explain. It presented itself nearly as in normal mice, i.e. short duration of infection, rapid increase of parasitæmia, death. The only difference was that the mice we used were all over four months of age; but this does not seem a sufficient explanation; because in mice, unlike rats, infection with *P. berghei* has the same lethal course in young and in adult animals.

As already observed by Hawking (1953), we ascertained that the addition of PABA to milk diet destroys the effect of the diet. What we could not explain was why six new-born rats, three days old, fed by the mother and inoculated with a small dose of parasites, all died in 11-15 days with very high parasitæmia. Hawking (1954) believes that to obtain positive results with sucklings it is necessary to keep the mothers at milk diet or at glucose diet. But the milk we and other workers used in the experiments with mice and rats was cow milk, that is of an animal kept at a normal diet. It is therefore not clear why the milk of the mother has no action on the new-born. It might be that younger animals can synthesize from simpler stuff the metabolites which favour the development of the parasite in the blood.

V. EFFECT OF SPLENECTOMY.

The observations of Galliard and Lapierre (1950) and of Fabiani *et al.* (1951) on the effect of splenectomy in rats infected with *P. berghei* led us to go over again the same experiments and search the effects of milk diet in splenectomized rats. The work is still proceeding and the results will be published by Dr. Carrescia, who is following the experiments.

Splenectomy modifies the course of infection in the adult rat, which loses its resistance to infection. Four adult rats, inoculated after splenectomy, developed heavy infections with high parasitæmia, macrocytosis, erythroblastosis and severe anæmia. All the rats died within two to four weeks. Hence the spleen seems to be an essential factor in bringing about the resistance to primary infection in the adult rat. Milk diet applied to splenectomized rats inoculated with *P. berghei* has given, up to now, varying results; in some cases milk diet seems to be effective even in splenectomized animals.

SUMMARY.

Investigations conducted at the *Istituto di Malariologia* of Rome on infection with *P. berghei* in mice and rats reached conclusions mainly in agreement with those of other researchers.

The course of infection in adult and young rats differs. During the first two months of age, the infection in rats is always lethal; after the third month it becomes milder and adult rats never die from infection, but recover spontaneously and acquire a complete and lasting immunity against reinfection. Subinoculation, performed two months after the disappearance of parasites from the blood, is never infective Splenectomy, performed about 80 days after recovery, does not bring the reappearance of parasites in the blood. Attention is drawn to this difference in the behaviour of the infection in relation to the age of the animal, which never occurs in other types of malarial infections.

It is striking how quick the strong anæmia, peculiar to the infection, sets in in mice ; the more striking as *P. berghei* seems to develop only in the reticulocytes and the increase in the number of parasites in the blood seems to follow the increase of reticulocytes and of the young red blood cells. It seems that anæmia is brought about not only by the destruction of red cells by the parasite, but also by some unknown hæmolitic factor.

Immunity in mice establishes itself very slowly and comes through only in mice, which, owing to milk diet, have a prolonged infection. An infection lasting from two to three weeks, and interrupted by therapy, gives no immunity against reinfections. In rats of more than three months of age, infection seems to confer a lasting and complete immunity.

Milk diet prolongs the infection in mice till, sometimes, a complete recovery is reached. This diet seems to be particularly effective when begun 2-14 days before inoculation with parasites. It resulted completely ineffective in eight mice maintained at milk diet for 45 days before inoculation; in those mice infection was

quickly lethal and it is difficult to understand the reason of it. The infection was rapidly lethal also in six rats of three days of age fed by the mother.

Splenectomy in adult rats deprives them of any resistance towards infection, which acts as in mice and in young rats and has a fatal ending. Spleen seems, therefore, to be an essential factor in bringing about the resistance to primary infection in adult rats.

REFERENCES.

BALDI, A. (1950) ... ... *Riv. Malariol.*, **29**, p. 349.
Idem (1952) ... ... *Ibid.*, **31**, p. 41.
CORRADETTI, A. (1950) ... ... *Riv. Parassitol.*, **11**, p. 201.
CORRADETTI, A. and VAROLINI, F. (1951) ... *Ibid.*, **12**, p. 69.
FABIANI, G., VARGUES, R., GRELLET, P. and CLAUSSE, J. (1951) ... ... *C.R. Soc. Biol.*, **145**, p. 1131.
FABIANI, G., VARGUES, R., GRELLET, P., PULCHIRON, G. and VERAIN, A. (1952*a*) *Bull. Soc. Path. Exot.*, **45**, p. 524.
Idem (1952*b*) ... ... *Ann. Inst. Pasteur*, **82**, p. 721.
FAIRLEY, N. H. and BROOMFIELD, R. J. (1933) *Trans. Roy. Soc. Trop. Med. Hyg.*, **27**, p. 289.
GALLIARD, H. (1949) ... ... *Bull. Soc. Path. Exot.*, **42**, p. 341.
GALLIARD, H. and LAPIERRE, J. (1950) ... *C. R. Soc. Biol.*, **144**, p. 402.
Idem (1951) ... ... *Bull. Soc. Path. Exot.*, **44**, p. 185.
GEIMAN, Q. M. and MACKEE, R. W. (1948) *Sci. Monthly*, Washington, **67**, p. 217.
HAWKING, F. (1953) ... ... *Brit. Med. J.*, **I**, p. 1210.
Idem (1954) ... ... *Ibid.*, **I**, p. 425.
MAEGRAITH, B. (1948) ... ... *Pathological process in malaria and blackwater fever.* Blackwell Scientific Publications, Oxford.
MAEGRAITH, B., DEEGAN, T. and SHERWOOD, J. E. (1952) ... ... *Brit. Med. J.*, **II**, p. 1382.
MERCADO, T. and COATNEY, G. R. (1951) ... *J. Parasitol.*, **37**, p. 479.
RAFFAELE, G. (1952) ... ... *Riv. Malariol.*, **31**, p. 1.
RAFFAELE, G. and BALDI, A. (1950) ... *Ibid.*, **29**, p. 341.
RAFFAELE, G. and CARRESCIA, M. (1954) ... *Ibid.*, **33**, p. 47.
RAMAKRISHNAN, S. P. (1953) ... ... *Ind. J. Mal.* **7**, p. 53.
RAMAKRISHNAN, S. P. and PRAKASH, S. (1950) *Ibid.*, **4**, p. 361.
SHORTT, H. E. and GARNHAM, P. C. C. (1948) *Brit. Med. J.*, **I**, p. 1225.
ZUCKERMAN, A. and YORLI, M. (1954) ... *J. Infec. Dis.*, **94**, p. 225.

Indian Journal of Malariology, **8**, 4, December 1954.

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

*XX. A physiological change observed in sulphadiazine resistant strain.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B.(Edin.), D.P.H.(Eng.), D T.M. & H. (London),

S. P. RAMAKRISHNAN,

SATYA PRAKASH

AND

V. N. BHATNAGAR.

(Malaria Institute of India, Delhi.)

(November 10, 1954)

HAWKING (1953) showed that retardation of growth of *P. berghei* in rats fed on an exclusive milk diet was restored if PABA was added to the milk. This investigation also demonstrated that PABA is an essential nutrilite for the growth and development of *P. berghei*. Ramakrishnan *et al.* (1953) came to a similar conclusion in their experiments on administration of pure nutrilites to infected starved rats. Fulton (1954) has confirmed the partial activity of PABA in his investigations on feeding infected rats on synthetic diets. Thurston (1953) suggested that *P. berghei* can utilize PABA to synthesize folic acid.

P. berghei is known to be highly susceptible to sulphadiazine (Hill, 1950 ; Thurston, 1950 ; Ramakrishnan *et al.*, 1951 ; Mudrow-Reichenow, 1951 ; Rollo, 1951). It was also demonstrated independently by Thurston (1950), Hill (1950) and Mudrow-Reichenow (1951) that as in the case of bacterial organisms, action of sulphonamides was due to its antagonism to PABA. Thus, by direct as well as indirect evidence, it has been established that PABA is essential for the growth of *P. berghei*.

* The investigation was financed by a grant from the Indian Council of Medical Research.

It has been possible to develop in the laboratory, strains of *P. berghei* which are highly resistant to sulphadiazine (Rollo, 1951 : 1952 ; Thurston, 1953 ; Krishnaswami *et al.*, 1954). It would appear justifiable, therefore, to infer that a strain of *P. berghei* resistant to sulphadiazine does not require PABA for its growth. The results of experiments to test the validity of such an inference are recorded in this paper.

MATERIAL AND METHODS.

The parasite.—Two strains of the parasite were used. One was the strain originally received in 1952 through the courtesy of Brigadier J. S. K. Boyd of the Burroughs Welcome Laboratories Ltd., London. It has since been maintained in adult albino rats by blood passage and had undergone 146 passages at the commencement of the current experiment.

The second strain of *P. berghei*, resistant to sulphadiazine, was developed from the normal strain referred to above. Since the sulphadiazine resistant strain described by Krishnaswami *et al.* (*loc. cit.*) was destroyed, it became necessary to develop another similar strain. This was accomplished by passage of the normal strain at its 112th rat transfer, to mice successively, which were treated by gradually increasing doses of sulphadiazine. The sulphadiazine used, the technique of development of resistance, and estimation of the degree of resistance were the same as described previously by Krishnaswami *et al.* (*loc. cit.*), with the exception that drug administration in the present case was not oral, but parenteral, once daily through the intraperitoneal route. At the time of the experiment, the degree of resistance was estimated to be more than 800 times the minimum effective dose of the drug required by the normal strain for clearance of parasitæmia from the peripheral blood.

Throughout the experiment, the dose of inoculation was five million parasites per mouse. The route of inoculation was always intraperitoneal.

The vertebrate host.—Eighteen male albino mice, each 24 weeks old, were used. They were all from the mice colony maintained at the Institute. Throughout the experiment, they were placed in individual cages so constructed that the animals could have no access to their excreta.

The eighteen mice were divided into two equal groups (I and II) of three batches (1,2,3,4,5 and 6) consisting of three animals each. One of the batches (1 and 4) from each group was fed on the standard diet.* The animals belonging to the remaining four batches (2, 3, 5 and 6) were fed on an exclusive milk diet commencing two days prior to inoculation. In addition to this diet, a solution containing 0·2 mg. sulphadiazine per 20 gm. body-weight per mouse was administered orally every day to animals of two batches (2 and 5).

*Standard diet consists of :—

Whole wheat flour	...	70 parts.
Skimmed milk powder	...	16 ,,
Pea nut ...	...	8 ,,
Dry brewers' yeast	...	2 ,,
Shark liver oil ...	...	2 ,,
Calcium carbonate	...	1 ,,
Table salt	...	1 ,,

The object of sulphadiazine administration was to inhibit any possible intestinal synthesis of PABA in mice which were denied PABA on account of the milk diet.

The milk preparation used was 'Nespray' of Nestle Company Ltd. The powder was mixed in water to form a thick paste and served in a metal cup. The quantity offered to each mouse once a day was in excess of its daily consumption.

Five million parasites of the normal strain were inoculated to each of the mice of Group I and a similar inoculum of the resistant strain was given to each of the mice of Group II on the same day. Thin blood smears, obtained daily from each mouse, were examined after being air dried, fixed in methyl alcohol and stained by J. S. B. stain. Smears, in which no parasites could be seen in 100 consecutive oil immersion fields, were declared negative. In positive smears, the number of parasites per 10,000 erythrocytes were enumerated.

RESULTS.

The course of infection in mice on different diet regimens, infected respectively with the normal and sulphadiazine resistant strains, is shown in Tables I and II. The infections due to the normal and resistant strains in the respective groups of animals fed on balanced diet appear to be identical. In other words, the normal and the resistant strains behave in the same way in mice when their PABA intake or its synthesis was not interfered with in any way.

There is an appreciable difference in the course of the two infections in the two groups of mice which were fed on an exclusive milk diet. The normal strain showed extremely retarded growth confirming the now well-known fact that a milk diet is deficient in PABA in the absence of which the parasite cannot grow. The resistant strain on the other hand, seemed to grow equally well in animals fed on an exclusive milk diet and in animals fed on balanced diet. The absence of PABA in the milk diet which has an inimical effect on the normal strain, did not in any way seem to affect the resistant strain.

When intake as well as the synthesis of PABA were eliminated by milk and sulphadiazine administered to mice, the behaviour of not only of the resistant and normal strain differed, but also that of the resistant strain was different in the batches of mice on milk alone and milk and sulphadiazine, respectively. The normal strain showed extremely scanty parasitæmia and soon disappeared from the peripheral blood just as it did in mice fed on milk alone. The resistant strain on the contrary developed in two animals almost normally upto the thirteenth and fourteenth days of patency and then the parasitæmia showed a decline and finally disappeared from the peripheral blood from the twentieth day. In the third animal the parasitæmia was comparatively mild from the tenth day of patency and disappeared from the peripheral blood from the eleventh day.

DISCUSSION.

The results of the experiments show that the progress of infection due to the resistant strain is similar to that of the normal strain in animals fed on a balanced diet (Table I and II). In other words, the course of infection due to the resistant strain is not altered from that of the normal strain in animals where the intake or any

possible intestinal synthesis of PABA is not interfered with in any way. The infection due to the resistant strains also developed in a normal fashion in animals to which the intake of PABA was highly or totally restricted by the milk diet. The resistant strain, therefore, would appear to be physiologically different from the normal strain in respect of its requirement of PABA.

The nature of such a physiological change in the nutritional requirement of the resistant strain lends itself to two explanations. Firstly, it is possible that the strain has learnt to do altogether without PABA. Secondly, it is possible that the resistant organism has been altered in such a way that it is able to synthesize its PABA requirement, which is absent in its *milieu*. The former is considered likely because the resistant strain developed and multiplied almost normally for the first several days in animals which were given sulphadiazine and denied PABA by the milk diet (Table II).

The object of sulphadiazine administration to mice, in addition to the milk diet, as already stated, was to suppress any possible intestinal synthesis of PABA. But the course of infection due to the normal strain was found to be no different in the mice fed exclusively on milk from that in mice which were given sulphadiazine in addition to milk (Table I). It would, therefore, appear that there was hardly any intestinal synthesis of PABA in the stock of mice used in the experiments.

In view of the above, the course of infection due to the resistant strain in mice fed on milk as well as sulphadiazine is unexpected. In general, the parasitæmia in the three mice was milder than in the other six. In one, it increased up to the thirteenth day of patency and declined thereafter. In the second animal, the infection was milder and the decline was apparent from the ninth day of patency. In both these animals, the peripheral blood was negative to parasites from the twentieth day. In the third, the parasitæmia was even milder than in the other two and disappeared from the peripheral blood from the eleventh day onwards.

These unexpected results are not likely to be due to any direct action of the sulphadiazine on the strain which is already resistant to the drug. The total amount of the drug administered per mouse in the batch up to the thirteenth day of patency is 2·6 mg. per 20 gm. body weight. This dose is only about 22 times the dose required by the normal strain for clearance of peripheral blood (Krishnaswami *et al.*, 1954) and as already pointed out the degree of resistance of the strain used in the current experiment is very much higher than 800-fold.

In view of the above, the question arises whether the action of prolonged daily administration of small doses of sulphadiazine is different from the administration of much higher doses of the drug for shorter periods which is without any effect on the resistant strain. This would appear to be unlikely especially when the total dose of drug administered daily over the prolonged period is much less than a single dose to which the organism is totally resistant.

It has already been shown that the restriction of PABA intake by the milk diet does not in any way interfere with the growth of the resistant strain (Table II). The unusual behaviour of the resistant strain in mice which were given both milk and sulphadiazine, could only be attributable to some action of the drug. In view of the strong evidence that the resistant organism is indifferent to the presence

TABLE I.

The course of parasitæmia in albino mice infected with the normal strain of P. berghei *and fed on the different diet regimens.*

Diet regimen	Mouse number	PARASITE PER 10,000 ERYTHROCYTES FROM THE FIRST DAY OF PATENCY																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Colony diet	M-1214	12	132	140	280	296	320	600	800	600	1080	1400	1600	1560	1800	1960	> 2000	D					
	M-1215	8	88	192	428	440	596	496	608	800	1180	1600	1800	> 2000	1960	> 2000	> 2000	> 2000					
	M-1216	16	68	180	320	360	1200	1080	1200	1600	1800	> 2000	> 2000	> 2000	D								
Milk diet	M-1211	8	4	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	M-1212	4	6	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	M-1213	N	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Milk diet and sulpha-diazine	M-1217	2	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	M-1218	1	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	M-1219	2	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	D

D = Died N = Negative > = Greater than

TABLE II.

The course of parasitæmia in albino mice infected with sulphadiazine resistant strain of P. berghei *fed on different diet regimens.*

Diet regimen	Mouse number	Parasites per 10,000 erythrocytes from the first day of potency																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Colony diet	M-1205	18	12	20	264	780	880	960	1080	1200	1800	> 2000	> 2000	D									
	M-1206	72	320	480	468	340	960	1500	1600	D													
	M-1207	88	280	400	480	340	320	1040	1180	1200	1600	1680	1600	1800	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000D	
Milk diet	M-1202	0	240	320	298	240	180	148	128	108	600	960	1020	1200	1800	1960	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000
	M-1203	12	284	296	240	160	180	240	300	160	480	1080	1180	1280	1400	1796	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000
	M-1204	16	272	280	180	120	160	340	400	520	460	880	920	1000	1260	1880	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000
Milk diet and sulphadiazine	M-1208	46	72	64	160	180	480	720	1080	680	800	1040	1200	1400	600	300	280	60	6	1	N	N	N
	M-1209	32	84	72	260	248	260	480	500	320	280	220	180	320	420	280	168	72	8	1	N	N	N
	M-1210	28	52	48	98	96	40	20	80	2	1	N	N	N	N	N	N	N	N	N	N	N	N

D = Died
> = Greater than
N = Negative

or absence of PABA in its *milieu*, it would appear justifiable to presume that sulphadiazine may act on some nutrilite other than PABA not so essential to the normal strain but very essential to the resistant organism. Whatever the factor may be, it would appear that it is considerably less sensitive to sulphadiazine than PABA. A great deal of further work will be necessary to elucidate the explanation for this finding.

SUMMARY.

It has been established that a sulphadiazine resistant strain is indifferent to the presence or absence of para-aminobenzoic acid in mice fed on a balanced diet as well as mice fed on an exclusive milk diet. This would indicate a physiological change in the resistant organism with regard to its nutritional requirement of PABA. The possibility of the resistant organism becoming capable of synthesizing its own PABA requirement is also considered.

The infection due to the sulpha resistant strain in mice fed on milk as well as sulphadiazine was much milder than in mice fed only on milk. This raises a question of a possible action of sulphadiazine on nutrilite(s) other than PABA, possibly required by the resistant strain, but not by the normal strain.

REFERENCES.

FULTON, J. D. (1954) ... ... *Lancet*, **1**, p. 162.
HAWKING, F. (1953) ... ... *Brit. Med. J.*, **1**, p. 1201.
HILL, J. (1950) ... ... *Ann. Trop. Med. Parasitol.*, **44**, p. 291.
KRISHNASWAMI, A. K., SATYA PRAKASH and RAMAKRISHNAN, S. P. (1954) ... *Ind. J. Mal.*, **8**, p. 9.
MUDROW-REICHENOW, L. (1951) ... Quoted by Thurston (1953). *Z. Tropenmed. U. Parasitol.*, **2**, p. 471.
RAMAKRISHNAN, S. P., KRISHNAWAMI, A. K. and SATYA PRAKASH (1951) ... *Ind. J. Mal.*, **5**, p. 455.
RAMAKRISHNAN, S. P., SATYA PRAKASH, KRISHNASWAMI, A. K. and CHANAN SINGH (1953) ... ... *Ibid.*, **7**, p. 225.
ROLLO, I. M. (1951) ... ... *Nature*, **168**, p. 332.
Idem (1952) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, p. 174.
THURSTON, J. P. (1950) ... ... *Brit. J. Pharmacol.*, **5**, p. 409.
Idem (1953) ... ... *Exp. Parasit.*, **2**, p. 311.

Indian Journal of Malariology, **8**, 4, December 1954.

PRELIMINARY OBSERVATIONS ON ESTROGEN IN *P. BERGHEI* INFECTION.

BY

N. K. RAY

AND

A. N. BOSE.

(Bengal Immunity Research Institute, Calcutta.)

(September 30, 1954.)

Reticulo-endothelial system is the normal abode of malarial parasites during the pre and exo-erythrocytic stages of their development. Estrogenic substances stimulate this system. Whether the stimulation of reticulo-endothelial system by estrogenic substances can affect the growth of malarial parasites was thus thought to be of interest.

Our inbred laboratory white mice and rats show estrus every fifth or sixth day. The estrus cycle in them covers a period of five to six days. *P. berghei* blood-induced infection in our laboratory mice and rats requires five to six days to reach the peak density of infection. It is thus quite evident that whatever be the position of the estrus cycle at the time of inoculation with *P. berghei*, the parasites during their whole process of development, to reach peak parasitæmia, shall encounter all the phases of the estrus cycle. It was therefore thought to be worth while to study the effect of the cycle in blood-induced *P. berghei* infection in our laboratory mice and rats as the work might throw some light on the interrelationship between parasitic growth, reticulo-endothelial activity and concentration of estrogen in blood which varies during different phases of the estrous cycle.

Observations were carried out with inbred laboratory mice and rats brought up under same nutritional and environmental condition. Animals of same age and approximately of equal weight were used in the experiments. There were two sets of animals, one consisting of normal females separated from males, few days after birth, and the other on which bilateral ovariecotomy had been performed while still immature. Animals weighing 20-25 grammes in the case of mice and 50 to 60 grammes in the case of rats were used. By frequent examination of vaginal smear, animals showing estrus regularly every five or six days were sorted out. In case of ovariectomized animals vaginal smear was examined continuously for three weeks to ensure complete absence of ovarian action. Ten units of standard

estrogen was then given subcutaneously to each of the animals under observation. Animals showing peak estrus on the third day of medication were used for the purpose of inoculation with *P. berghei.*

In case of normal animals, inoculation of infected r.b.c. 10^6 per gm. body weight was made during three different stages of the cycle viz. anestrus, proestrus and estrus. In case of ovariectomized animals subcutaneous injection of ten units of standard estrogen was made and ioculation done with infected r.b.c. 10^6/ gm. body weight on three successive days on three different groups. The mode of inoculation was in all cases intraperitoneal. Examination of blood for estimation of parasitic density as compared with the control was made on the fifth day of inoculation by thick and thin film procedures using Giemsa stained preparations of blood from the tail vein of the experimental animals. The density of parasitæmia was determined by the number of infected r.b.c. per 1,000 r.b.c.

The results of some experiments on mice are given below in Tables I and II as regards density of parasitæmia, prepatent period and survival time after inoculation.

TABLE I.

Normal animals.

	Number of animals used in each group	Average density on the fifth day/ 1,000 r.b.c.	Survival time after inoculation	Prepatent parasitæmia
Inoculation during anestrous ...	10	276·0	9–15 days	2
Inoculation during pre-estrous ...	10	285·0	6–13 days	2
Inoculation during estrous ...	10	291·4	6–7 days	2
Inoculation in ovariectomized animals	10	107·2	More than three weeks	2
Male animals of same age and weight	10	239·3	6–12 days	2

TABLE II.

Ovariectomized animals.

	Number of animals used in each group	Prepatent period	Average density on the fifth day/ 1,000 r.b.c.	Survival time after inoculation. (days)
Inoculated on the first day of estrogen administration ...	5	3	109	14–21
Inoculated on the second day of estrogen administration ...	5	2	142·5	10–16
Inoculated on the third day of estrogen administration ...	5	2	193·4	7–10
Normal female in anestrous ...	5	2	268·4	6–10

From the results given above and from similar other studies, results of which have not been given here it seems that our inbred laboratory mice inoculated intraperitoneally with one million infected r.b.c. per gramme body weight show no marked difference as regards development of parasitic density when inoculated with *P. berghei* during different phases of the estrus cycle. Early death when infected during estrous period is however of considerable significance as difference in parasite density is not such as would explain this difference in survival period. The way the ovariectomized mice react when infected with inoculum of same strength creates some interest. Density of parasitæmia remains at a considerable lower level suggesting that the absence of ovarian hormone might upset the metabolic pattern in a way not favourable for parasitic growth. Table II further shows that the effect of permanent absence of ovarian hormone cannot be neutralized by injection of ten units of standard estrogen, which though, is quite sufficient to start estrus. The fact that the prepatent parasitæmia is same in all the animals of the two groups suggest that under no circumstances the appearance of the parasite in the systemic blood from their natural abode (reticulo-endothelial system) is hindered. Reticulo-endothelial activity cannot affect the normal growth in blood-induced infection in our inbred mice. Similar results were observed with rats. It is very often not possible to draw any inference as regards human behaviour from animal experiment hence less severity of malarial infection in females after menopause, and good results sometime observed in chronic malarial patients treated with antimalarial drugs and testosterone requires extensive study.

SUMMARY.

1. Stimulating effect of estrogenic substances on the reticulo-endothelial system do not affect the growth of *P. berghei* in our laboratory mice and rats.

2. Inoculation with one million infected r.b.c. per gramme body weight during different phase of the estrus cycle has almost the same results as regards parasitæmia but mortality is earlier when inoculation is made during estrous.

3. Density of infection remains at a considerable lower level in ovariectomized mice and rats when inoculated with same number of infected r.b.c. per gramme, body weight.

ACKNOWLEDGMENT.

Thanks are due to Dr. U. P. Basu, D.Sc., F.N.I. for taking interest in this investigation.

Indian Journal of Malariology, **8**, 4, December 1954.

SOME HOST-PARASITE RELATIONSHIPS IN *PLASMODIUM BERGHEI* INFECTIONS.

BY

JOSEPH GREENBERG

AND

G. ROBERT COATNEY.

(*National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases, Bethesda* 14, *Maryland, U.S.A.*)

(December 8, 1954.)

In the study of malaria, there are many advantages in utilizing the pronounced susceptibility of the mouse (*Mus musculus*) to *Plasmodium berghei*. Among these advantages is the availability of many highly inbred strains of laboratory mice. Animals of such strains are considered to be genetically homogeneous and therefore the element of genetic variability should be small in such populations. Furthermore, if different reactions to *P. berghei* should be found among these various inbred strains, the influence of heritable factors on the course of the infection could be studied. We have found that such differences do exist.

Table I is a summary of data, taken from our published reports (Greenberg, Nadel and Coatney, 1953 : 1954), on the survival of various inbred strains of mice after inoculation with the parasite. C57 black mice survived twice as long as *SWR* (inbred Swiss) and *A* mice. A statistical analysis of the data revealed that the various strains fell into four or five categories. In each cateogry there were no significant differences in terms of survival; but survival within each category differed significantly from all others. Only the *RIL* mice spanned two categories.

TABLE I.

Survival of mice of various inbred strains inoculated, when 2 months old, with 1 million erythrocytes parasitized with P. berghei (*Kasapa strain*)

Strain	Number of mice	Mean survival (days)
A	38	8·38
SWR ...	21	8·81
C3H ...	23	9·65
DBA ..	76	9·90
STR	77	10·45
Brsunt ...	31	10·71
C58	17	10·40
BALB/C ...	65	12·51
C57 Brown ...	18	12·33
RIL	18	14·39
C57 Leaden ..	16	15·00
C57 Black ..	51	17·60

Course of the infection in 13 hybrids of some of the strains listed in Table I has been examined and a summary of published and unpublished findings are shown in Table II. Again the difference in survival between the shortest—and the longest-lived strains is at least two-fold. Comparing the data in this table with those in the previous table, it is apparent that all but three hybrids survived significantly longer than the longest-lived parent. The hybrids not exhibiting increased survival over the longest-lived parent were those resulting from crosses between closely related strains. The analogy with the results of hybridizing corn, for example, is obvious; heterosis was obtained in the hybrids unless the parents were closely related. One could presume that the explanation for heterosis in other hybrids would apply to mice infected with malaria; the results arising in consequence of a favourable recombination of many allelic genes. Heterosis in the mice was lost in the F_2 hybrid (*C*57 Black × *DBA* F_2) and when the hybrid was back-crossed to the shorter-lived parent [(*C*57 Black × *DBA*) × *DBA*], but it was not lost when the backcross was made to the longer-lived parent [(*C*57 Black × * *DBA*) × *C*57 Black]. These results would seem to eliminate such environmental factors as maternal influence. The evidence strongly suggests genetic influences on the survival of mice infected with malaria; but it also suggests that many genes are involved in a complicated manner.

In the studies just summarized it was noted that while distinct differences in survival occurred among various inbred strains of mice and between them and

* This is the symbol for "crossed with".

TABLE II.

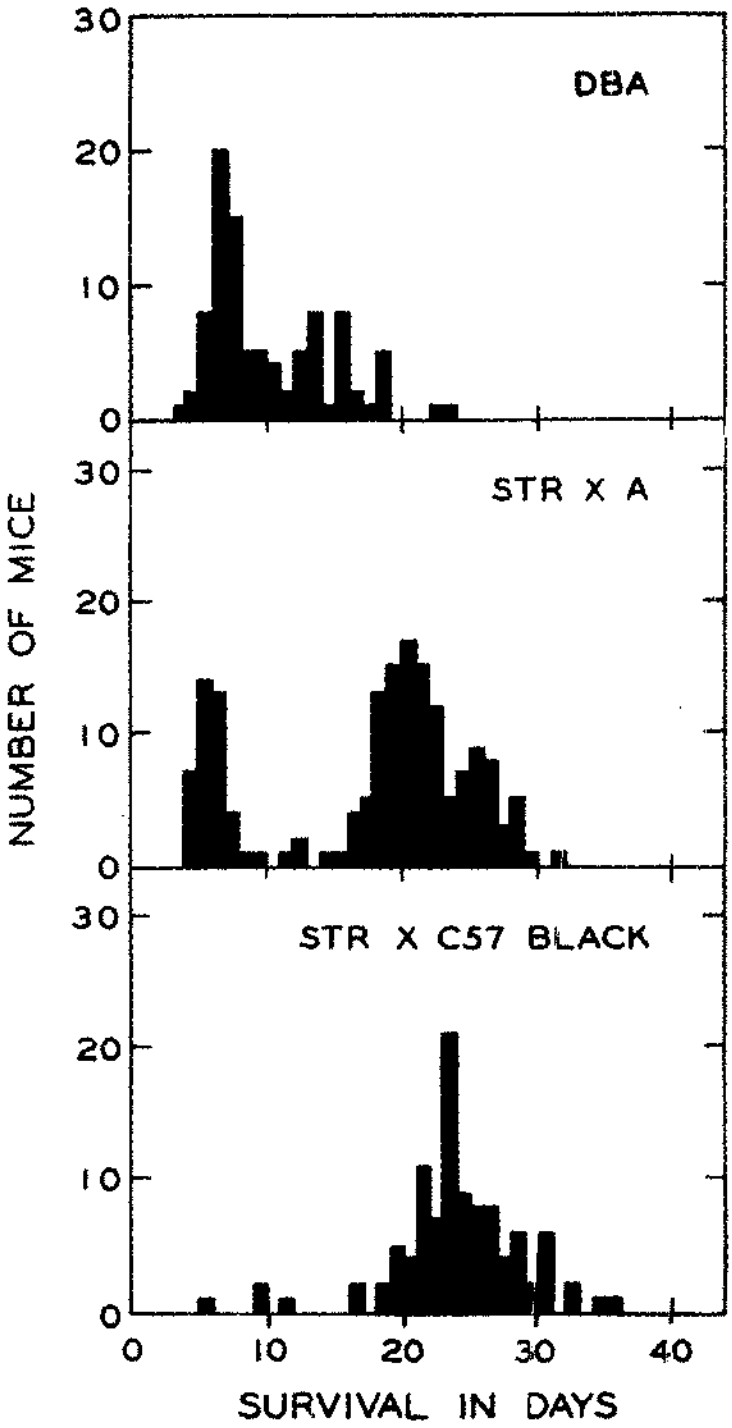
Survival of various hybrid mice inoculated, when 2 months old, with 1 million erythrocytes parasitized with P. berghei *(Kasapa strain).*

Hybrid	Number of mice	Mean survival (days)
C57 Black × *C57* Leaden ...	25	11·28
BALB/C × *A*	30	13·23
C57 Black × *C57* Brown ...	58	15·05
BALB/C × *C3H* ...	30	15·10
A × *DBA*	57	15·07
BALB/C × *DBA* ...	13	16·38
STR × *DBA**	135	17·85
STR × *A**	165	18·21
C57 Leaden × *DBA** ...	140	19·25
C57 Leaden × *A* (*LAF*) ...	31	19·29
C57 Black × *DBA* ...	44	21·23
C57 Black × *A* ...	34	22·65
STR × *C57* Black* ...	103	24·45
C57 Black × *DBA* F_2* ...	174	17·14
(*C57* Black × *DBA*) × *DBA** ...	169	13·83
(*C57* Black × *DBA*) × *C57* Black*	81	20·01

*Data from Nadel, Greenberg, Jay and Coatney (unpublished).

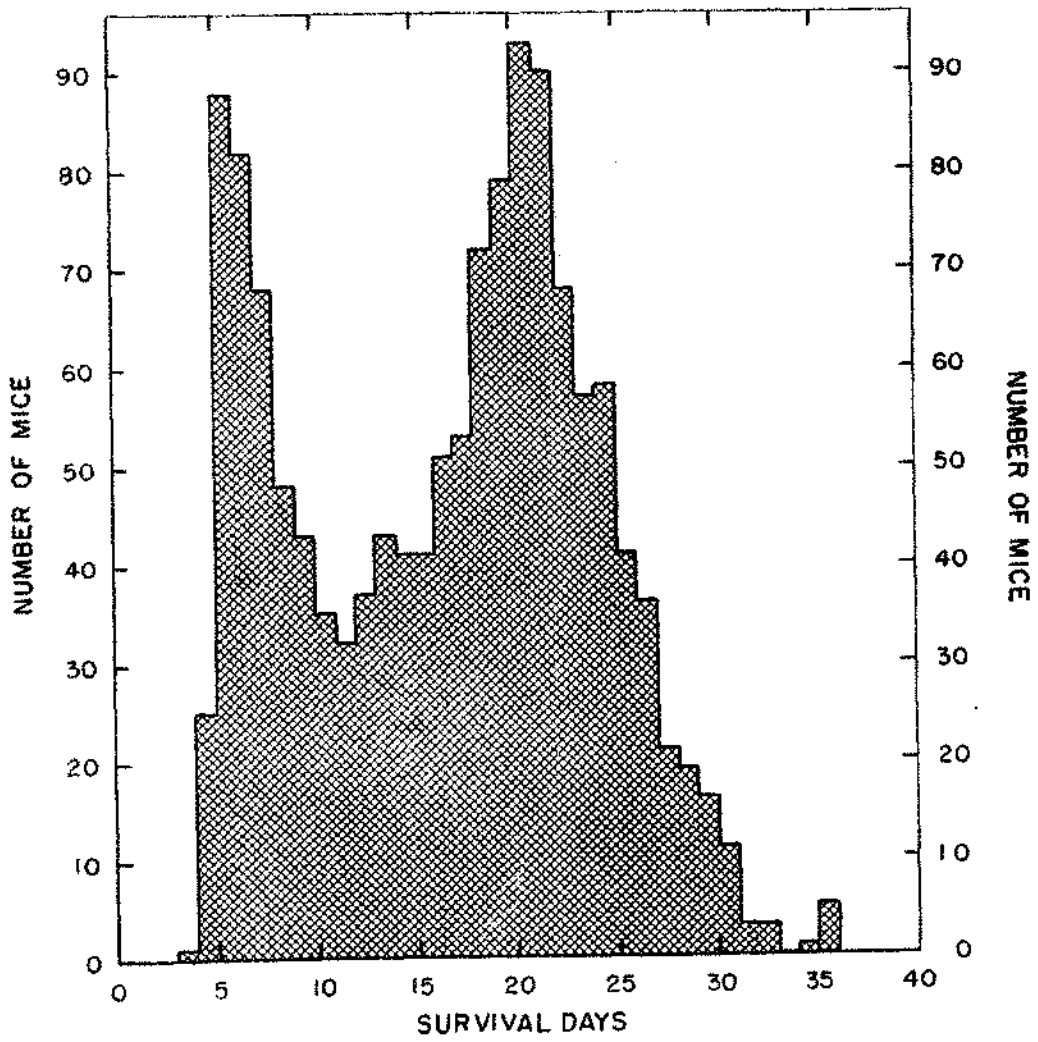
their hybrids, there was usually an almost complete overlap in the range of individual deaths. The longest-lived groups had individuals which died early (six or seven days after inoculation), and the shortest-lived groups had individuals which survived a long time. Among *DBA* mice, for example, (Chart 1), most individuals died in the first week of the infection, but some survived into the third week. In many strains and their hybrids the distribution of deaths was distinctly bimodal (Chart 1), *STR* × *A* and in others (*STR* × *C57* Black) there was only a single wave of deaths. The bimodal distribution of deaths becomes more apparent when large numbers of mice of various extractions are pooled (Chart 2). There is one distinct peak of deaths around Day 6 and another around Day 21. All of the 1,740 mice involved in these studies died by Day 36. The mean values for survival which have been presented in the tables are in essence a reflection of the proportion of the mice in each group which survived the first peak or wave of deaths.

CHART 1.



Survival of some inbred and hybrid mice infected with *Plasmodium berghei.* (Nadel, Greenberg, Jay and Coatney, unpublished).

CHART 2.



Summary of the survival of mice of various genetic backgrounds after inoculation with *Ptasmodium berghei*. (Nadel, Greenberg, Jay and Coatney, unpublished).

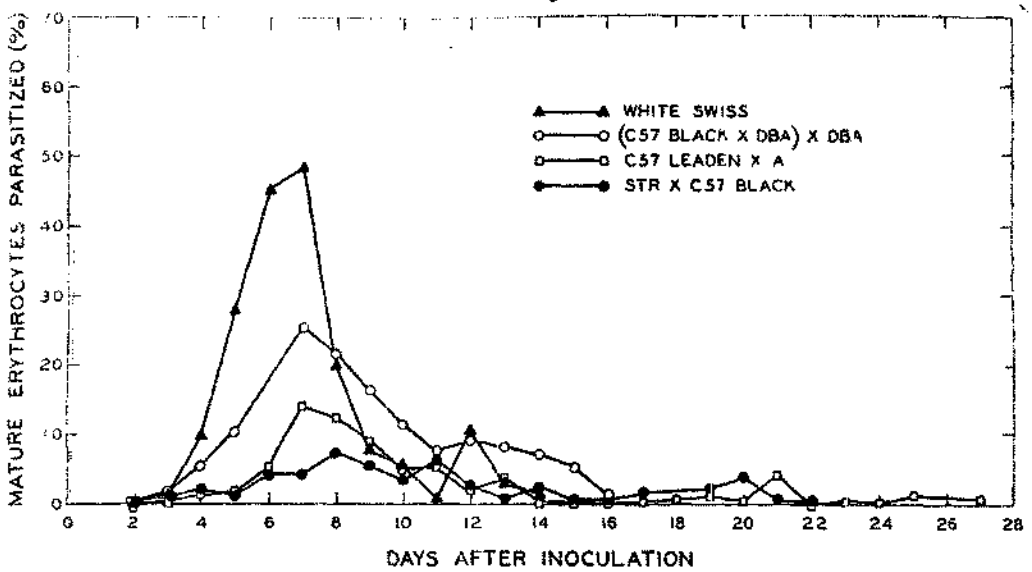
An explanation for this bimodal death curve was sought in an examination of the pathological changes produced in short-(white Swiss) and long-lived mice (C_{57} Leaden $\times$ *A*, *LAF*) by *P. berghei* (Highman, Greenberg and Coatney, 1954). By the end of the first week after inoculation, mice of both groups exhibited pathological changes which were, however, more severe in the white Swiss than in the *LAF* mice. Some pathological changes present at the end of the first week in *LAF* mice, such as fatty infiltration of the heart muscle, disappeared thereafter. It

was felt that the Swiss mice, most of which died within eight to ten days after inoculation, died of toxæmia or shock brought on by the rapid loss of erythrocytes. The *LAF* mice, most of which survive to the third week, died probably of anoxic anæmia.

It was also found during the examination of tissues for pathological changes that there were distinct differences between the two strains in the course of the infection as measured by the number of mature and immature erythrocytes infected at various stages. In both groups, the infection during the first eight days was predominantly in mature erythrocytes. In Swiss mice, up to 84 per cent of the mature erythrocytes were infected by Day 8; in *LAF* mice, less than 26 per cent of these cells were infected. In the latter, the infection in mature cells declined after Day 8 until by the third week less than 12 per cent were infected. Accompanying this decline in the infection of mature erythrocytes there was in *LAF* mice an increasing predominance of young erythrocytes in the total population. These young erythrocytes were heavily parasitized (about 90 per cent) throughout the course of the infection. In *LAF* mice, then, the initial infection was predominantly in mature erythrocytes, the later infection predominently in young erythrocytes. In Swiss mice, the second phase did not occur because of the death of the animals.

A more complete study of the type of cells invaded at various stages of the infection was then made (Greenberg, Highman and Coatney, unpublished), using four strains of mice which, in our experience, had a distinctly different mean survival time: white Swiss (about eight days), (*C*57 Black × *DBA*) × *DBA* backcross (13 days), *C*57 Leaden × *A* (20 days), and *STR* × *C*57 Black (25 days). In all the mice which survived sufficiently long (ten *plus* days), there was a distinct peak of infection in mature erythrocytes on days 7 to 9. Thereafter the count in mature cells dropped and remained low throughout the life of the mouse (Chart 3). The

CHART 3.

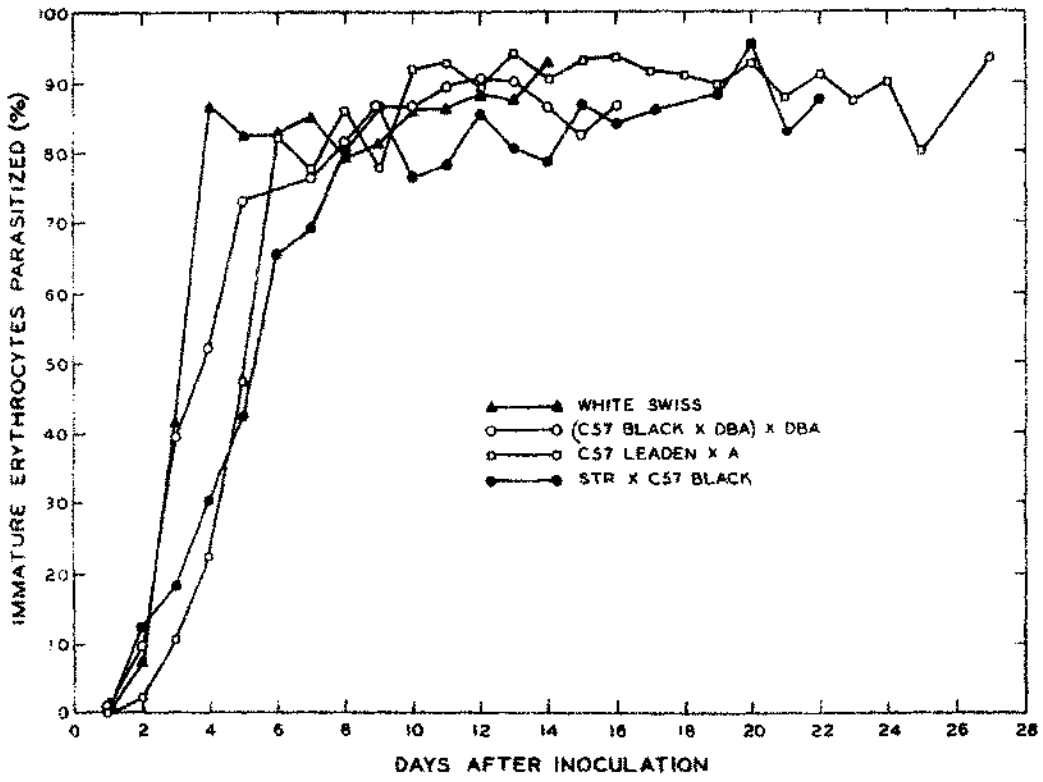


The course of *P. berghei* infection in mature erythrocytes of four genetically distinct strains of mice.

rate of increase of the infection in mature erythrocytes, as well as the ultimate peak achieved, varied among the strains inversely as the survival of the strains based on our previous experience. In the shortest-lived (Swiss) mice, about 50 per cent of the mature erythrocytes were infected at the peak; in the longest-lived (*STR* ×*C*57 Black), the peak was less than ten per cent. The correlation between the highest infection in mature erythrocytes and survival was not as simple when the fate of individual mice was considered. Individual mice with the highest infections died earliest, but those with the lowest infections did not die latest. Even in a rather limited series, such results would indicate that while there was very likely causal correlation between high infections of mature erythrocytes and early deaths, the effect of the infection in mature erythrocytes upon animals which survived the first ten days, is not as clear.

In all strains, the infection in immature erythrocytes (Chart 4) rose rapidly to about 90 per cent by Day 3 and remained at this level throughout the course of the infection. In all strains the proportion of immature to mature cells began

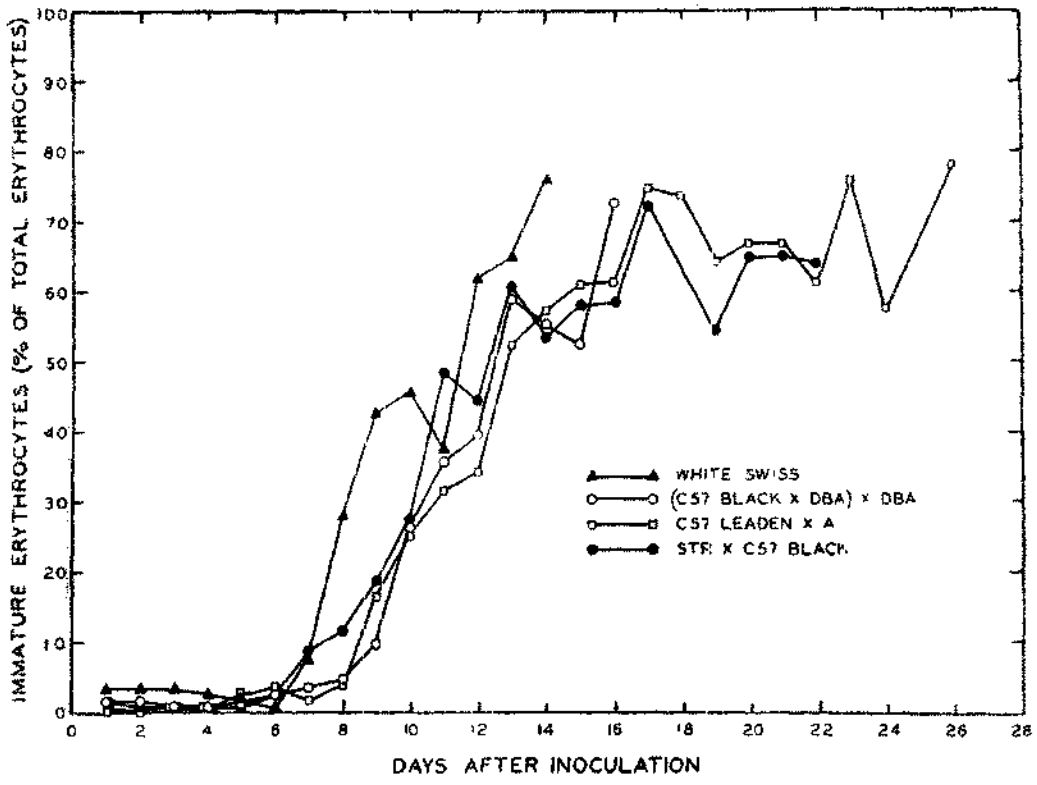
CHART 4.



The course of *P. berghei* infection in immature erythrocytes of four genetically distinct strains of mice.

to increase on Day 7, and by Day 13 reached a stable level of 50 to 60 per cent (Chart 5). The total red cell count (Chart 6) began to drop on Day 3, from 9 to 11 million to about one million in all mice which survived sufficiently long. There were differences in the rate of fall in the erythrocyte count between Swiss and the other mice, but not among the others. If any differences existed among the strains in the rate of increase of the infection in immature cells or the rate of increase of the proportion of immature cells, they were too small to be significant in our sample.

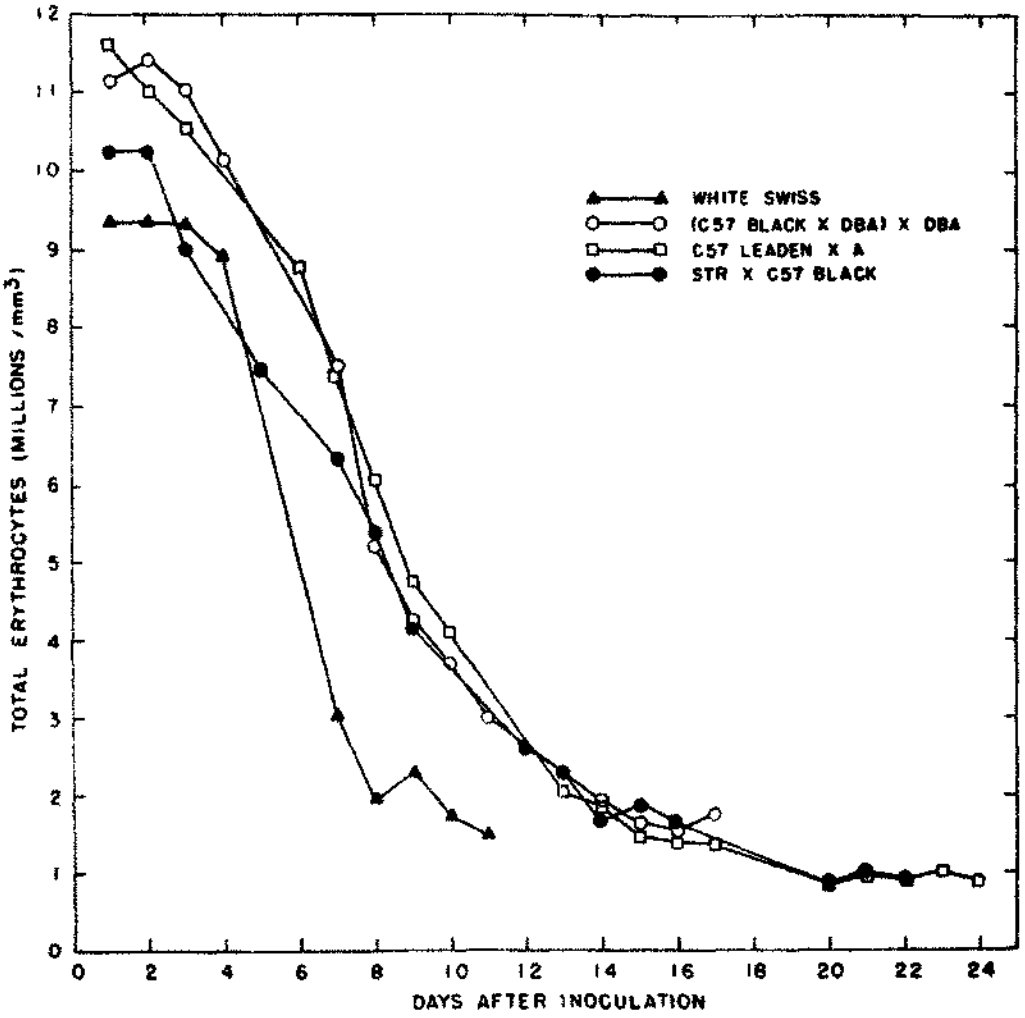
CHART 5.



The proportion of immature erythrocytes to total erythrocytes during the course of a *P. berghei* infection in four genetically distinct strains of mice.

Total parasitæmia was, then, actually a summation of three probably interrelated events: (1) the rise and fall of the infection in mature erythrocytes; (2) the almost constant high rate of infection in immature cells; and (3) the eventual increase of the proportion of young erythrocytes in the population to a more or less steady level of 50 to 60 per cent. The resultant is depicted in Chart 7.

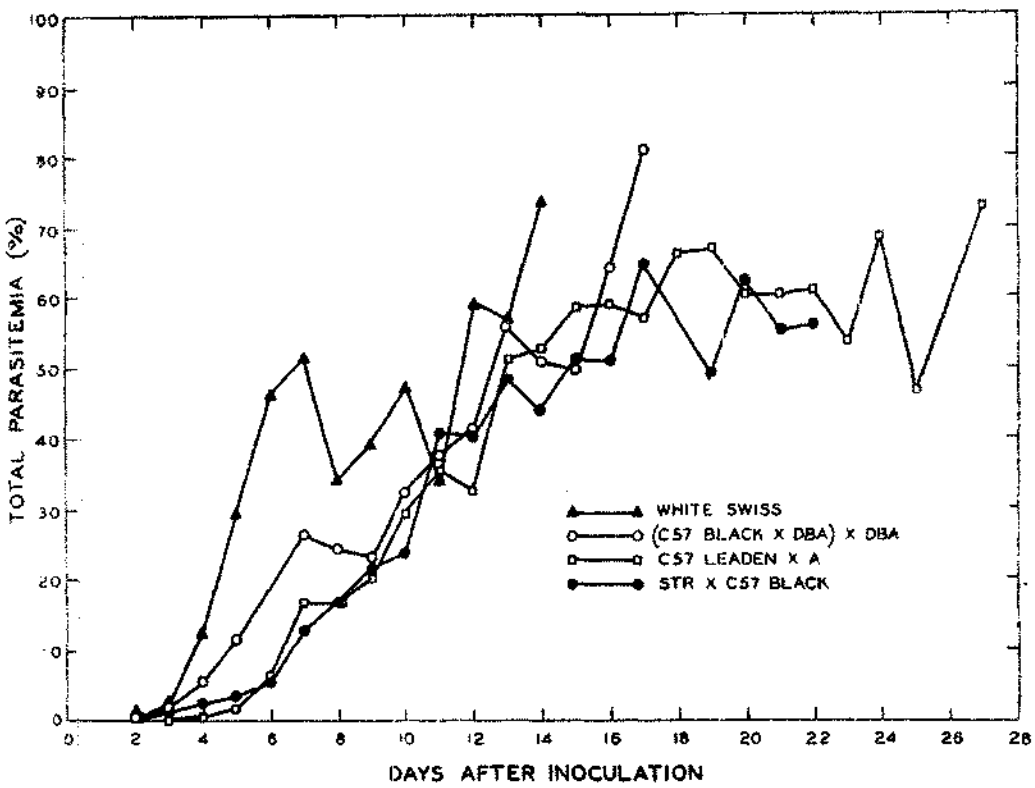
CHART 6.



Total red cell count in four genetically distinct strains of mice infected with *P. berghei*.

Data have been presented in several reports to show that *P. berghei* has a predilection for immature erythrocytes (Ramakrishnan and Prakash, 1950 ; Baldi, 1950 ; Corradetti and Verolini, 1951 ; Ramakrishnan, Prakash and Krishnaswami, 1951 ; Hsu and Geiman, 1952 ; Fabiani, Clausse and Fulchiron, 1952). It is obvious from our experiments that this predilection is relative and varies with the strain of mouse and in the same mouse with the stage of the infection. This observation brings up some pertinent points. It is obvious that even some of the shortest-lived mice can control the infection in mature erythrocytes,

CHART 7.



The course of *P. berghei* infections in all erythrocytes of four genetically distinct strains of mice.

but none of the mice examined seemed able in any significant way to limit the infection in immature erythrocytes. The mechanism of such a highly selective immunological response would seem worthy of further investigation.

Several explanations could be offered for the decline of the infection in mature cells : (1) There may be two different types of mature erythrocytes, only one of which is vulnerable to attack by the parasite. The proportion of these types of cells may vary from strain to strain ; (2) The host is able to make a certain proportion of its mature erythrocytes refractory in response to the initial invasion ; (3) The mouse acquires the ability to remove the mature erythrocytes as soon as they are invaded. While none of these explanations may be correct, we favour the last because it explains certain supplementary anomalies of the infection in mice.

The total erythrocyte count dropped rapidly, in *STR* × *C57* Black mice for example, even though less than ten per cent of these cells were invaded at the peak of the infection. This would imply that destruction of mature erythrocytes continued even though infected cells were difficult to find in the circulation. The destruction of the erythrocytes could have been the result of a non-specific toxemia. However, it did not appear from the data that the rate of destruction of mature

erythrocytes was related in any way to the total number of infected erythrocytes, mature or immature. It seems anomalous that a mouse, which limited the infection in mature cells to less than ten per cent, should continue to lose sufficient cells to call forth the decided reticulocyte response. It might be, however, that the mice in some manner acquire the ability to remove mature erythrocytes from the circulation as soon as they are invaded, but do not acquire the same ability regarding immature cells and these act as a reservoir for the further invasion of mature cells.

This hypothesis can be examined in the light of observations on other rodents as hosts of *P. berghei*. Appendix I is a summary, not definitive, of mammalian hosts which have been tested for infectivity by *P. berghei*. These animals seem to fall into four general groups, with some minor overlapping, as regards their response to the parasite. Mice and hamsters develop an acute and fatal infection (Group 1) ; voles and young rats (Group 2) develop an infection which is fatal to many but not all. Animals in Group 3 exhibit an infection which is benign and self-limiting; animals in Group 4 may or may not become infected, but if they do it is only under extremely special conditions.

Studies in the young rat (Corradetti and Verolini, 1951) indicate that the infection in mature erythrocytes rarely exceeds three per cent, while in the immature erythrocytes it approaches 90 per cent. The young rat resembles, then, the long-lived strains of mice. The same peculiar anomaly applies to the young rat as to the long-lived mouse; why, in the face of a two to three per cent infection in mature cells, is there sufficient destruction of these cells to cause an anæmia and a decided reticulocyte response ? The rat may be depicted as having the innate ability to limit the infection in mature cells by some filtering process which removes these infected cells, but is lacking in the ability to control the infection in immature cells. If this is true, the immature cells may act as a reservoir for further infection of the mature cells.

Little information is available on the cell preference of *P. berghei* in animals of Group 3. These animals would appear to be able to limit the infection in both mature and immature erythrocytes. However, it is significant that after splenectomy, animals in this group behave like those in Group 2 : the infections become acute and in certain specific instances the parasites are found predominantly in immature erythrocytes (Adler, Yoeli and Zuckerman, 1950 ; Zuckerman and Yoeli, 1951 ; Fabiani, Clausse and Fulchiron, 1952).

Our conclusions might be summed up as follows : There are in rodents two mechanisms for the control of *P. berghei* ; one specifically removes infected mature cells, the other infected immature cells. Some rodents, such as the mouse, possess neither mechanism inherently but can acquire one, the ability to remove infected mature cells. Strains of mice differ among each other in their ability to call forth the one immunological response they have. This difference accounts to some extent for the differences in survival among short- and long-lived strains of mice.

Some rodents, such as young rats, possess innately, or rapidly acquire, the ability to limit the infection in mature erythrocytes but most of them lack the ability to remove infected immature erythrocytes. In this respect they resemble long-lived mice.

Other rodents possess or acquire both types of defence and can remove mature or immature cells as they become infected. A reservoir for the reinfection of mature cells does not exist and there is no rapid loss of red cells, no anæmia, and no reticulocytosis. However, these animals, when splenectomized, behave like young rats.

Though this thesis may be speculative, it presents several areas for further investigation. Especially of interest to us is the possible genetic influence on the ability of the host to control the infection in specific types of cells.

REFERENCES.

ADLER, S., YOELI, M. and ZUCKERMAN, A. (1950) ... ... *Nature*, **166**, p. 571.
BALDI, A. (1950) ... ... *Riv. di malariol.*, **29**, p. 349.
Idem (1952) ... ... *Ibid.*, **31**, p. 41.
BRAY, R. S. (1951) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **44**, p. 362.
CORRADETTI, A. (1950) ... ... *Riv. di parassit.*, **11**, p. 201.
CORRADETTI, A. and VEROLINE, F. (1951) ... *Ibid.*, **12**, p. 69.
DESCHIENS, R. and LAMY, L. (1951) ... *Bull. Soc. Path. Exot.*, **44**, p. 405.
DURAND, P. and MATHIS, M. (1950) ... *Compt. Rend. Acad. d. Sc.*, **231**, p. 633.
Idem (1951) ... ... *Compt. Rend. Soc. de Biol.*, **145**, p. 29.
FABIANI, G., CLAUSSE, J. and FULCHIRON, G. (1952) ... ... *Compt. Rend. Acad. d. Sc.*, **235**, p. 274.
GALLIARD, H. and LAPIERRE, J. (1951) ... *Bull. Soc. Path. Exot.*, **44**, p. 185.
GREENBERG, J., NADEL, E. M. and COATNEY, G. R. (1953) ... ... *J. Infect. Dis.*, **93**, p. 90.
Idem (1954) ... ... *Ibid.*, **95**, p. 114.
HIGHMAN, B., GREENBERG, J. and COATNEY, G. R. (1954) ... ... *Riv. Parassit.* (in press).
HILL, J. (1950) ... ... *Ann. Trop. Med. Parasitol.*, **44**, p. 291.
HSU, D. Y. M. and GEIMAN, Q. M. (1952) ... *Amer. J. Trop. Med. Hyg.*, **1**, p. 747.
MERCADO, T. I. and COATNEY, G. R. (1951*a*) *J. Nat. Malaria Soc.*, **10**, p. 359.
Idem (1951*b*) ... ... *J. Parasit.*, **37**, p. 479.
Idem (1953) ... ... *Amer. J. Trop. Med. Hyg.*, **2**, p. 30.
RAFFAELE, G. and BALDI, A. (1950) ... *Riv. di Malariol.*, **29**, p. 341.
RAMAKRISHNAN, S. P. and PRAKASH, S. (1950) *Ind. J. Mal.*, **4**, p. 369.
Idem (1951) ... ... *Nature*, **167**, p. 533.
RAMAKRISHNAN, S. P., PRAKASH, S. and KRISHNASWAMI, A. K. (1951) ... *Ind. J. Mal.*, **5**, p. 447.
VAN RIEL, J. (1950) ... ... *Ann. Inst. Pasteur*, **79**, p. 772.
RODHAIN, J. (1949) ... ... *Ann. Soc. Belge de Med. Trop.*, **29**, p. 483.
Idem (1951) ... ... *Ibid.*, **31**, p. 289.
SCHNEIDER, J., DECOURT, PH. and MONTEZIN, G. (1949) ... ... *Bull. Soc. Path. Exot.*, **42**, p. 449.
SCHNEIDER, J. and MONTEZIN, G. (1950) ... *Bull. Soc. Path. Exot.*, **43**, p. 144.
SCHNEIDER, J. and SCHNEIDER, C. R. (1950) *Ibid.*, **43**, p. 324.
SERGENT, Ed. and PONCET, A. (1950) ... *Arch. Inst. Pasteur d'Algerie*, **28**, p. 323.
Idem (1951) ... ... *Ibid.*, **19**, p. 269.
THURSTON, J. P. (1950) ... *Brit. J. Pharmacol.*, **5**, p. 409.
VINCKE, I. H. and LIPS, M. (1948) ... *Ann. Soc. Belge de Med. Trop.*, **28**, p. 97.
VINCKE, I. H., and VAN DEN BULKE, M. A. (1949*a*) ... ... *Ibid.*, **29**, p. 525.
Idem (1949*b*) ... ... *Ibid.*, **29**, p. 537.
Idem (1949*c*) ... ... *Ibid.*, **29**, p. 545.
ZUCKERMAN, A. and YOELI, M. (1951) ... *J. Infect. Dis.*, **89**, p. 130.

Appendix I.

Types of reactions to infections with P. berghei *by various hosts.*

Class I—Acute, fatal

Mus musculus	...	Vincke and Lips, 1948 ; Schneider, Decourt and Montézin, 1949 ; Vincke and van den Bulke, 1949*a* ; Thurston, 1950 ; Schneider and Schneider, 1950 ; Schneider and Montézin, 1950; Mercado and Coatney, 1951*b*.
Mesocricetus auratus	...	Hill, 1950 ; Adler, Yoeli and Zuckerman, 1950 ; Durand and Mathis, 1950 : 1951.

Class II—Acute, some fatal

Rattus rattus (young)	...	Vincke and van den Bulke, 1949*b*; Corradetti, 1950 ; Raffaele and Baldi, 1950; Baldi, 1950; Ramakrishnan and Prakash, 1950 ; Ramakrishnan, Prakash and Krishnaswami, 1951 ; Galliard and Lapierre, 1951 ; Mercado and Coatney, 1951*a*;
Microtus gunthери	...	Adler, Yoeli and Zuckerman, 1950; Zuckerman and Yoeli, 1951.
Microtus pennsylvanicus	...	Mercado and Coatney, 1953.
Thamnomys surdaster		Vincke and van den Bulke, 1951.
Oryzomys palustris	...	Mercado and Coatney, 1953.
Perognathus penicillatus	...	Mercado and Coatney, 1953.
Perognathus baileyi	...	Mercado and Coatney, 1953.
Perognathus intermedius	...	Mercado and Coatney, 1953.
Dipodomys spectabilis	...	Mercado and Coatney, 1953.
*Dipodomys merriami**	...	Mercado and Coatney, 1953.
Acomys cahirinis	...	Mercado and Coatney, 1953.

Class III—Benign, self-limiting

Sygmodon hispidus	...	Rodhain, 1949 ; Mercado and Coatney, 1953.
Clethriomonys glareolus	...	Bray, 1951.
Sciurus palmarum	...	Ramakrishnan and Prakash, 1950; 1951.
Meriones shawi	...	Sergent and Poncet, 1950; 1951.
Roussettus leachi	...	van Riel, 1950.
Rattus rattus (old)	...	Raffaele and Baldi, 1950 ; Galliard and Lapierre, 1951.

Class IV—No infection or extremely transient

Cavia cobaya	...	Vincke and Lips, 1948 ; Raffaele and Baldi, 1950; Deschiens and Lamy, 1951; Baldi, 1952.
Lepus caniculus	...	Vincke and Lips, 1948 ; Raffaele and Baldi, 1950 ; Deschiens and Lamy, 1951.

* Classification in question due to insufficient data.

Indian Journal of Malariology, **8**, 4, December 1954.

MALARIA AND NUTRITION WITH SPECIAL REFERENCE TO *PLASMODIUM BERGHEI* INFECTIONS IN RATS.

BY

S. P. RAMAKRISHNAN.

(*Malaria Institute of India, Delhi.*)

(October 29, 1954.)

MANKIND has from time immemorial been interested in the relationship between nutrition and resistance to disease. The establishment of infective ætiologic agents for specific diseases has in no way minimized the effort at an understanding of the effect of nutrition on the various stages of host parasite relationship. The effect of nutrition, perhaps, plays very little part in acute infections with dramatic onset, a rapid course and its termination by crisis with the establishment of a more or less solid immunity against the pathogen among survivors. Even in such acute infections, especially of a viral origin, the effect of nutrition of the host on the pathogen is well recognized. Nutrition plays a more prominent part in the establishment of host parasite relationship in the case of those infective agents which exhibit a period of primary attack, latency, relapses and end in death of the host, or a more or less complete eradication of infection. Certain effects of nutrition were partially assumed on *a priori* considerations and others deduced from epidemiological observations. But there is a comparative paucity in literature to determine the effect of nutrition with precise experimental methods, at all events, with respect to the host parasite relationship in mammalian malaria. Such relationship has no doubt been studied between pathogens other than the malarial parasite and their hosts. A fair amount of work has been carried out to study such relationship in avian malaria,* and to a lesser extent, in the case of simian malaria.†

In the ultimate analysis, the effect of nutrition on host parasite relationship in human malaria is of the most vital importance. *Plasmodium berghei* malaria in rodents offers an unprecedented opportunity to study the effect of nutrition on mammalian host parasite relationship and correlate the experimental findings with epidemiological observations in human malaria. This paper records a review of the author's work and the results.‡

*Brackett, Waletzky and Baker (1946) ; Brooke (1945) ; Roos. Hegsted and Stare (1946) ; Seeler and Ott (1944 : 1946) ; Seeler, Ott and Gundel (1944) and Trager (1943 : 1947*a* : 1947*b* : 1949).

†Bray and Garnham (1953); Jaswant Singh *et al.* (1953) ; McKee and Geiman (1948) and Passmore and Sommerville (1940).

‡This paper forms part of a thesis submitted for the degree of Doctorate in Public Health.

The ultimate aim of the investigation is to attempt to furnish an answer to the following questions: (1) What effect does adequate nutrition or its inadequacy in quality or in quantity has on the degree of multiplication of the malarial parasites in man ? (2) To what extent does nutrition affect man's ability to contend against the primary infection ? (3) What rôle does nutrition play on the relapse patterns ? (4) What effect does nutrition play on the effects of parasitic multiplication in the stage of acute parasitæmia and during relapses on man ? Finally (5) what practical suggestions have the results of experiments to offer in determining the strategy of malaria control under conditions of semi or acute starvation ?

In the fulfilment of these aims by experimental methods, the choice has naturally to be made of a suitable host and a suitable parasite which would give the best approximation to conditions of host parasite relationship in human malaria. Other considerations such as the ease of manipulation of the laboratory animal and the maintenance of the virulence of the parasite without any change throughout the course of the experiment have also to be borne in mind. The bulk of the hosts used in the experiments consists of rats; the plasmodial species selected, namely *berghei* kills off a certain proportion of the rats during acute infections, a good number of the rats survive such infections, and after suffering one or more relapses, there is an almost complete eradication of the infection. Amongst human plasmodia, *vivax, malariæ* and *ovale* do not have any direct fatal effect on man. *Falciparum*, on the other hand, runs a fatal course even during acute parasitæmia in a certain proportion of cases. This species has a tendency to relapse much fewer times than *vivax*. The capacity of the host to bring about a complete eradication of the infection is manifest to a greater extent in infections of this species than with *vivax*.

Mice have also been included as hosts in a very limited number of experiments since *P. berghei* runs a very rapid course of acute parasitæmia in this host with an invariable fatal termination. In experiments with this host, an attempt has been made to ascertain the basic nutritional requirements of the parasite. But for a study of the more important effects of nutrition on the various stages which follow infection and establish different degrees of host parasite relationship, the rat has been chosen as the more useful host for purposes of experiment.

The experiments were planned to make a broad-based study of nutrition and its effects on mammalian malaria. Specific problems that have been investigated include a consideration of the effects of total starvation, under-nourishment, different diets adequate in quantity but varying in quality, and specific deficiency of two vitamins on host parasite relationship in the various stages which follow an acute infection. An attempt has also been made to determine to what extent a few specific nutrients are necessary for parasitic multiplication or for the effective operation of innate immunity on the part of the host.

In the course of critical review of the investigations on the subject of nutrition and disease, Clark *et al.* (1949) have discussed several flaws in different experimental models. Some of these are important like age, genetic homogeneity, uninfected controls on experimental diet, and ensurement of constancy of the virulence of pathogen throughout the investigations. These were given due consideration by the author in his experiments.

The criteria chosen for assessment of the effect of diet on the course of infection, namely, average daily parasitæmia, peak and duration of patent parasitæmia, immunity to challenge reinfection and the average enlarged spleen volume of animals were comprehensive and enabled the author to interpret the two-fold effects of diet separately on the parasite, and the host defence mechanism both against the parasite as such and its 'toxic'* effects.

The results of the experiments described by the author (Ramakrishnan, 1953) show that even with starvation for as long as ten days the infection is not completely eradicated. On account of the extreme starvation, however, the parasites are unable to multiply sufficiently long and go into latency at a fairly early stage during the primary acute attack. After subsequent adequate nourishment, the latent phase gives place to manifest parasitæmia, but the course of infection is much less virulent than in animals fed throughout. Conditions of acute starvation do not normally occur in man, except during acute famine. These experimental results are in accord with the general findings during the Bengal famine in 1943 (Famine Inquiry Commission Report on Bengal, 1945). During the period of acute famine, the number of men showing parasites in their peripheral blood was extremely small. Presumably on account of acute emergency, there is no factual record of such a phenomenon in literature. This, however, has been the common experience of the workers in Calcutta as frequently referred to by them during the course of scientific discussions. There is also evidence for this phenomenon in that direct deaths due to malaria recorded during the acute period of famine, were very low indeed.

Starvation for only five days and subsequent feeding brings out parasitic relapse more prominently than in the case of starvation for ten days before feeding. But even under such a condition the course of parasitæmia reached is milder than in the case of animals fed throughout on normal diet. When the animal is starved for five days and then experimentally inoculated and fed on normal diet, the subsequent course of parasitæmia is much more severe than in the case of an animal fed throughout on a normal diet. This shows that conditions of complete starvation before infection greatly undermine the operation of the innate defensive mechanism of the host against the parasite. This has a bearing on famine conditions where, though there may be a very low degree of parasitæmia during the period of acute famine, on establishment of facilities for feeding, the course of parasitæmia is likely to be much more severe than in a community fed on normal diet throughout. It is this factor that has to be specially borne in mind in providing facilities for rehabilitation after acute famine. During the Bengal famine of 1943, it has been recorded that mortality due to malaria after establishment of feeding centres was very much higher than in the previous quinquennial average. While it is obvious that facilities for feeding would have to be established during famine, it would also seem necessary that, in order to mitigate the more virulent course of infection which is likely to follow the provision of feeding facilities, simultaneous arrangements should also be made by way of providing suitable antimalarial drugs on a mass scale along with the provision of feeding facilities. Such arrangements would seem particularly necessary for the younger age groups.

*No true toxin has yet been demonstrated in the case of the malarial parasite, but for purposes of convenience its effects on the host are called 'toxic' effects.

The results of the experiments recorded by Ramakrishnan, Satya Prakash *et al.* (1953*a*) show that methionine, and perhaps to a lesser extent PABA, are essential nutrilites for *P. berghei.* Starvation apparently reduces the availability of these two nutrients below the critical requirements of the parasite. It is also shown that probably starvation does not deplete glucose and biotin below the critical threshold necessary for parasite multiplication. The author considers that administration of pure nutrilites, singly and in combination, to infected starving animals is a useful technique for the study of physiology and metabolism of malarial parasites in different hosts.

It was observed that a ketogenic diet of a high fat content does not favour parasitic growth to the same extent as a balanced diet. Ketosis of starvation probably plays a rôle in addition to the lack of essential nutrilites during starvation which are responsible for poor parasitic growth in starved rats (Ramakrishnan, 1954*a*).

The results recorded by Ramakrishnan (1954*b*) are of considerable interest. Under-nourishment of the host affects the parasite much more than the host during the stage of primary parasitæmia. Hence, in human malaria, communities with inadequate nourishment would probably exhibit a less virulent course of parasitæmia than communities with adequate diet. If under-nourishment played no other rôle in the subsequent course of the infection, one would perhaps not feel pessimistic about the lack of nutrition in so far as the malarial infection is concerned, but the results of the investigation also show that in under-nourished animals parasitic densities are much higher during relapses than in well-fed animals. It is even more important that the host reacts very unfavourably to such relapses even though the parasitic density reached during such relapses is not as high as the parasitic density reached during the course of primary parasitæmia in well-fed animals. In other words, relapses in under-nourished hosts would appear to increase the mortality due to malarial infections. As the human malarial parasite has a fair degree of tendency to relapse, the effects of parasitic multiplication on the host during such relapses are of vital significance in planning the strategy of malaria control. There is, therefore, no warrant to assume that, taking an over-all picture, under-nourishment is a favourable factor so far as the host is concerned in the case of malaria infection. Fortunately modern methods of malaria prevention are so well-defined and so well within the competence of even rural communities in under-developed countries, that they are being adopted on a large enough national scale.

The effect of milk diet on malaria is now a topic of world interest. The author was one of the earliest to investigate the effect of milk diet on rodent malaria and found that milk was probably partially deficient in some essential nutrilites required by the parasite (Maegraith, Deegan and Jones, 1952 ; Ramakrishnan, Satya Prakash *et al.*, 1953*b*). The author and his colleagues have subsequently shown that this effect, however, is variable with a different plasmodium (Ramakrishnan, Bhatnagar *et al.*, 1953). In the case of *P. gallinaceum*, a pure milk diet was very favourable for parasitic multiplication. On the whole, therefore, the effect of milk diet on the course of parasitæmia would seem to depend not so much on its dietary value but rather on certain constituents required by certain species of plasmodia being present or absent in a milk diet.

Diets may be largely grouped into three broad divisions namely, (1) a pure vegetarian diet, (2) a lacto-vegetarian diet, and (3) a mixed diet containing meat.

In the case of *P. berghei* infections in rat (Ramakrishnan, 1954*c*), a lacto-vegetarian diet was found to favour the host better than a vegetarian diet or a mixed diet of high calorific value made up of a high proportion of meat. On the other hand, a mixed diet with a low proportion of meat and isocalorific with a lacto-vegetarian diet seemed to favour the host even better. With a high meat diet, not only was the course of primary parasitæmia more severe, but the fatality of the host in chronic infections was also of a higher order. With a pure vegetarian diet, though the course of primary parasitæmia was more severe than in the case of a lacto-vegetarian diet, it did not prevent the acquisition of specific immunity by the host as shown by the survivors being resistant to a challenge infection almost to the same extent as survivors on a lacto-vegetarian diet. On the whole, therefore, these experiments would appear to establish that a mixed diet with a low proportion of meat would be the most ideal. A lacto-vegetarian diet would be the next best, but a pure vegetarian diet would seem to be better than a mixed diet containing a high proportion of meat.

The results of unpublished experiments (Ramakrishnan, 1953) show that in the case of mice which are highly susceptible to *P. berghei*, biotin deficiency does not affect the course of parasitæmia, but the mice succumbed earlier than animals on a diet containing biotin, and after only smaller peak densities were reached. In the case of rats, which are less susceptible than mice, biotin seemed to affect the forces of natural innate immunity during the acute primary attack and the degree of immunity acquired towards its end. Pyridoxin has been found to be a more essential requirement for *P. berghei* (Ramakrishnan, 1954*d*).

Man's primary interest in nutrition and resistance to disease is indefinably associated with an abstract hope—not unlike that of an alchemist—that by a suitable manipulation of diet alone he may achieve freedom from disease. In so far as resistance to parasitic diseases is concerned, the concept of specificity of host parasite relationship has been so repeatedly demonstrated that a universal resistance to all parasitic diseases is to be definitely considered as impossible. Indeed, it is well known that resistance developed towards one species of a parasite is not available against another pathogenic species of the same genus or even strains of the same species.

Good and even buoyant health, provided by good nutrition and other factors, is no insurance against malaria. Contrary to popular opinion, available evidence indicates that better the nutrition of an individual, the greater are the facilities offered to malaria organisms to grow and multiply during the primary attack. It is, however, a fact that freedom from malaria promotes good health, human endeavour and output in agriculture, industry, etc. In a relapsing disease such as malaria one should not only consider the course of primary parasitæmia but also its relapses and their effects on man. These are the effects that mainly contribute to mortality due to malaria both directly and indirectly. These effects are very considerably affected by malnutrition either due to inadequacy or wrong quality.

Susceptibility to parasitic disease of a species and even a race of the same species is governed by laws of heredity. Nutrition on the other hand is an attribute of the environment. But the investigator whose problem is nutrition and disease, has to consider heredity and environment together, as both these are the two aspects of a single phenomenon, namely, the Natural World (Schneider, 1949).

In experiments on this problem "The interfering agents are so varied, some exceedingly simple, potassium and phosphorus, and others more complex, vitamins, vitamins analogues, and metabolites in differing phases of nutrition, and some of the results are so striking with almost 100 per cent differences between controls and experimental animals, that one must urge more and continuous study along many of the suggestive leads" (Clark, 1949).

Investigations on nutrition and parasitic diseases are no more undertaken to find a single principle of diet which will afford immunity to all parasitic diseases. They are conducted as in the author's series of experiments for an understanding of the natural history of disease. Adequate methods of prevention or interception of transmission of parasitic and infectious diseases are available today to an extent that many of these can be eliminated or at least kept under adequate control irrespective of the nutritional status of a population. Indeed, as referred to earlier, there is sufficient evidence that a disease like malaria promotes poverty, reduces the productive effort of man and results in under-nutrition (Sinton, 1935*a*: 1935*b* : 1936).

REFERENCES.

Brackett, S., Waletsky, E. and Baker, M. (1946) ... ... *J. Parasit.*, **32**, pp. 453-462.
Bray, R. S. and Garnham, P. C. C. (1953) *Brit. Med. J.*, May 30, pp. 1200-1204.
Brooke, M. M. (1945) ... ... *Amer. J. Hyg.*, **41**, pp. 81-108.
Clark, P. F. (1949) ... ... *Bact. Rev.*, **31**, pp. 122-127.
Clark, P. F., McCuing, L. S., Pinkerton, H., Price, W. H., Schneider, H. A. and Trager, W. (1949) ... ... *Ibid.*, **31**, pp. 99-134.
Famine Inquiry Commission Report on Bengal (1945) ... ... Government of India Press, New Delhi, p. 118.
Jaswant Singh, Nair, C. P., Ramakrishnan, S. P. and Ray, A. P (1953) ... *Ind. J. Mal.*, **7**, pp. 253-260.
Maegraith, B. G., Deegan, T. and Jones, E. S. (1952) ... ... *Brit. Med. J.*, Dec. 27, pp. 1382-1384.
McKee, R. W. and Geiman, Q. H. (1948) ... *Parasitic infections in man*, edited by Harry Most, Columbia University Press, New York.
Passmore, R. and Sommerville, T. (1940) ... *J. Mal. Inst. Ind.*, **3**, pp. 447-455.
Ramakrishnan, S. P. (1953) ... ... *Ind. J. Mal.*, **7**, pp. 53-60.
Idem (1954*a*) ... ... *Ibid.*, **8**, pp. 85-88.
Idem (1954*b*) ... ... *Ibid.*, **8**, pp. 89-95.
Idem (1954*c*) ... ... *Ibid.*, **8**, pp. 97-105.
Idem (1954*d*) ... ... *Ibid.*, **8**, pp. 107-113.
Ramakrishnan, S. P., Bhatnagar, V. N., Satya Prakash and Misra, B. G. (1953) *Ibid.*, **7**, pp. 261-265.
Ramakrishnan, S. P., Satya Prakash, Krishnaswmi, A. K. and Chanan Singh (1953*a*) ... ... *Ibid.*, **7**, pp. 225-228.
Idem (1953*b*) ... ... *Ibid.*, **7**, pp. 61-65.
Roos, A., Hegsted, D. M. and Stare, P. J. (1947) ... ... *J. Nutrition*, **32**, pp. 473-484.
Schneider, H. A. (1949) ... ... *Bact. Rev.*, **31**, pp. 101-105.
Seeler, A. O. and Ott, W. H. (1944) ... *J. Inf. Dis.*, **75**, pp. 175-178.
Idem ... ... *Ibid.*, **77**, pp. 181-184.
Seeler, A. O., Ott, W. H. and Gundel, M. E. (1944) ... ... *Proc. Soc. Expt. Biol. Med.*, **55**, pp. 107-109.
Sinton, J. A. (1935*a*) ... ... *Rec. Mal. Surv. Ind.*, **5**, pp. 223-264.
Idem (1935*b*) ... ... *Ibid.*, **5**, pp. 413-439.
Idem (1936) ... ... *Ibid.*, **6**, pp. 91-169.
Trager, W. (1943) ... ... *J. Expt. Med.*, **77**, pp. 557-582.
Idem (1947*a*) ... ... *Proc. Soc. Exp. Biol. & Med.*, **64**, p. 129.
Idem (1947*b*) ... ... *J. Exp. Med.*, **85**, pp. 663-683.
Idem (1949) ... ... *Bact. Rev.*, **31**, pp. 105-110.

Indian Journal of Malariology, **8**, 4, December 1954.

EXPERIMENTAL STUDY ON THE IMMUNOLOGY OF MALARIA DUE TO *PLASMODIUM BERGHEI.**

BY

EDMOND SERGENT

(*Directeur de l'Institut Pasteur d'Algerie, Alger.*)

(September 21, 1954.)

EXPERIMENTAL study of immunity in rodent malaria due to *Plasmodium berghei* has been investigated only in animals inoculated with infected blood. It has not been possible up to the present time to employ sporozoite-induced infections for such studies. One exception, however, was the study of de Smet (1954). The present paper gives a brief summary of work published up to September, 1954, on innate and acquired resistance and on the mechanism of the organic defence against the parasite.

PART I. INNATE RESISTANCE.

The expression, innate resistance, is preferable to 'natural immunity' to describe congenital absence of susceptibility to an infectious disease. We believe that the term 'natural immunity' is wrong. On the one hand this term (natural immunity) is much too restricted since an immunity resulting from a spontaneous recovery from a disease is also a natural immunity. On the other hand this term is often related to various restrictive states which must be differentiated (Sergent and Parrot, 1935). Papers published up to now enable one to draw a list of animal species which are susceptible, though in various degrees, to experimental inoculation of *P. berghei*. Similarly it is possible to draw a list of animal species which have been shown to be completely resistant to this parasite. The tables below are based on papers published by Satya Prakash, Krishnaswami and Ramakrishnan (1952), Mercado and Coatney (1953) and Thurston (1953).

* The editor is grateful to Dr. L. J. Bruce-Chwatt, Malaria Service, Medical Department, Nigeria for kindly translating the original paper written in French into English.—*Editor*.

TABLE I.

Animal species susceptible to P. berghei. *The susceptible species are predominantly rodents.*

Experimental animal	Author	Experimental animal	Author
Thamnomys surdaster ...	Vincke and Lips (1948).	*Meriones shawi*	Sergent and Poncet (1950).
Rattus rattus var. *frugivorus*...	,, ,,	*Graphiurrus murinus* ...	Vincke (1950).
var. *alexandrinus*	,, ,,	*Clethrionomys glareolus britannicus*	Bray (1951).
Mus musculus var. *albinos* ...	,, ,,	*Mus musculus spretus* ...	Durand and Mathis (1951).
Signodon hispidus ...	Rodhain (1949).	*Dipodillus campestris* ...	,, ,,
Pelomys frater	Vincke (1950)	*Microtus pennsylvanicus pennsylvanicus*	Mercado and Coatney (1953).
Rattus concha	,, ,,	*Oryzomys palustris* ...	,, ,,
Rattus tullbergi	,, ,,	*Perognathus penicillatus* ...	,, ,,
Rattus calbino	,, ,,	*Perognathus baileyi* ...	,, ,,
Lophuromys rita ...	,, ,,	*Perognathus intermedius* ...	,, ,,
Seccostomus sp.	,, ,,	*Dipodomys spectabilis* ...	,, ,,
Dendromus mumilio ...	,, ,,	*Dipodomys merriami* ...	,, ,,
Tatera myasoe	,, ,,	*Acomys cahirinus*	,, ,,
Mastomys concha	,, ,,	*Cricetomys ansorgei* ...	de Smet (1954).
Legadda sp.	,, ,,		
Mesocricetus auratus ...	Durand and Mathis (1950).		
Microtus guentheri ...	Adler, Yoeli and Zuckerman (1950).		
Sciurus palmarum	Ramakrishnan and Satya Prakash (1950*b*).		

Apart from rodents, some bats have been shown to be susceptible.

Experimental animal	Author	Experimental animal	Author
Roussettus leachi	van Riel (1950).	*Epomophorus haldemani hallowell*	Rodhain (1952).
Eidelon helvum	Rodhain (1952).	*Micropteropus pusillus* ...	,, ,,

It must be pointed out that all the authors have observed a far greater susceptibility of young animals of the same species than of the fully grown animals. This follows the general rules in parasitology.

According to some authors, some adult mice cannot be infected on the first inoculation and often not even on repeated inoculations. Ramakrishnan and Satya Prakash (1950*a*) reported that 56 per cent males and 24 per cent females resisted the first inoculation but could be infected on the third, fourth or fifth attempts. Matilla *et al.* (1954) found that out of more than 1,500 white mice inoculated with *P. berghei*, 10 resisted repeated attempts to infect them by various routes. Sergent (1954*b*) found that out of 1,450 mice inoculated with *P. berghei*, there was not a single case of resistance. All the mice developed a fatal infection.

Fabiani, Vargues, Fulchiron, Grellet and Verain (1951) recorded that the first attempt to inoculate 12 white rats through the subcutaneous route resulted in a scanty infection only in two, which showed very few parasites in the peripheral blood for a few days only. During investigations on hundreds of white rats,

Sergent (1954*a*) did not find a single instance of resistance to infection through the intraperitoneal route. On the other hand, he found that 13 per cent of rats inoculated through the subcutaneous route did not develop the infection.

2. ANIMAL SPECIES REFRACTORY TO *P. BERGHEI*.

Among small laboratory rodents the guinea-pig and rabbit show an absolute innate resistance to *P. berghei* (Vincke and Lips, 1948 ; Raffaele and Baldi, 1950).

Sergent and Poncet (1951*b*) found the guinea-pig to possess absolute innate immunity to *P. berghei*. They sacrificed a guinea-pig by exsanguination two days after it was inoculated. The blood was inoculated intraperitoneally into 21 mice of which five developed the infection. A second guinea-pig was exsanguinated four days after inoculation and the blood was inoculated intraperitoneally into 26 white mice. Not a single one of these developed the infection. Thus the parasites in the guinea-pig had completely disappeared between the second and fourth day after inoculation.

Deschiens and Lamy (1951) were able to infect new-born rabbits but not born guinea-pigs. Apart from guinea-pigs and rabbits, the following animal species have been shown to be totally refractory to *P. berghei* infection in order of publication.

Man	...	Durand and Mathis (1950).
Monkey	...	,, ,, ,,
Lamb	...	Durbin (1951)
Puppy	...	,, ,,
Kitten	...	,, ,,
Chick	...	,, ,,
Piglet	...	,, ,,
Monkey	...	Satya Prakash, Krishnaswami and Ramakrishnan (1952).
Bandicoot	...	,, ,, ,, ,, ,, ,,
Mongoose	...	,, ,, ,, ,, ,, ,,
Pigeon	...	,, ,, ,, ,, ,, ,,

3. INNATE PSEUDO RESISTANCE.

Sergent (1954) observed that occasionally adult white rats inoculated subcutaneously do not develop the infection and when re-inoculated on several occasions (up to six times), never show parasites in the peripheral blood. When these animals were submitted to "complete proof of infection", that is, when they were sacrificed and their blood and tissues were inoculated into susceptible animals, the latter at times developed the infection. The rats were, therefore, carriers of a latent infection from the commencement (*infection latente d' emblee*). In other words, the infection in the rats was established after inoculation without producing any morbid manifestation establishing a state of premunition. This concept of cryptic infections leading to premunition may explain the negative results obtained by Vincke and van den Bulcke (1949*c*) on a " great number " of *Thamnomys surdaster* which they inoculated with *P. berghei*. *Thamnomys* constitutes a reservoir of infection of *P. berghei* in nature. One could think that the inoculated animals were carriers

of a latent infection and, therefore, were premunised against new infection. In conclusion, there are cases of innate pseudo resistance of premunition due to the presence of a latent infection acquired previously.

PART II. ACQUIRED RESISTANCE.

Experimental inoculation of *P. berghei* to susceptible animals has shown that those which survive the acute infection develop a resistance to re-inoculation. Some authors in their reports indicate the period between the date of the last day of primary parasitæmia and the day of re-inoculation. The earlier date is considered as the date of recovery. Other authors indicate the period between the date of primary and re-inoculation. This latter method seems to be more logical as it is based on two precise dates while the last day of primary parasitæmia or the day of disappearance of parasites from the peripheral blood varies considerably in individuals of the same species. Besides, one often sees parasitic relapses several weeks after the primary parasitæmia in animals considered to have recovered. The observations on acquired resistance have not been based on mice which die of acute infection. The observations have nearly always been on white rats. Since the duration of primary parasitæmia in white rats is always less than one month, it may be possible to standardize the statistical tables to add one month to the period shown as dividing the end of the acute attack from the date of re-inoculation. Resistance to re-inoculations following first attacks may last for a long time. Vincke and van den Bulcke (1949*b*) recorded very considerable resistance for many months after inoculation. Raffaele and Baldi (1950) found that re-inoculation at the end of 90 days after the primary parasitæmia was not successful. Corradetti (1950 : 1952) recorded re-inoculation with negative results 205 days after the end of the primary parasitæmia.

Vargues and Fabiani (1951), Fabiani and Vargues (1951) and Fabiani, Vargues, Grellet, Fulchiron and Verain (1952) found that rats re-inoculated a few weeks after the primary parasitæmia, showed in their peripheral blood rare parasites for a few days or often did not show any at all. Ramakrishnan, Satya Prakash and Krishnaswami (1951) observed the effect of re-inoculation on nine rats in which the infection was in the latent or metacritical stage. The longest period of observation was seven months after the first inoculation. One of the rats which resisted the first re-inoculation five months after the primary attack, resisted a second re-inoculation also 11 months after the first inoculation. Zuckerman (1953) investigated the problem in Palestinian voles (*Microtus guentheri*). Eight of them were re-inoculated through the intraperitoneal route 8 to 41 days after the end of the primary parasitæmia. Three of them showed a few parasites. Six other voles were re-inoculated 57 to 70 days after the primary parasitæmia and only one showed a patent infection. The degree of resistance to re-inoculation bore no relationship with the parasite intensity of the first attack. Sergent (1954*b*) re-inoculated thirty-seven rats recovered from primary parasitæmia on the second and 27th month from the first inoculation. Twenty-five of them showed complete resistance to the re-inoculation. On the other hand, eleven other rats also recovered from primary attack re-inoculated between the eighth and 26th month after the first inoculation, had showed an attack of premunition type. One

single rat, however, re-inoculated during the 23rd month after the primary re-inoculation, showed an infection of moderate intensity.

Specific acquired resistance.—Fabiani and Fulchiron (1952) compared two strains of *P. berghei* by means of cross-inoculation technique of Laveran and Mesnil. One strain was obtained from Professor Schneider of Paris who in turn had received it from Professor Garnham of London, to whom it had been given by Professor Rodhain of Antwerp. The second strain designated Keyberg-173 was received from Professor Dubois, Director of the Institute of Tropical Medicine, Antwerp. The two strains investigated were found to have reciprocal cross-resistance.

Transmission of acquired resistance through milk to young suckling rats.—Bruce-Chwatt (1954) investigated the passive transference of immunity to the offspring through milk of lactating mother previously infected. The gravid rats which resisted the first attack were re-inoculated. They delivered litters which they nursed and the young rats showed a certain amount of tolerance to infection by *P. berghei*. Infection in control young rats born of normal mothers on the other hand was found to be usually fatal. The conclusion arrived at was that the milk of immune mothers transmitted a relative tolerance to their litters.

PART III. THE NATURE OF RESISTANCE ACQUIRED THROUGH PREMUNITION.

Since experiments have proved that first attack of *P. berghei* infection produces a resistance to re-infection, the question remains whether this acquired immunity is due to a true immunity accompanied by a sterilization of the organism or to premunition due to a state of latent infection. To find an answer to the question, one will have to determine whether malaria due to *P. berghei*, like all other known malarias, has a pre-patent stage or incubation period followed by a stage of acute infestation and a stage of latent or metacritical infection.

The existence of a stage of latent metacritical infection can be proved by the appearance of parasitic relapses seen by blood examination, but the amount of blood which can be examined under the microscope is minute even if carried out for a long time and only the positive results can be taken into account. Negative results of blood investigation even of several such cannot be taken as proof that the animal has a sterilizing immunity. The best way to find the presence of latent infection is to inoculate into susceptible animals a considerable amount of blood of the animal under investigation. This is what we call the infectivity test*. But we must distinguish two types of infectivity test. In one type we take the peripheral blood of a living animal and inoculate it into a susceptible animal. The amount of blood obtained from a living animal like the rat from its tail is not considerable; since the amount of blood is small the test is only a partial infectivity test. A second and better method is to inoculate into susceptible animals the total mass of blood of the animal under investigation sacrificed by exsanguination and also to include

*Some authors use the term sub-inoculation which is erroneous from our point of view as it gives rise to ambiguity. For instance, we often say that an experimental subject is inoculated or re-inoculated. To say he was sub-inoculated has no meaning at all because this subject is not inoculated. His blood is taken and another subject is inoculated with his blood.

in the inoculum fragments of the internal organs of the donor animal. This is the total infectivity test. For each sacrificed rat, about 20 experimental rats or mice will be required for the test for intraperitoneal inoculation (Sergent 1954*a*). The unequal value of different methods of detection of latent infections explains the diversity of results obtained by several workers. The superior value of the infectivity test as compared to the simple examination of peripheral blood was shown by several workers* of whom one is quoted here.

In one series of experiments (de Smet, 1954), the blood inoculation gave positive results while at the same time the blood examination gave constantly negative results. Thrice in *Cricetomys ansorgei* and thirteen times in white rats, blood was obtained by partial exsanguination for inoculation into susceptible animals. The positive results would have been even more numerous if the blood had been obtained by total exsanguination. This sample shows that we must not believe that an animal which has overcome his acute primary parasitæmia and showed no more parasites by microscopical examination, has made a radical recovery. He has actually recovered partially but has not been de-parasitized. The table below gives a few data referring to the duration of the latent metacritical infection recorded by various observers. The figures given in the table refer to the period of latency reckoned from the date of experimental inoculation.

DURATION OF THE LATENT METACRITICAL INFECTION.

For white rats.—	
Raffaele and Baldi (1950) ...	14 months.
Fabiani, Vargues, Grellet and Clausse (1951); Fabiani, Vargues, Grellet, Fulchiron and Verain (1952); Fabiani and Fulchiron (1954*b*) ...	Several weeks.
Black (1951)	10 months.
Ramakrishnan, Satya Prakash and Krishnaswami (1951) ...	At least six months.
Sergent (1954*b*)	Up to ten months.
For Rattus rattus.—	
Vincke and van den Bulcke (1949*b*)	Up to one year.
For other rodents.—	
Rodhain (1949 : 1951)... ...	For cotton rat (*Sigmodon hispidus hispidus*) latent infection is of a very long duration.
Vincke and van den Bulcke (1949*c*)	For *Thamnomys surdaster surdaster*, is of long duration.

Arch. Inst. Pasteur d' Algerie, **28**, pp. 1-70 (1950).
Ibid, **30**, pp. 203-239 (1952).

Adler, Yoeli and Zuckerman (1950)	A latent stage observed in the Palestinian vole *Microtus guentheri.*
Bray (1951)	Also a latent stage in the English vole (*Clethriomonys glareolus britannicus*).
Ramakrishnan and Satya Prakash (1951)...	For the squirrel (*Sciurus palmarum*), latent infection is of at least six months duration after the first inoculation.
Durand and Mathis (1951*b*) ...	For a small rodent of Tunisia (*Dipodillus campestris*), the latent infection is of five months duration after inoculation.
Sergent and Poncet (1950 : 1951*a*)	In the *Meriones shawi*, rodent of North Africa, it is of 11 months duration after the inoculation.
Mercado and Coatney (1953) ...	For the field mouse (*Microtus pennsylvanicus*), it is of 19 to 51 days ; in the rice rat (*Oryzomys palustris*) it is of 54 days.

In Section III, the data relating to the duration of latency produced by splenectomy in infected animals are discussed. Animals surviving the primary parasitæmia, show resistance to re-inoculation for a long time. In a given animal species, the duration of acquired resistance is usually in relation to the period of latent metacritical infection (Sergent, 1954*b* ; Ramakrishnan, Satya Prakash and Krishnaswami, 1951). We are justified in thinking that rodent malaria due to *P. berghei* is a disease with premunition like all other malarias. The essential character of premunition is that it is related to the presence of latent infection. Therefore, when the infection disappears either *per se* or in response to treatment, the organism becomes susceptible to a re-infection. This was shown by experiments on mice which were first infected and then treated with an antimalarial. They were not more resistant to a re-infection than new animals. Vincke and van den Bulcke (1949*a*) treated one infected mouse by three injections of Aralen and the mice recovered from infection. Three months after the cure, the animal was re-inoculated resulting in a fatal infection. Baldi (1952) cured the infection in eleven mice employing various antimalarials (sulphonamides, pentaquine and resochin). At the end of the treatment and two to four months thereafter, parasites were not found in the peripheral blood. On re-inoculation, all of them developed a fatal infection.

Sergent (1954*b*) found by total infectivity test that infection in mice can be radically cured by nivaquine. Fifteen infected mice were treated by nivaquine and were re-inoculated 8 to 32 days after the treatment. All of them developed an acute fatal infection similar to that produced in controls. Lapierre (1954) cured by nivaquine the acute and subsequent relapsing infections in four mice. The animals were re-inoculated on 200th, 140th, 30th and 20th days, respectively, after disappearance of parasitæmia from the peripheral blood. Out of these, three developed an acute attack while the fourth re-inoculated 30 days after the disappearance of parasites, died 11 days later without showing any parasites in its

blood taken from the heart, liver and spleen smears. Similar experiments were carried out on white rats. We know that in contrast to mice, first infection in rats leads to acquired resistance. Fulchiron (1952) recorded that quinine causes the disappearance of parasites from the peripheral blood of infected rats but does not interfere with the development of acquired resistance. We have already referred to under the heading " Innate pseudo resistance " that an infection latent from the commencement leads to the establishment of premunition in the same way as a metacritical infection (Sergent, 1954*a*).

Maintenance of the parasites in the mammalian host.—In what developmental form does *P. berghei* maintain itself during its long periods of latent infection ? Vincke and van den Bulcke (1949*a*) stated, " we must admit that either submicroscopic amounts of parasites remain in the blood or there is a persistent exoerythrocytic cycle ". Galliard and Lapierre (1950) removed the spleen from a rat with latent infection, 20 days after the disappearance of parasites from the peripheral blood. The spleen was inoculated to clean rats which did not develop any infection. The splenectomized rat, however, in course of time showed a parasitic relapse. They concluded that parasites during latent infection do not maintain themselves in the spleen, but elsewhere.

Much more work is still needed to solve the problem of the maintenance of parasites during latent infections : (1) What are the organs or tissues or body fluids, in which the parasites maintain themselves ? and (2) Can *P. berghei* maintain itself during its latent phase in a stage which is not constantly infective and which recovers its virulence only under certain conditions, as for instance, some changes in the medium in which it passes its vegetative phase ?

PART IV. ORGANIC DEFENCE.

The spleen.—As in other plasmodial infection, the spleen in animals infected with *P. berghei* shows a pronounced reaction. Hill (1950) recorded that in rats sacrificed during their acute attack, the spleen shows a considerable enlargement while the liver often remains normal. Ramakrishnan (1952) has observed that during the latent metacritical infection in the rat, the spleen increases in volume and does not revert to normal before three months after the end of the primary parasitæmia.

Baldi (1952) splenectomized a guinea-pig and observed that when it was inoculated with *P. berghei*, there was no change in its absolute innate resistance.

Rodhain (1949 : 1951) carried out experiments on cotton rats to study the effect of splenectomy before inoculation with *P. berghei*. He found that cotton rats splenectomized prior to inoculation showed after inoculation an intense parasitaemia and died rapidly. The parasitæmia was similar to that seen normally in white mice. Exceptionally, however, a splenectomized animal may survive and remain a carrier of scanty parasites or even show an apparent complete cure. Voles splenectomized before or during the acute attack were found to die invariably (van Riel, 1950).

The infection was violent in rats splenectomised before inoculation (Galliard, 1950).

Some field mice, splenectomized before or during acute attack, died (Zuckerman and Yoeli, 1951).

Fulchiron (1952) observed that not a single rat out of 28 splenectomized before inoculation or during the primary parasitæmia, recovered from the infection. Fabiani and Fulchiron (1954*a*) concluded on the basis of experiments on 44 white rats that the spleen plays no part in the innate resistance to malaria and that the spleen is indispensable for acquiring specific immunity.

Matilla *et al.* (1954) investigated the effect of splenectomy on mice which did not develop the infection after inoculation. Seven white mice which resisted the first inoculation with *P. berghei* were splenectomized. They were re-inoculated one month later. All developed an acute fatal attack after a short incubation period.

SPLENECTOMY AFTER THE ACUTE ATTACK.

Galliard and Lapierre (1950) were the first to report that, splenectomy carried out five to ten days after the disappearance of parasites from the peripheral blood, is followed by a parasitic relapse which has the same characters of an acute attack. Fabiani, Vargues, Grellet and Clausse (1951) and Grellet (1951) splenectomized 15 rats between two days to one month after recovery from primary parasitæmia. Eleven of these showed a severe relapse and ten died in two to ten days. Three of the rats showed a short benign relapse. The remaining one rat showed no relapse at all. Zuckerman and Yoeli (1951) found that the Italian voles (*Microtus guentheri*) splenectomized during the first five days of their latent metacritical infection, invariably died. If the splenectomy was carried out between the 5th and 15th day of latent infection, the resulting relapse was found to be fatal in some and in others of long duration up to 150 days. Occasionally splenectomy showed no relapse whatsoever. If the operation was carried out 15 days after commencement of latency, no relapse resulted.

The authors concluded that splenectomy interferes with acquired immunity and also with innate resistance to *P. berghei*. Rodhain (1949 : 1951) splenectomized cotton rats which had apparently got rid of its parasites and found that in some a fatal relapse was the result while in others the result was a prolonged chronic patent parasitæmia. Corradetti (1951) splenectomized albino rats which had recovered from primary parasitæmia and found that the operation was not followed by relapse. But when the splenectomized animals were re-inoculated with a homologous strain, the resultant patent parasitæmia lasted as long as 222 to 255 days after re-inoculation. Black (1951) observed that 25 rats which were splenectomized after recovery from primary parasitæmia, generally suffered from parasitic relapse of varying intensity. The relapses were of low severity if the operation were carried out prior to the 52nd day after inoculation. Fabiani, Clausse and Fulchiron (1952*b*) attributed the rapid increase of parasitæmia in the early stage of relapse produced by splenectomy in rats recovered from primary parasitæmia to the concurrent reticulocytosis. Similarly, the intense parasitæmia seen after massive re-inoculation of splenectomized rats previously resistant to *P. berghei* is also attributed to the increased reticulocytes. Zuckerman (1953) observed patent parasitæmia for several months in voles (*Microtus guentheri*) which

were splenectomized after recovery from primary prasaitæmia and re-inoculated. This indicated that the splenectomized voles had a greater degree of immunity than those with intact spleen. Nevertheless the survival and low parasitæmia in the splenectomized animals showed that there was a certain degree of immunity.

Summarizing the conclusions of the various authors on the effect of splenectomy on malaria due to *P. berghei*, we must admit that their results are rather inconsistent. More research is still necessary on this problem.

REMOVAL OF SUPRA-RENALS.

Fabiani and Izzo (1952*b*) found that removal of supra-renals between one hour and two days prior to inoculation or at the very commencement of infection in 27 white rats had no effect on the course of primary parasitæmia. When supra-renelectomy was carried out at peak parasitæmia, the animals died in less than 24 hours of the operation. When the operation was performed during the declining phase of parasitæmia the animals died in one to two days of the operation, occasionally after a parasitæmiac relapse. The same authors (1952*a*) studied the functional state of supra-renal cortex by Thorn's test on 24 white rats infected with *P. berghei*. (Thorn's test is positive if injection A.C.T.H. is followed by absolute decrease in eosinophile leucocytes). The test was positive at the commencement of the acute attack, and became negative when the infection progressed and remained negative during the period of high parasitæmia. It, however, became positive again when the parasitæmia was on the decline. No effect on the course of parasitæmia was found when A.C.T.H. was injected at the commencement of or during the attack. Injection of the hormone during the late phase of the attack was found to result in increased parasitæmia.

PHAGOCYTOSIS.

Fabiani and Fulchiron (1953*b*) observed intense phagocytosis of parasites introduced into the peritoneal cavity of rats with acquired immunity.

RETICULOCYTOSIS.

Several authors have confirmed the observation of Galliard (1949) that *P. berghei* has a peculiar affinity for young erythrocytes. Moreover, Baldi (1950) and also Fabiani, Clausse and Fulchiron (1952*a* : 1952*b* : 1952*c*), Fabiani, Fulchiron and Clausse (1952) and Fulchiron (1952) observed a correlation between the high susceptibility of mice to *P. berghei* and the presence of a high proportion of reticulocytes in mice blood. Young animals whose blood has higher number of reticulocytes are also more susceptible than adults. But, according to Galliard, Lapierre and Golvan (1954) reticulocytosis is not the cause of acute course of infection with *P. berghei* but rather it is one of the effects of infection. Reticulocytosis is the result of the bone marrow compensating the intense destruction of red blood cells infected with parasites.

SEROLOGY.

Vargues (1951) observed that the titre of complement in rat blood decreased slightly during the acute attack followed by recovery. But the complement disappeared completely when the animal was moribund and there was no detectable complement soon after death of the animal. The ambocepter was found to remain intact in the blood of all rats at the end of the acute attack, but its titre was found to be low in fatal cases. The serum of rats recovered from the infection was found to fix complement strongly in the presence of antigen. The titre of the ambocepter began to decrease one month after recovery.

Vargues, Fabiani and Fulchiron (1951) observed that antibodies appeared in the blood about the 10th day of infection and increased up to the 15th day and remained at a constant level for about two months. Six months after inoculation, a low titre of antibodies was present. Pautrizel and Nguyen-Vinh-Nien (1953) in their experiments found that antibodies appeared after the 12th day of infection and were at their maximum between the 15th and 30th day. They could still be found after three months. In re-inoculated rats, their titre was very high.

Vargues and Fabiani (1952) studied the euglobulin content of serum in white rats infected with *P. berghei* by a method modified from that of Sandor. They found that there was an increase of euglobulin of the alkaline isoelectric point (gama globulin) and the appearance of a pathological euglobulin which was not seen in the serum of normal rats.

Fabiani and Fulchiron (1953*a*) demonstrated the presence of a specific protective factor in the serum of rats recovered from the acute infection. This test of sero-protection was carried out in 21 rats immediately before the first infection or at its very commencement and showed that the action of serum is more obvious when it was obtained from rats re-inoculated after recovery. The action of the serum is of a short duration and can never completely prevent the development of infection.

Fabiani, Vargues and Fulchiron (1953) carried out Henry's reaction with iron melanin or hemozoin antigen. They found the test positive about the sixth day of the acute attack in white rats and the titre increased during the following days. It was strongly positive during the first few days after recovery and reached its maximum about the end of the first week after the disappearance of parasites. Then it decreased but did not become negative until three to four months later. During relapses following splenectomy, the titre of Henry's reaction test showed a considerable increase.

SUMMARY.

The innate resistance to *P. berghei* shows a striking dissimilarity in various animal species. In the guinea-pig, there is an innate absolute sterilizing resistance. In the white mouse the susceptibility is at its extreme leading to fulminating attacks. In white rats there is no resistance when the inoculation is made through the intraperitoneal route, but an obvious, though varying, resistance is seen when

the inoculation is made through the subcutaneous route. Cases of innate pseudo resistance are due to an infection latent from commencement which leads to premunition.

Acquired resistance against re-inoculation of *P. berghei* in such rodents as white rats which survive the acute attack of first inoculation lasts for several months. On the other hand, experience shows that in white rats the acute attack is followed by a latent stage of some duration.

Refractory state following re-inoculation and latent metacritical infection leads to the conclusion that in malaria due to *P. berghei*, like all other malaria, acquired resistance is not caused by a true sterilizing immunity, but related to a state of premunition.

REFERENCES.

ADLER, S., YOELI, M. and ZUCKERMAN, A. (1950) ... ... *Nature*, **166**, p. 571.
BALDI, A. (1950) ... ... *Riv. Malariol.*, **29**, pp. 349-356.
Idem (1952) ... ... *Ibid.*, **31**, pp. 41-52.
BLACK, R. H. (1951) ... ... *Ann. Trop. Med. Parasit.*, **45**, pp. 199-206.
BRAY, R. S. (1951) ... ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **44**, p. 362.
BRUCE-CHWATT, L. J. (1954) ... ... *Nature*, **173**, pp. 353-354.
CORRADETTI, A. (1950) ... ... *Riv. Parassit.*, **11**, pp. 201-209.
Idem (1952) ... ... *Rendiconti Ist Sup. Sanita*, **15**, pp. 1165-1172.
DESCHIENS, R. and LAMY, L. (1951) ... *Bull. Soc. Path. Exot.*, **44**, pp. 405-409.
DURAND, P. and MATHIS, M. (1950) ... *C. R. Acad. Sci.*, **231**, pp. 633-635.
Idem (1951*a*) ... ... *C. R. Soc. Biol.*, **145**, pp. 29-31.
Idem (1951*b*) ... ... *Tunisie Medicale*, **29**, pp. 699-705.
DURBIN, C. G. (1951) ... ... *Proc. Helminth. Soc. Washington*, p. 108.
FABIANI, G., CLAUSSE, J. and FULCHIRON, G. (1952*a*) ... ... *C. R. Soc. Biol.*, **146**, pp. 1580-1584.
Idem (1952*b*) ... ... *Ibid.*, **146**, pp. 1587-1589.
Idem (1952*c*) ... ... *C. R. Acad. Sci.*, **235**, pp. 274-276.
FABIANI, G. and FULCHIRON, G. (1952) ... *C. R. Soc. Biol.*, **146**, pp. 435-437.
Idem (1953*a*) ... ... *Ibid.*, **147**, pp. 99-103.
Idem (1953*b*) ... ... *Ibid.*, **147**, pp. 103-106.
Idem (1954*a*) ... ... *Ibid.*, **148**, pp. 530-533.
Idem (1954*b*) ... ... *Ibid.*, **148**, pp. 673-675.
FABIANI, G., FULCHIRON, G. and CLAUSSE, J. (1952) ... ... *Ibid.*, **146**, pp. 1584-1586.
FABIANI, G. and IZZO, M. A. (1952*a*) ... *Ibid.*, **146**, pp. 1153-1155.
Idem (1952*b*) ... ... *Ibid.*, **146**, pp. 1155-1157.
FABIANI, G. and VARGUES, R. (1951) ... *C. R. Acad. Sci.*, **233**, pp. 502-504.
FABIANI, G., VARGUES, R. and FULCHIRON, G. (1953) ... ... *C. R. Soc. Biol.*, **147**, pp. 430-432.
FABIANI, G., VARGUES, R., FULCHIRON, G. and GRELLET, P. (1951) ... ... *Ibid.*, **145**, pp. 1156-1157.
FABIANI, G., VARGUES, R., GRELLET, P. and CLAUSSE, J. (1951) ... ... *C. R. Soc. Biol.*, **145**, pp. 1131-1134.
FABIANI, G., VARGUES, R., GRELLET, P., FULCHIRON, G. and VERAIN, A. (1952) ... *Bull. Soc. Path. Exot.*, **45**, pp. 524-539.
FABIANI, G., VARGUES, R., GRELLET, P. and VERAIN, A. (1951) ... *Bull. Soc. Path. Exot.*, **44**, pp. 580-591.
FULCHIRON, G. (1952) ... ... *Etude de l' immunite dans le paludisme experimental du rat blanc. These de Medecine*, Alger, pp. 1-162.
GALLIARD, H. (1949) ... ... *Bull. Soc. Path. Exot.*, **42**, pp. 431-433.
GALLIARD, H. and LAPIERRE, J. (1950) ... *C. R. Soc. Biol.*, **144**, pp. 402-403.
GALLIARD, H., LAPIERRE, J. and GOLVAN, Y. (1954) ... ... *C. R. Acad. Sci.*, **238**, pp. 741-743.

GRELLET, P. (1951)	*Le paludisme experimental du rat blanc par* Plasmodium berghei. *These de Medecine*, Alger, pp. 1-168.
HILL, J. (1950)	*Ann. Trop. Med. Parasit.*, **44,** pp. 291-297.
LAPIERRE, J. (1954)	*Bull. Soc. Path. Exot.*, **47,** pp. 380-387.
LEVADITI, C. and VAISMAN, A. (1950) ...	*Ibid.*, **43,** pp. 693-697.
MATILLA, V., APARICIO GARRIDO, J., PRIETO LORENZO, A. and FERNANDEZ NAFRIA, A. (1954)	
MERCADO, T. I. and COATNEY, G. R. (1953)	*Medicina Colom.*, **23,** pp. 8-11.
PAUTRIZEL, R. and VINH-NIEN, N. (1953) ...	*Amer. J. Trop. Med. Hyg.*, **2,** pp. 39-46.
RAFFAELE, G. and BALDI, A. (1950) ...	*Bull. Soc. Path. Exot.*, **46,** pp. 671-673.
RAMAKRISHNAN, S. P. (1952)	*Riv. Malariol.*, **29,** pp. 341-347.
RAMAKRISHNAN, S. P. and SATYA PRAKASH (1950*a*)	*Ind. J. Mal.*, **6,** pp. 189-198.
Idem (1950*b*)	*Ibid.*, **4,** pp. 361-367.
Idem (1951)	*Ibid.*, **4,** pp. 369-375.
RAMAKRISHNAN, S. P., SATYA PRAKASH and KRISHNASWAMI, A. K. (1951) ...	*Nature*, **167,** p. 533.
VAN RIEL, J. (1950)	*Ind. J. Mal.*, **5,** pp. 147-154.
RODHAIN, J. (1949)	*Ann. Inst. Pasteur*, **79,** pp. 772-776.
Idem (1951)	*Ann. Soc. Belge, Med. Trop.*, **29,** pp. 483-496.
Idem (1953)	*Ibid.*, **31,** pp. 289-296.
SATYA PRAKASH, KRISHNASWAMI, A. K. and RAMAKRISHNAN, S. P. (1952) ...	*Bull. Soc. Path. Exot.*, **46,** pp. 315-318.
SERGENT, ED. (1954*a*)	*Ind. J. Mal.*, **6,** pp. 175-182.
Idem (1954*b*)	*C. R. Acad. Sci.*, **239,** pp. 524-525.
SERGENT, ED. and PARROT, L. (1935) ...	*Unpublished paper.*
SERGENT, ED. and PONCET, A. (1950) ...	*Arch. Inst. Pasteur d'Algere*, **13,** pp. 279-319.
Idem (1951*a*)	*Arch. Inst. Pasteur d'Algerie*, **28,** pp. 323-334.
Idem (1951*b*)	*Ibid.*, **29,** pp. 269-272.
SERGENT, ED. and SERGENT, ET. (1950) ...	*Ibid.*, **29,** pp. 273-276.
Idem (1952)	*Arch. l'Inst. Pasteur d'Algerie*, **28,** pp. 1-70.
DE SMET, R. M. (1954)	*Ibid.*, **30,** pp. 203-239.
THURSTON, J. P. (1953)	*Unpublished paper.*
VARGUES, R. (1951)	*Exp. Parasit.*, **2,** pp. 311-332.
VARGUES, R. and FABIANI, G. (1951) ...	*C. R. Soc. Biol.*, **145,** pp. 1134-1136.
Idem (1952)	*Ibid.*, **145,** pp. 1521-1523.
VARGUES, R., FABIANI, G. and FULCHIRON, G. (1951)	*Ibid.*, **146,** pp. 1161-1164.
VINCKE, I. H. and VAN DEN BULCKE, M. A. (1949*a*)	*Ibid.*, **145,** pp. 1298-1300.
Idem (1949*b*)	*Ann. Soc. Belge Med. Trop.*, **29,** pp. 525-535.
Idem (1949*c*)	*Ibid.*, **29,** pp. 537-543.
VINCKE, I. H. and LIPS, M. (1948) ...	*Ibid.*, **29,** pp. 545-548.
VINCKE, I. H., PEETERS, E. M. E. and FRANKIE, G. (1953)	*Ibid.*, **28,** pp. 97-101.
ZUCKERMAN, A. (1953)	*Ibid.*, **33,** pp. 269-282.
ZUCKERMAN, A. and YOELI, M. (1951) ...	*J. Infec. Dis.*, **92,** pp. 205-223
	Ibid., **89,** pp. 130-142.

Indian Journal of Malariology, **8**, 4, December 1954.

IMMUNOLOGY OF *PLASMODIUM BERGHEI.**

BY

G. FABIANI.

(*The Faculty of Medicine, University of Algiers, North Africa.*)

(October 8, 1954.)

In the first publication of Vincke and Lips (1948) in which they described infection of wild young rodents by a new blood parasite they named *Plasmodium berghei*, the same authors pointed out that the infection in rats may undergo a spontaneous cure, while it was invariably fatal in mice. This parasite is of great interest immunologically. In certain kinds of animals, it is capable of setting in motion some organic reactions resulting in immunity and among others a feeble defence. Vincke and Lips (1948) also reported that natural resistance to infection is found in some of the rodents as the attempts to infect the guinea-pig and rabbit were unsuccessful.

Another feature of interest in the immunological study of this parasite is the possibility of quantitative measurement of the immunity. It is easy to determine the absolute number of erythrocytes per c.mm. of blood and the proportion of parasitized cells. We shall see later that it is also possible to determine precisely the proportion of reticulocytes to normal blood cells; reticulocytes are the cells that are highly susceptible to infestation.

Since 1948 numerous investigations have been undertaken on *P. berghei* infections and have indicated the principal characteristics of immunity against the infection. We will summarize the essential features of the researches while studying the techniques, natural and acquired resistance and the defence mechanism.

The disease is caused experimentally by inoculation of trophozoites. It is, therefore, an artificially-induced malaria in the sense described by Boyd and Kitchen. Attempts to induce infection through sporozoites and to study immunity

*The editor is grateful to Mr. K. Gopalakrishnan, Assistant Director-General, Indian Post and Telegraphs, Delhi, for translating the original paper written in French into English.

in such infections have met with limited success (Vincke, Peeters and Frankie, 1953). The existence of secondary exo-erythrocytic forms has not been demonstrated with certainty and their presence has not been proved. We will, therefore, consider in this malaria, only immunity to erythrocytic forms.

TECHNIQUES OF STUDY OF IMMUNITY.

We will indicate the techniques which appear to us useful and which are classical in immunology. We would like to emphasize at the outset, the necessity for precise techniques in order to obtain comparable results.

Inoculation.—It is preferable to inoculate a known number of parasitized cells through the intra-peritoneal route.

Observation of the course of infection.—The most accurate method is to determine the proportion of parasitized red cells and their absolute number per c.mm. of blood. Fabiani and Grellet (1952) have shown that by determining the percentage of parasitized cells, a curve can be established to indicate the intensity and progress of infection from which the establishment of cure or otherwise can be predicted. The blood examination should be carried out daily or on alternate days. To detect a latent infection it is necessary either to inoculate the blood into a new animal or sacrifice the experimental animal and pulverize the organs and infect into fresh animals, or splenectomize the experimental animal which usually provokes a relapse of latent infection.

ASSESSMENT OF IMMUNITY.

(*a*) *Reinoculation of known massive quantities of parasitized cells.*—A detectable parasitæmia is thus produced very rapidly and the fate of the cells introduced in the general circulation can be followed.

(*b*) *Sero-protection test.*—The protective power of immune serum can be estimated by injecting it intra-peritoneally into a fresh animal, into which an hour afterwards a known number of parasitized cells are also injected. Fabiani and Fulchiron (1953*a*) have indicated that such a technique is suitable to rats.

(*c*) *Estimation of the opsonising action of immune serum.*—In the course of the above-mentioned test the peritoneal fluid is tapped and examination of several stained films of it will enable one to enumerate the phagocytosed parasites.

(*d*) *Enumeration of reticulocytes in the blood.*—The reticulocytes are preferentially invaded by *P. berghei*. Polychromatophilic erythrocytes are considered to be reticulocytes by some authors. It is preferable to estimate the reticulocytes as it is accurate. A drop of blood is taken on a slide with which is mixed one drop of citrated brilliant cresyl blue solution. After a minute, the mixture is dried and stained by May-Grunwald or Geimsa stain.

THE VIRULENCE OF THE PARASITE.

It does not appear that various strains of *P. berghei* present great difference in virulence. Perhaps, however, one strain maintained in albino rats for several

months does not provoke more than a small mortality in these animals. The sporozoites appear to possess a less infecting power than trophozoites to laboratory bred young rats. A small number of parasitized cells suffice to induce a consistent infection while failure to infect by sporozoites is frequent. Finally we emphasize that the quantity of inoculated parasites does not influence the course of infection, but only the duration of pre-patent period.

NATURAL IMMUNITY.

The list of animal species susceptible to *P. berghei* is described elsewhere. We will now confine ourselves to a list of animals which exhibit an absolute resistance to infection, as for example, the guinea-pig which is refractory at birth and even after splenectomy (Vincke and Lips, 1948; Raffæle and Baldi, 1950; Deschiens and Lamy, 1951; Satya Prakash *et al.*, 1952). Other animals, as the rabbit, have also very great resistance to *P. berghei* infection (Vincke and Lips 1948; Raffæle and Baldi, 1950; Satya Prakash *et al.*, 1952). This resistance can perhaps be overcome as the rabbit can be infected from birth till it is 15 days old (Deschiens and Lamy, 1951). In such studies it should be remembered that parasitized erythrocytes of one animal are inoculated into an animal belonging to another species. The foreign cells are very often rapidly destroyed before the parasites contained in them have had a chance to develop. In this way, susceptibility—even though feeble but real—to *P. berghei* can be missed.

We must also emphasize the fact that an animal refractory to this parasite does not destroy it always rapidly. The blood of a guinea-pig inoculated two days prior through the subcutaneous route was found to be infective to mice (Sergent and Poncet, 1951*b*). The blood of splenectomized bandicoot was found to contain viable parasites for five days after inoculation (Satya Prakash *et al.*, 1952).

A large number of animals susceptible to this parasite belong to the orders *Rodentia* and *Chiroptere*. But even among them, there appears to be a certain degree of natural resistance to free progress of infection. It is, therefore, important to determine the absolute number of parasites as indicated by Fabiani and Orfila (1954*a*). The growth is not so rapid as it should be if every merozoite resulting from a 24-hour schizogony cycle survived. At the commencement of infection in rats and mice, we have noticed that when parasitæmia has reached one or two per cent erythrocytes, it often remains stationary for three or four days. These facts prove very well the destruction of the majority of merozoites, thus indicating an undisputed presence of innate immunity.

FACTORS INFLUENCING VARIATIONS IN NATURAL IMMUNITY.

Greenberg *et al.* (1953) have studied the course of infection among two pure races of mice and their hybrids. They proved that the average mortality occurs earlier in one than in the other and the mortality in hybrids is much more delayed. As a result of precise studies, the authors concluded that the increase in the rate of parsitæmia was not related to innate resistance but to a greater or less

tolerance to the parasitæmia for long periods. The same observation applies to the influence of sex, for the mortality is earlier among the males than females.

The conclusion of Greenberg *et al.* (1953) seems applicable to a certain number of other experiments on natural immunity in which the tolerance to infection has not been given sufficient consideration. Only a precise study of the parasitæmia and its comparison with those of controls enables one to know if the infection is of slow development or slow to appear in the peripheral blood, which indicates the resistance of the animal to the invasion by the parasite. But death, occurring sooner or later, shows a diminished or increased tolerance. Death can also signify absence of specific immunity.

Does an individual resistance to malaria exist in a susceptible species ? The question arises from the observation of Vincke and Lips (1948) who found that when a large number of mice was inoculated, some proved refractory to the infection. The proportion of refractory mice, however, scarcely exceeded one per cent. But when the animals were re-injected, they appeared almost always susceptible. Matilla *et al.* (1954) were the only others who reported that some mice resist several re-inoculations up to 12 through different routes.

The influence of age is indisputable, the young animals being distinctly more susceptible than adults. This fact has been observed in several species of animals. Among the young rats, the parasitæmia is very high and the infection is almost always severe which contrasts with the disease which often undergoes spontaneous remission in adult rats (Raffæle and Baldi, 1950; Galliard and Lapierre, 1951; Hsu and Geiman, 1952). We have noticed the same during inoculation of malaria to new-born rats by the subcutaneous route (Fabiani, Vargues, Grellet and Clausse, 1951). When the infection is passaged serially through young hamsters (*Mesocricetus auratus*) less than a year old, it exhibits a severe course, while the adult hamster is refractory to the infection (Durand and Mathis, 1951*a*; Hsu and Geiman, 1952). The infection is rapidly fatal in the vole (*Meriones shawi*) when it is very young (Durand and Mathis, 1951*b*; Greenberg *et al.* 1953). We have already seen that the rabbit is susceptible only if inoculated soon after its birth (Deschiens and Lamy, 1951). Finally let us note the curious but not detailed observation of Hsu and Geiman (1952), " The mice that were two or three months old showed a low infection rate or failed to develop detectable parasitæmia. Of these mice those that died, showed a low grade of parasitæmia ".

NUTRITIONAL FACTORS.

Vitamin A deficiency.—Fabiani and Grellet (1952) have shown that among the deficient rats the infection is mild and of brief duration and that the relapses after splenectomy are benign. When white rats are subjected to starvation, the infection is very mild or does not appear (Ramakrishnan, 1953).

Milk diet.—A very interesting study by Maegraith *et al.* (1952) has shown that among rats fed on an exclusive milk diet (with the addition of vitamins) and infected, the parasitæmia is very mild or does not appear. It was not a question of destruction of the parasites but of a suppressive effect probably due to decreased multiplication. Very soon afterwards, Hawking (1953), Ramakrishnan *et al.*

(1953*a*) and Mackerras (1953) confirmed the work of Maegraith *et al.* (1952). Hawking (1953) attributes the low PABA content of milk to explain its action and points out that the infection develops normally in rats fed on milk to which PABA or folic acid is added. According to him, other deficient diets (solution of glucose) is equally inhibitory to the development of *P. berghei* malaria. Thus he confirms the conclusions made by Ramakrishnan *et al.* (1953*b*).

Among the white mice, the results are less regular. Maegraith *et al.* (1952) indicate without going into details that milk diet suppresses the infection in mice. But Rodhain (1953*a*) observed a feeble parasitæmia in mice similarly treated. Hawking (1953) proves that the suppression is less complete than among rats. Finally, Schneider (1953) do not observe any effect due to milk diet in infected mice.

The action of milk diet thus appears to be complex. This is what Fabiani and Orfila (1954*c*) proved in a series of experiments carried out on 32 white mice by determining in a regular manner the percentage of infected erythrocytes and of reticulocytes, that is to say, of the susceptible erythrocytes, that in some cases the milk diet prevents the increase of reticulocytes or provokes irregular variation in them and that the parasitæmia faithfully follows the reticulocyte variations. Diet, therefore, would appear to act not on the parasite, but on the host which is made less receptive. At other times, the parasitæmia is mild in spite of increased proportion of reticulocytes indicating that in such cases the milk has an inhibiting action on the parasites. Finally we shall see that the milk diet can render mice capable of acquiring a specific immunity to *P. berghei* infection.

THE NATURE OF BLOOD CELLS.

Galliard and Lapierre (1949) observed that *P. berghei* preferably infests young erythrocytes of the rat and mouse. This was confirmed by Baldi (1950), Corradetti and Verolini (1951), Ramakrishnan and Satya Prakash (1951). Baldi (1950) observed that such a tropism is not only obligatory but necessary for massive multiplication of *P. berghei* and he thought that it explains a certain number of biological facts : the great susceptibility of mice may be linked with an intense erythropoetic reaction. The greater resistance of rats, and even perhaps the increased susceptibility of splenectomized rats, may be attributable to the extent to which reticulocytes are mobilized.

Fabiani, Clausse and Fulchiron (1952*a*) have attempted to study precisely the affinity of this parasite for young erythrocytes. They have preferred to study the reticulocytes as it is more accurate to do so, although their identity in general is the same as polychromatophilic cells. They have proved among the rats the parallelism of the reticulocyte and parasitæmia curves up to the moment of parasitic crisis. As a result of these experiments, the authors have wondered if the plateau, sometimes present in the parasitic curve, may not be due to the low numbers of reticulocytes in the blood during that period. They also proceeded to study the course of infection in animals in which the reticulocytes were increased or decreased by other means. Injection of phenyl hydrazine or excessive bleeding and administration of vitamin B12 induces considerable reticulocytosis. Increase of reticulocytosis can be prevented by daily transfusion of blood. By these studies it was

shown that parasitæmia follows the reticulocyte curve faithfully. At the commencement of infection in rats the parasitæmia remains stationary which appears extraordinary since parasite multiplication is taking place at the same time. This is explainable by the fact that the bone marrow liberates at this period only a small number of reticulocytes.

The severe character and intensity of relapse in infected splenectomized rats would appear to be unexpected, since when fresh rats are splenectomized and then infected, the infection develops gradually. This paradoxical susceptibility of the immune but splenectomized rat can also be explained by the fact that there is already a considerable reticulocytosis [removal of the inhibiting effect of the spleen on the marrow and reawakening of latent *Hæmobartenolis* (Fabiani, Clausse and Fulchiron, 1952*b*)].

The above has been confirmed by Hsu and Geiman (1952) who show that an infection of *Hæmobartonella muris* provokes a strong reticulocytosis and aggravates the malaria infection. Jones and Maegraith (1953) and Ramakrishnan, Satya Prakash and Krishnaswami (1953) have observed that reticulocyte is preferentially selected by *P. berghei*.

Fabiani and Orfila (1954*a*) have made some identical observations in the mice. During the first few days of patent parasitæmia and throughout the infection it is the result of reticulocytosis which influences the blood infection. However, during two or three days of the commencement of the patent period, the parasitæmia increases without any increase of reticulocytes and parasites are found in mature erythrocytes. This is not due to a sudden and extraordinary change in the affinity of parasites to the mature cells. But the parasites invade the young cells in the bone marrow which is always active, and the reticulocytes mature very rapidly. Fabiani and Orfila (1954*b*) actually observed an increased rate of maturation of reticulocytes in the plasma.

Singer (1953) reported that infected mice exposed to x-rays showed a decreased or no patent parasitæmia. This is explained by the inhibitory action of the rays on hæmatopoesis. Singer (1954*a*: 1954*b*) also observed that the malaria of splenectomized mice treated with cortisone results in a relatively low infection despite the increase in reticulocytes.

All observations in white rats and mice demonstrate the elective affinity of *P. berghei* for the immature erythrocytes. These observations are of great importance. Firstly, they enable us to understand that in malaria there is a veritable vicious circle in which the infection provokes a medullary reaction (reticulocytosis) which enhances the infection up to a fatal termination unless the development of a specific immunity arrests it. Secondly, *P. berghei* infection represents one of the rare infectious diseases in which one can estimate an essential element of the host (reticulocytes) in which the infection develops. It is possible to determine the number of susceptible erythrocytes, i.e. reticulocytes. The conclusion is that the variations of the reticulocytes must always be studied when one studies the action of a pharmacological agent or of an immunizing factor in *P. berghei* infections. In such investigations one can observe the effect of the agent under study on the parasite directly or indirectly by some modification of a host

element (erythorcytes) essential to the parasite. It is sufficient to recall that an increase in reticulocytes indicates a reduction of natural immunity. This reduction can be measured.

The tropism of the parasite for the young red cell has a chemical basis. The reticulocytes and polychromatophilic cells contain more ribo-nucleic acid than mature red cells.

RÔLE OF THE SPLEEN.

The well-known rôle which the spleen plays in the resistance of the animal to the infection, and in particular to malaria, has stimulated investigations to determine its influence on natural resistance. Rodhain (1949 : 1951) removed the spleen from cotton rats before inoculation. He found that the infection progressed rapidly in them and attained the same intensity as in mice. The infection in intact animals is mild and of a brief duration. Galliard (1949) showed that splenectomized rats have an intense and severe infection and that, in mice infection as well as anæmia, is of extreme degree. Galliard and Lapierre (1950 : 1951) experimented on three rats weighing 50 to 60 gm. and four rats weighing 100 to 120 gm. All the animals of the former group and three of the latter died.

Among the field voles (*Microtus guentheri*), splenectomy performed prior to infection or during the first few days of patent parasitæmia results in a much greater severe infection than in controls (Zuckerman and Yoeli, 1951).

Matilla *et al.* (1954) have observed ten mice presenting an innate resistance to malaria (resistance to several re-inoculations). They showed that after splenectomy all the mice contracted the infection. This interesting observation must be reconciled with the observations of Rodhain (1949), but a similar effect is not applicable for the investigations of Satya Prakash *et al.* (1953) who found that a splenectomized bandicoot was no more susceptible than an intact animal. Similarly Raffæle and Baldi (1950) were not able to render guinea-pigs susceptible to *P. berghei* by removal of spleen.

Fabiani and Fulchiron (1954*a* : 1954*b*) investigated the influence of splenectomy on the parasite curve of two naturally susceptible animal species. They found that in 24 rats splenectomized prior to infection and on 20 others during the infection (before the intervention of specific immunity), there was no evidence for the spleen being responsible for natural immunity. Fabiani and Orfila (unpublished results) observed that the infection in 30 splenectomized mice was no more severe than among the controls. On the contrary, consequent to a low reticulocyte count in splenectomized animals, the parasitæmia was found to rise only moderately and remain constant. Singer (1954*a*) showed that the mice splenectomized prior to infection, developed a less severe infection than intact animals. The reticulocytes also were not found to increase. It can, therefore, be concluded that the spleen plays a different rôle in the natural resistance of animals to other plasmodial infections. The rôle would appear to vary in the different animal species. It may be an important one, to a totally unimportant or an indifferent rôle.

ENDOCRINE GLANDS.

Supra-renalectomy of infected rats caused their death rapidly but had no appreciable effect on the parasitæmia (Fabiani and Izzo, 1952). Fabiani and Orfila (unpublished reports) indicate that the effects of splenectomy are the same in mice. According to Findlay and Howard (1952), the injection of cortisone results in increased parasitæmia but the period of infection is reduced. Galliard (1953), however, found that cortisone does not produce increased parasitæmia in adult rats. Schneider (1953) does not observe any aggravation of infection in mice due to cortisone.

Our own experiments on mice confirm the finding of Schneider (1953). Thorn's test by injection of A.C.T.H. when positive, does not show any manifest effect of corticotrophic hormone. If in any animal death occurs earlier it would appear to be due to a bacterial septicæmia as evidenced by a greatly increased leucocytosis. The effect of cortisone on the rat appears more complex. We could not draw any definite conclusions from our experiments because these were confined to two rats only. In them, however, injection of cortisone resulted in a more severe and prolonged infection and appeared to influence the development of specific immunity, if A.C.T.H. is injected at the commencement of infection or during the established primary infection. It should also be remembered that Thorn's test is often negative at the commencement of infection. On the contrary, during the declining phase of infection or at its crisis, administration of A.C.T.H. results in a fresh increase of parasitæmia.

Hypophysis.—Galliard and Lapierre (1953) recorded, "hypophysectomy confers on the rat very distinct resistance to the infection. This resistance is augmented by somatotrophine and neutralized by A.C.T.H." They have also shown that the somatotrophic hormone exercises a delaying and distinctly neutralizing action in the mice : prolongation of prepatent period and the stabilization of parasitæmia at a low level with complete disappearance of multiplying forms.

Fabiani and Orfila (unpublished reports) observed the effect of somatotropine hypophysaine combined with acetate of Desoxycorticosterone (D.O.C.A.) on the malaria of mice. Since the investigation was confined to 12 mice only, no conclusive results can be drawn.

ACQUIRED IMMUNITY.

In a certain number of animal species, the acute infection by *P. berghei* can be controlled and clinical recovery or cure is not followed immediately by the total disappearance of parasites but by a period of latent infection lasting some months, during which relapses can occur either spontaneously or be induced by splenectomy. The cured animal resists re-inoculation. We will, therefore, analyse the principal manifestations of acquired immunity, its general characteristics and its mechanism. We shall consider mainly the researches conducted on white rats for this animal has been studied the most.

I. PRINCIPAL MANIFESTATIONS OF ACQUIRED IMMUNITY.

(*A*) Cure of acute infection.

Among the animals sufficiently old and susceptible to spontaneous cure, the parasitæmia undergoes a progressive increase which is more or less regular, up to a maximum. If death does not occur during this period, the parasitæmia decreases very rapidly. A 50 per cent cell infection, for example, fell in a day to ten per cent, then to five per cent and later to one per cent. In a few days, parasites disappear from the peripheral blood. This parasitic crisis has been observed and described in white rats by Corradetti (1950) and confirmed by Fabiani, Vargues, Grellet, Fulchiron and Verain (1952). It is also seen distinctly in the diagramatic representation of the course of parasitæmia, made by Black (1951) and the figures reported by Hsu and Geiman (1952) and Ramakrishnan, Satya Prakash and Krishnaswami (1951). It must, however, be noted that Mercado and Coatney (1951*b*) describe a progressive dimunition of the blood infection.

The abrupt fall in parasitæmia which demonstrates the intervention of specific immunity has been reported in *Microtus guentheri* by Zuckermann and Yoeli (1951) and Mercado and Coatney (1953).

Role of the spleen.—Rodhain (1949 : 1951) recorded that three out of four cotton rats splenectomized prior to infection, died of the disease. Galliard and Lapierre (1951) made similar observations in four young rats and three of an older age group. One of the latter, however, recovered after a prolonged parasitæmia. Zuckerman and Yoeli (1951) removed the spleen from 26 voles (*Microtus guentheri*) prior to inoculation or during patent parasitæmia (before the intervention of specific immunity). All the animals died. Fabiani and Fulchiron (1954*a*) splenectomized 44 white rats prior to inoculation or during patent infection and all of them died. The spleen, therefore, plays a very important rôle and is almost indispensable for the development of acquired immunity.

(*B*) Latent infection.

The parasites persist in the animal after it recovers from the primary parasitæmia, from a few weeks to months. The latent character of the disease shows that the immunity is not sufficient to destroy all the parasites. It is, however, sufficient to prevent patent parasitæmia. During the period of latency, it is not rare to demonstrate the presence of occasional parasites in the blood, either by frequent examination of the blood, or by inoculation of the animal's blood into susceptible animals. The latter proof would appear to be more sensitive than the former. The occasional parasitæmia during latency is often transitory and is always low and hardly deserves the term of relapse by which it is commonly called.

Sergent and Poncet (1950) demonstrated the presence of parasites in the blood of white rats, a few days after recovery from primary parasitæmia. Levaditi and Vaisman (1950) proved that 18 or 42 days after clinical cure, blood or viscera of animals can be infective although the blood films are negative. Raffæle and Baldi (1950) observed that parasites were patent in the blood infrequently during a period of 100 days after inoculation. Corradetti (1950) examined the blood of ten rats daily during a period of 28 to 122 days. He found that only three of them

had any parasitic recrudesences which became absent after the 50th day. Black (1951) observed 25 rats daily and recorded mild relapses of one to five days' duration in the three weeks following primary parasitæmia. Galliard and Lapierre (1951) record similar results.

Fabiani and Grellet (1952) observed that the blood of three out of nine animals is infective one week after recovery, but not later. A great point of interest is, Black (1951) in his experiments sacrificed a rat two months after it recovered from infection, and demonstrated that its blood and viscera were infective to a fresh rat. Similar procedure, however, in a second similar rat killed two weeks later, showed that its blood and tissues did not contain any parasites. Coudert and Chastel (1952) report results similar to those of other authors.

It would appear, therefore, that among the majority of the rats a latent infection was of a brief duration which does not exceed two or three months. However, other studies show that at least occasionally the latent period can be of longer duration. Ramakrishnan, Satya Prakash and Krishnaswami (1951) observed two relapses ; one seven months and the other eight months after the inoculation. They also showed that blood can remain infective up to six months. Vincke, Peeters and Frankie (1953) record, " The longest period in which we have obtained positive sub-inoculations is 150 days after the end of the critical period. However, sub-inoculation from another rat was found to be negative after the 37th day. The white rat can conserve parasites in the blood for long periods, but can also lose them rapidly ".

In animals other than the rat, the period during which the blood contains latent parasites is very variable. No one seems to have observed reappearance of parasites in the blood of *R. rattus*. Parasites were seen in the blood for a few days in bats (*Roussette*) and more often for some months among *Thamnomys surdaster* (Vincke *et al.*, 1953), *Oryzomys palustris* (Mercado and Coatney, 1951*b*) and in the Gerbille, the Merion (Durand and Mathis, 1951*b*). In the last mentioned animal, Sergent and Poncet (1951*a*) have proved the presence of parasites in the viscera, ten months after the infection. Let us note finally the existence of spontaneous relapses with intensive parasitæmia in *Cricetomys ansorgei* (Vincke *et al.*, 1953).

Relapses provoked by splenectomy.

Relapses are frequent in rat malaria as in that of man, monkey and bird, when it is splenectomized during latent infection (Galliard and Laiperre, 1950; Black, 1951 ; Ramakrishnan, Satya Prakash and Krishnaswami, 1951 ; Hsu and Geiman, 1952 ; Chastel, 1952). But this result is obtained only if the splenectomy is performed shortly after the primary phase. It is thus that Hsu and Geiman (1952) obtain a relapse in the week which follows the cure only as long as the spleen on removal is found to be enlarged. Black (1951) in his experiments splenectomized 24 rats in the course of his experiments. He found that relapses did not occur if splenectomy were performed 52 days after the inoculation. Finally, Corradetti (1952) did not observe in ten rats recovered from the infection any relapse on splenectomy which was performed during 28 to 122 days. The post-splenectomy relapse would appear to vary in character and duration. It may end with death of the host, or be overcome completely or partially when a low infection may persist for a long time.

The experiments of Fabiani, Vargues, Grellet and Clausse (1951) and Fabiani and Fulchiron (1954*b*) were carried out on 60 rats. They observed 50 relapses. But it is essential to distinguish the results according to the intervals separating the end of the primary parasitæmia from the splenectomy. If the interval was less than a month, the relapse was constant. In 37 animals of this series, recovery was obtained for less than 20 days. In 26 of them, the relapses were severe. In nine others, the relapse was not severe even if splenectomy occurred between 20th and 29th days. Fourteen rats were splenectomized 30 days after recovery from primary parasitæmia. Only four of them had relapses and in three out of these four other factors like supra-renalectomy and excessive bleeding may have contributed towards the relapse.

Zuckerman and Yoeli (1951) have obtained comparative results among *Microtus guentheri*. The occurrence of post-splenectomy relapse and its intensity are the function of the interval which separates the removal of the spleen and the commencement of the latent period. Finally, Rodhain (1951) obtained relapse in three cotton rats following splenectomy after prolonged chronic infection.

The spleen, therefore, is very useful in the maintenance of immunity to infection, but the latter can reappear after splenectomy ; such a relapse can terminate completely or incompletely, in which case it becomes a chronic infection. In proportion to the period that elapses between splenectomy and recovery from primary parasitæmia, we have seen that the extra-splenic factors play a rôle of increasing importance in establishing persistence or re-establishment of immunity.

Other factors influencing latent infection.

The bilateral supra-renalectomy, injection of adrenaline or hæmolytic serum can induce a relapse of *P. berghei* malaria. The blockage of the reticulo-endothelical system, bleeding, barometric depression and gestation do not appear to have any effect on the immunity during latent infection (Fabiani, Izzo and Grellet, 1951).

C. Resistance to re-inoculation.

This classical fact, well-known in the different plasmodial infections, was first recognized with reference to *P. berghei* malaria in *R. rattus* by Vincke and van den Bulcke (1949*b*). Re-inoculation of 22 immune animals resulted in a feeble parasitæmia only among four animals. The persistence of the original infection during a minimum period of seven months was demonstrated by massive inoculation of blood and organs of some of the animals into another series of fresh animals in which they observed " progressive parasitæmia rising with fatal issue ". " The last ten were cured after a brief infection ". Some of these immune animals in the experiments had recovered from their primary parasitæmia more than a year earlier. Raffæle and Baldi (1950) note the failure of re-inoculation in a rat 90 days after the primary infection. Corradetti (1950) re-inoculated 11 rats which had recovered from primary parasitæmia at varying periods, from 76 to 205 days previously. Four rats presented a low parasitæmia which lasted for three to five days. Ramakrishnan, Satya Prakash and Krishnaswami (1951) report the failure of re-inoculation in nine instances. Black (1951) points out that after re-inoculation only one or two parasites in 50 microscopic fields were observed.

Fabiani and collaborators (unpublished reports) re-inoculated known quantities of parasitized cells. Out of 21 immune rats which were re-inoculated with 0·1 million to 5 million parasitized cells, only four had patent parasitæmia for a brief period. Fabiani, Vargues and Fulchiron (1952) inoculated large quantities of parasites of the order of 300 million per rat through the intra-cardiac route or intra-peritoneal route. The technique of massive inoculations results in an appreciable patent parasitæmia rapidly or even immediately in the receptor animal. Such a technique of re-inoculation may indicate the degree of acquired immunity. Besides, it will be possible to observe the presence or absence of parasite multiplication in the receptor animal. The parasitized polychromatophilic cells of the donor can be recognized in the receiving animal, and may make it possible to distinguish the donor infection from that of the recipient which, if present, will be found at the commencement of infection in mature erythrocytes. By these techniques, the authors have proved resistance to re-inoculations in immune rats for as long periods as 10, 11 and even 15 months after recovery from primary infections. They have also been able to show that different degrees of acquired immunity can be distinguished and have been able to measure it quantitatively. It is in this fashion that they have been able to assess in a precise manner the reinforcement of immunity by successive reinoculations in hyper-immunized rats.

Krishnaswami *et al.* (1953) report some comparable results by employing very high doses (up to two milliards of parasites). Zuckerman (1953) re-inoculating coles arrived at identical conclusions.

Reinoculation of immune but splenectomized animals.

Re-inoculation of immune animals which did not suffer from any relapse after splenectomy, or recovered from the post-splenectomy relapse, generally results in a chronic infection of low intensity (Black, 1951 ; Zuckerman and Yoeli, 1951 ; Fabiani and Fulchiron, 1952). This would indicate that there is a persistence of a certain degree of specific immunity even after splenectomy, since the parasitæmia remains low. This persistence of specific immunity is also demonstrated in a splenectomized animal by the chronic patent infection remaining unaltered by re-inoculation.

General characteristics and mechanism of immunity.

The period of immunity.—Re-inoculation experiments have shown that acquired immunity in rats can last at least one year after the recovery from primary infections. As the latent infection is hardly prolonged in general for more than a few months, it appears probable that the immunity can persist even after the latent infection ends. Likewise the disappearance of the parasites by nivaquine does not suppress the immunity.

Cross immunity.—A cross immunity exists more or less between two different strains of *P. berghei* (Vincke and van den Bulcke, 1949*a* ; Fabiani and Fulchiron, 1952 ; Krishnaswami *et al.*, 1953).

Passive immunity of maternal origin.—When rats born of immune females are inoculated some days after birth, the resulting infection gets cured spontaneously. This is contrary to the course of infection similarly produced in rats of the same age born of non-immune females (Fabiani and Fulchiron, 1952).

Mechanism of immunity.—Both humoral and cellular mechanisms of immunity are present in *P. berghei* infections. Phagocytes play an important rôle in the natural resistance and above all in the acquired resistance during parasitic crisis. Several workers have observed it. Fabiani and Hadjeres (unpublished report) have studied its topographical localization and its mode of appearance in the spleen and liver. The humoral mechanism also comes into play, but is more complex. Some visible signs of it appear in the course of the disease (Vargues, Fabiani and Fulchiron, 1951; Pautrizel and Nein, 1953), but its immunizing rôle is not proved. On the contrary, a humoral factor described by Talliaferro (1944) in simian malaria which effects a decrease in the number of merozoites during the parasitic crisis, would seem to be present in rats also. The protective power of serum can also be demonstrated by the sero-protection test (Fabiani and Fulchiron, 1952). These antibodies exercise an opsonising effect which is demonstrated by the presence of phagocytosed parasitized cells in the peritoneal fluid withdrawn after successive injections of immune serum and parasitized cells respectively (Fabiani and Fulchiron, 1952). The sero-protective power is of brief duration and cannot prevent for long the development of malaria in a fresh animal. It does not appear to have any action in mice.

The rôle of the spleen is characteristic in the immunity to *P. berghei* infection as to other plasmodial infections, particularly to erythrocytic infection. Its rôle in malaria is evident, whatever the precise physiological mechanism may be. Investigations on *P. berghei* infections in voles and white rats permit us to state more precisely the immunizing function of the spleen. At the commencement of the disease, the spleen is indispensable to the anti-plasmodial defence ; without it the immunity almost never appears. The spleen is thus an organ of primary response to blood infection. Later it ceases to be indispensable for some mechanism of substitution for the spleen develops at least partially. Our conclusions are similar to those of Zuckerman and Yoeli (1951) and in part to those of Talliaferro (1950) in the studies on the production of hæmolysins in rabbits.

The immunological problem of the mice.

The infection of the mice is always severe and fatal. Such is the opinion of every author who has studied it. We have demonstrated this in 200 mice which we infected. There is only one exception reported by Vincke, Peeters and Frankie (1953) who state " Only one mouse outlived nearly four months this infection and given a positive sub-inoculation of his blood 90 days after his critical phase ".

The causes of this absence of immunity in mice are not known. Death occurs even when the infection lasts several weeks either naturally or due to therapeutic intervention. Fabiani and Hadjeres (unpublished report) have proved that phagocytosis develops less quickly and more feebly in mice than in rats. The question remains, however, whether the phagocytic insufficiency causes the non-manifestation of immunity and nullifies the efficacy of antibodies. But in this connection it must be remembered that Fabiani and Orfila (1954*a*) have not been able to determine the existence of any protective power in the serum of mice in which the infection had been present for two weeks.

It has, however, been possible for us to obtain the appearance of specific immunity among the mice subjected to milk diet or to a treatment by sulphadiazine (Fabiani and Orfila (1954*c* : 1954*d*). In two mice fed on a milk diet, the patent parasitæmia disappeared. The mice were then fed on the normal diet and later re-inoculated and exhibited only a mild parasitæmia from which they recovered spontaneously. On the other hand, four infected mice which were treated with sulphadiazine showed either a transient relapse or underwent a spontaneous remission of parasitæmia. The sulphadiazine treatment was stopped after two or three weeks. Those that did not show repatency of parasites under treatment, were re-inoculated after the therapy with the result that a feeble and transient parasitæmia appeared after a long pre-patent period. In this instance also it appeared to demonstrate the existence of immunity to reinfection. Lapierre (1954) confirms the existence of immunity in mice treated with nivaquine due to the fact that relapses occurred in such mice at progressively increasing intervals. Investigations are being pursued in order to determine by what mechanism the milk food or the sulphadiazine treatment modify the host (mouse) and enable it to develop specific immunity.

CONCLUSION.

It is possible to classify the immunologial facts that have resulted from studies on *P. berghei* infections.

1. In susceptible animals inoculated : A lowering of the natural resistance (increase of reticulocytes).

2. In susceptible animals inoculated after splenectomy : Severe progress of infection (absence of acquired immunity).

3. In animals which recover spontaneously from the primary infection : Absence of relapse in spite of a latent infection ; resistance to re-inoculation ; relapses can be induced by certain procedures ; disappearance of latent infections.

4. In immune animals submitted to re-inoculations : Reinforcement of immunity.

5. In immune animals splenectomized : Relapse or a chronic infection or a re-appearance of an immunity.

6. In mice : Possibility of appearance of immunity under the influence of milk diet or sulphanamide therapy.

Investigations on immunity to *P. berghei* infections are of great interest for different reasons : possibility of quantitative assessment of immunity, and varying either the intensity of infection or a host element essential to parasites, namely, reticulocytes. Analysis of the rôle of spleen in the various manifestations of immunity. Effect of specific nutritional factors like milk on the establishment of specific immunity.

In conclusion, immunity to *P. berghei* infections offers an extremely wide range for study ; innate resistance, influence of age, the diet or infection itself, the greater or less tolerance of the host to the physio-pathological influence of the infection, the rapid or slow intervention or intense of mild intervention of specific immunity, efficacy and stability of acquired immunicy, causes for

individual variations in a susceptible animal species, the diversity of the course of infection in different animals, varying from inapparent infections in the vole (*Meriones*) to pernicious infections in mice or relapsing infections in *Cricetomys*. There is probably no other plasmodial species than *P. berghei* which provokes so great a diversity of infection patterns in spite of the uniformity of immunological reactions.

REFERENCES.

Baldi, A. (1950) ... ... *Riv. Malariol.*, **29**, p. 349.
Idem (1952) ... ... *Ibid.*, **31**, p. 41.
Black, R. H. (1951) ... ... *Ann. Trop. Med. Parasit.*, **45**, p. 199.
Chastel, C. (1952) ... ... *These Medicine*, Lyon.
Corradetti, A. (1950) ... ... *Riv. di Parasit.*, **11**, p. 201.
Idem (1952) .... *Rendiconti Istituto Superiore di Sanita.*, **15**, Part XI, p. 1164.
Corradetti, A. and Verolini, F. (1951) ... *Riv. di Parasit.*, **12**, p. 69.
Coudert, J. and Chastel, C. (1952) ... *J. de Medecine de Lyon*, p. 557.
Darrow, E. M., Gingrich, W. D. and Prine, J. H. (1952) ... ... *Amer. J. Trop. Med. Hyg.*, **1**, p. 927.
Deschiens, R. and Lamy, L. (1951) ... *Bull. Soc. Path. Expt.*, **44**, p. 405.
Deschiens, R. and Poirier, M. (1952) ... *Ann. Inst. Pasteur*, **83**, p. 725.
Durand, P. and Matiis, M. (1950) ... *C. R. Acad. Sci.*, **231**, p. 633.
Idem (1951*a*) ... ... *C. R. Soc. Biol.*, **145**, p. 29.
Idem (1951*b*) ... ... *Tunisie Medicale*, **29**, p. 699.
Fabiani, G. (1954) ... ... *Communication au 79e Congres Nationale des Societes Savantes*, Alger, April 14, 1954.

Fabiani, G., Clausse, J. and Fulchiron, G. (1952*a*) ... ... *C. R. Soc. Biol.*, **146**, p. 1580.
Idem (1952*b*) ... ... *Ibid.*, **146**, p. 1587.
Idem (1952*c*) ... ... *C. R. Acad. Sci.*, **235**, p. 274.
Fabiani, G. and Fulchiron, G. (1952) ... *C. R. Soc. Biol.*, **146**, p. 435.
Idem (1953*a*) ... ... *Ibid.*, **147**, p. 99.
Idem (1953*b*) ... ... *Ibid.*, **147**, p. 103.
Idem (1954*a*) ... ... *Ibid.*, **148**, p. 530.
Idem (1954*b*) ... ... *Ibid.*, **148**, p. 673.
Fabiani, G., Fulchiron, G. and Clausse, J. (1952) ... ... *C. R. Soc. Biol.*, **146**, p. 1584.
Fabiani, G. and Grellet, P. (1952) ... *Ibid.*, **146**, p. 441.
Fabiani, G. and Izzo, M. A. (1952) ... *Ibid.*, **146**, p. 1155.
Fabiani, G., Izzo, M. A. and Grellet, P. (1951) ... ... *Ibid.*, **145**, p. 1301.
Fabiani, G., Jaiber, H. and Fulchiron, G. (1951) ... ... *Ibid.*, **145**, p. 1155.
Fabiani, G. and Orfila, J. (1954*a*) ... *Ann. Inst. Pasteur*, **87**, p. 38.
Idem (1954*b*) ... ... *C. R. Soc. Biol.*, **148**, p. 670.
Idem (1954*c*) ... ... *Ibid.*, **148**, p. 1239.
Idem (1954*d*) ... ... *Ibid.*, **148**, p. 1390.
Fabiani, G. and Vargues, R. (1951*a*) ... *C. R. Acad. Sci.*, **231**, p. 633.
Idem (1951*b*) ... ... *C. R. Soc. Biol.*, **145**, p. 1523.
Fabiani, G., Vargues, R. and Fulchiron, G. (1952) ... ... *Ibid.*, **146**, p. 437.
Idem (1953) ... ... *Ibid.*, **147**, p. 430.
Fabiani, G., Vargues, R., Fulchiron, G., Grellet, P. and Verain, Mme. (1951) *Bull. Soc. Path. Exot.*, **44**, p. 580.
Fabiani, G., Vargues, R., Grellet, P. and Clausse, J. (1951) ... ... *C. R. Soc. Biol.*, **145**, p. 1131.
Fabiani, G., Vargues, R., Grellet, P., Fulchiron, G. and Verain, Mme. (1952) *Ann. Inst. Pasteur*, **82**, p. 721.
Galliard, H. (1949) ... ... *Bull. Soc. Path. Exot.*, **42**, p. 431.
Idem (1953) ... ... *Bull. Acad. Med.*, October 20, 1953.
Galliard, H. and Lapierre, J. (1950) ... *C. R. Soc. Biol.*, **144**, p. 402.
Idem (1951) ... ... *Bull. Soc. Path. Exot.*, **44**, p. 185.
Idem (1953) ... ... *C. R. Acad. Sci.*, **237**, p. 477.

Gallard, H., Lapierre, J. and Golvan, Y. (1954) ...	*C. R. Acad. Sci.*, **238**, p. 741.
Gallard, H., Lapierre, J., Larivière, P. and Berbonneau, R (1953.)	*Ibid.*, **236**, p. 1308.
Greenberg, J., Nadel, E. M. and Coatney, G. R. (1953)	*J. Inf. Dis.*, **93**, p. 96.
Grellet, P. (1951)	*These Medecine*, Alger.
Hadjeres, S. (1954)	*Ibid.*
Hawking, F. (1953)	*Brit. Med. J.*, **i**, p. 1201.
Idem (1954)	*Ibid.*, **i**, p. 425.
Hsu, D. Gin Ming and Geiman, Q. M. (1952)	*Amer. J. Trop. Med. Hyg.*, **1**, p. 747.
Jones, E. S., Maegraith, B. G. and Gibson, Q. H. (1953)	*Ann. Trop. Med. Hyg.*, **47**, p. 431.
Krishnaswami, A. K., Satya Prakash and Ramakrishnan, S. P. (1953)	*Ind. J. Mal.*, **7**, p. 303.
Lapierre, J. (1954)	*Soc. Path. Exot.*, April 7, 1954.
Lavaditi, C. and Vaisman, A. (1950)	*Bull. Soc. Path. Exot.*, **43**, p. 603.
Lorenzo, A. P. (1953)	*Medicina Colonial.*, **21**, p. 621.
Idem (1953)	*Ibid.*, **22**, p. 30.
Mackerras, M. J. (1953)	*Austral. J. Sci.*, **16**, p. 24.
Maegraith, B. G., Deegan, T. and Jones, E. S. (1952)	*Brit. Med. J.*, **ii**, p. 1389.
Matilla, V., Carrido, J. A., Lorenzo, A. P. and Napria, A. (1954)	*Medicina Colonial*, **23**, p. 8.
Mercado, T. and Coatney, G. R. (1951*a*)	*J. Parasit.*, **37**, p. 479.
Idem (1951*b*)	*J. Nat. Mal. Soc.*, **10**, p. 339.
Idem (1953)	*Amer. J. Trop. Med. Hyg.*, **2**, p. 39.
Pautrizel, R. and Nien, Nguyen Vinh (1953) ...	*Bull. Soc. Path. Exot.*, **46**, p. 671.
Rafaelle, G. and Baldi, A. (1950)	*Riv. Malariol.*, **29**, p. 341.
Ramakrishnan, S. P. (1953)	*Ind. J. Mal.*, **7**, p. 53.
Ramakrishnan, S. P. and Satya Prakash (1950*a*) ...	*Ibid.*, **4**, p. 361.
Idem (1950*b*)	*Ibid.*, **4**, p. 369.
Idem (1951)	*Nature*, **167** p. 533.
Ramakrishnan, S. P., Satya Prakash and Krishnaswami, A. K. (1951)	*Ind. J. Mal.*, **5**, p. 447.
Idem (1953)	*Ibid.*, **7**, p. 93.
Ramakrishnan, S. P., Satya Prakash, Krishnaswami, A. K. and Chanan Singh (1953*a*)	*Ibid.*, **7**, p. 61.
Idem (1953*b*)	*Ibid.*, **7**, p. 225.
Refaat, M. A., Bray, R. S. and Maegraith, B. G. (1953)	*Brit. Med J.*, **ii**, p. 1047.
Rodhain, J. (1949)	*Ann. Soc. Belge Med. Trop.*, **29**, p. 483.
Idem (1951)	*Idem.*, **31**, p. 289.
Idem (1953*a*)	*Bruxelles Medical.*, **33**, p. 1520.
Idem (1953*b*)	*Ann. Soc. Belge Med. Trop.*, **33**, p. 245.
Idem (1953*c*)	*Bull. Soc. Path. Exot.*, **45**, p. 315.
van Riel (1950)	*Ann. Inst. Pasteur.*, **79**, p. 772.
Satya Prakash, Krishnaswami, A. K. and Ramakrishnan, S. P. (1952)	*Ind. J. Mal.*, **6**, p. 175.
Schneider, J. (1953)	*Bull. Soc. Path. Exot.*, **46**, p. 1016.
Schneider, J. and Montezin, G. (1950)	*Ibid.*, **43**, p .44.
Idem (1953)	*Ibid.*, **46**, p. 947.
Sergent, Ed. and Poncet, A. (1950)	*Arch. Inst. Pasteur. Algerie*, **28**, p. 323.
Idem (1951*a*)	*Ibid.*, **29**, p. 269.
Idem (1951*b*)	*Ibid.*, **29**, p. 273.
Singer, I (1953)	*J. Inf. Dis.*, **92**, p. 97.
Idem (1954*a*)	*Idem.*, **94**, p. 159.
Idem (1954*b*)	*Idem.*, **94**, p. 164.
Taliaferro, W. H. and Taliaferro, L. G. (1944) ...	*Ibid.*, **75**, p. 1.
Idem (1950)	*Ibid.*, **87**, p. 37.
Thurston, J. P. (1953)	*Exper. Parasit.*, **2**, p. 311.
Vargues, R. (1951)	*C. R. Soc. Biol.*, **145**, p. 1134.
Vargues, R. and Fabiani, G. (1951*a*)	*Ibid.*, **145**, p. 1510.
Idem (1951*b*)	*Ibid.*, **145**, p. 1521.
Vargues, R., Fabiani, G. and Fulchiron, G. (1951) ...	*Ibid.*, **145**, p. 1298.
Vincke, I. H. and Lips, M. (1948)	*Ann. Soc. Belge Med. Trop.*, **28**, p. 97.
Vincke, I. H. and Bulcke, L. van den (1949*a*)	*Ibid.*, **29**, p. 524.
Idem (1949*b*)	*Ibid.*, **29**, p. 537.
Idem (1949*c*)	*Ibid.*, **29**, p. 545.
Vincke, I. H., Peeters, E. M. E. and Frankie, G. (1953)	*Ibid.*, **33**, p. 269.
Zuckerman, A. (1953)	*J. Inf. Dis.*, **92**, p. 205.
Zuckerman, A. and Yoeli, M. (1951)	*Ibid.*, **89**, p. 130.

Indian Journal of Malariology, **8**, 4, December 1954.

INVESTIGATIONS ON IMMUNITY TO *PLASMODIUM BERGHEI* INFECTION IN MICE.*

BY

H. GALLIARD

AND

J. LAPIERRE.

(Faculty of Medicine, Institute of Parasitology, Paris.)

(October 16, 1954.)

PLASMODIUM BERGHEI infections in mice are invariably fatal. The present investigations are attempts to determine whether it is possible to terminate or to modify in any manner the progress and fatal course of infection. We have tested, in the last four years, various substances administered to infected mice, for their action. Some among the substances have an antimalarial activity against the parasite. Others, with no specific antimalarial activity, were also of interest to us due to their modifying action of the host immunity to infection.

The investigations are described under three headings :—

(1) Biological attempts.
(2) Attempts with various non-specific substances.
(3) Attempts with antimalarials.

BIOLOGICAL ATTEMPTS.

The acquisition of immunity being the rule in infected rats, we considered that the presence of antibodies in the serum of immune rats could protect mice against *P. berghei* infection. We, therefore, injected the serum collected from rats, five days after the infection became latent, into mice. We found that this sero-protection is distinctly ineffective in either the prevention of infection or in modifying its course when once established.

We also injected immune rat serum preserved in ice for three days into another lot of mice in which the infection was well established. The course of infection in the mice was not modified in any way.

The problem was approached in another way. It is known that the parasites in the gut of *Rhodnius prolixus* fed on infected rats do not survive for more than

*Thanks are due to Mr. K. Gopalakrishnan, Assistant Director-General, Indian Posts and Telegraphs, for translating the original paper written in French into English.—*Editor*.

24 hours. The digested gut contents of *Rhodnius* were infected intradermally into mice and found of no protective value to them when infected.

Thus treatment with immune rat serum appeared to be distinctly inefficacious to protect mice from *P. berghei* infection. It has, however, to be borne in mind that it was not possible to administer large doses of immune rat serum to mice to confer passive immunity to them due to the mice being highly sensitive to heterogenous protein.

ATTEMPTS WITH VARIOUS NON-SPECIFIC SUBSTANCES.

We have investigated the action of para-aminosalycilic acid (PAS) in *P. berghei* infections in mice. Deschiens and Pick (1949) found favourable results in *P. gallinaceum* infections when treated with PAS. Three lots of mice have been used. The first lot received one mg. of PAS daily through the subcutaneous route for six days commencing from two days prior to the inoculation. Thereafter the dose was increased up to 50 mg. The mice died on the 11th, 12th and 13th day of infection similar to the controls of the third lot. The second lot of mice were treated at the height of infection (commencing from the 8th day) with three injections of 30 mg. of PAS in six days. The mortality was similar to that of the first lot. It appears, therefore, that PAS has no effect whatsoever on *P. berghei* infections.

We have also tried to protect infected mice by a diet of milk exclusively or milk enriched by vitamin complex (B_1, B_2, nicotinamide and calcium pantothenate). Like Schneider and Montezin (1950), we have not proved any influence of milk on the progress of infection. It is not known whether these results were not due to digestive disorders which are nearly always present in mice when submitted to such diets.

In order to reduce the anæmia due to *P. berghei* infection in mice, we have injected into them, both prior to and during infection, folic acid and vitamin B_{12}. The modest doses of vitamin B_{12} used at the beginning of our experiments were soon increased up to 100-1,000 gamma. By such treatment, we have obtained increased survival of infected mice by one to three days. But on the whole the difference between treated and untreated mice is not appreciably significant.

We have tested the effect of somatotrophic pituitary hormone (STH) in *P. berghei* infections, as it is known to stimulate tissue and metabolic growth, promote phagocytosis and at the same time is antagonistic to ACTH.

Inoculation and commencement of treatment took place simultaneously in the first series of experiments involving 14 mice. Injection of five units of the hormone every alternate day delayed death by three days in the experimental lot as compared to the controls (nine days against six days). The parasitæmia in the treated group was less than that of controls.

In the second series of animals (14 mice), the treatment commenced on the day after the inoculation and lasted for ten days. Each injection of the hormone given every alternate day, consisted of five units. The untreated controls died

in five to six days with 50 per cent cell infection while the treated animals died between the 9th and 11th day, without any patent parasitæmia, or occasionally with one parasite for every 1,000 to 3,000 red cells. The parasitæmia remained constant during the last three days. As against untreated controls which died in eight days with 40 to 60 per cent parasitized cells, some treated mice survived up to 24 days and had 8 to 15 per cent parasitized cells at death. The progress of infection in the treated mice has been characteristic in that the prepatent period was eight days as compared to two days in the untreated. Further, the course of patent parasitæmia was peculiar and characteristic. In three animals, the parasitæmia distinctly increased (30 to 40 per cent parasitized cells) up to the 15th day, then decreased to 20 per cent parasitized cells on the 17th day and five per cent on the 20th day when the animals died. In one animal, the injection of hormone was stopped on the 22nd day. The parasitæmia rose to ten per cent cell infection on the 24th day when the animal died. In all cases, the parasitæmia was low at death.

From the point of view of morphology of parasites, there is hardly any difference in the treated and untreated animals till the 15th day. The treatment produces a sudden dimunition of the parasitæmia which is characterized by a considerable reduction in the number of dividing forms, a complete absence of schizonts and merozoites both intra- and extra-cellular. The parasites are mainly those of the ring stage found in red cells which exhibit basophille granules. When the mice survive beyond 20 days and the treatment is withdrawn, dividing forms and schizonts immediately reappear with an increase in parasitæmia.

By way of recapitulation, pituitary STH hormone exercises a retarding and neutralizing action on *P. berghei* infection in mice, provided the animals are pre-conditioned by treatment prior to inoculation and continued after it. The course of infection is characterized by a prolongation of the prepatent period and the stabilization of parasitæmia at a low level with the disappearance of dividing forms. The reappearance of dividing forms and increase in the parasitæmia take place immediately the treatment is withdrawn.

We have described above the observed effect of pituitary STH hormone on the course of infection. The slightly longer survival of the treated animals, however, is difficult to interpret. It is possible that a diet, rich in protein, may counteract the increased metabolism due to the hormone and facilitate its action. To test such a hypothesis, we fed mice with a total extract of milk (hyperprotidine). When such mice are infected and treated with STH, the animals are found to have increased appetite and their infection is much more severe. The results varied in the different animals. In some, the infection was prolonged and persisted till after 28 days when the blood showed 5 to 20 per cent parasitized cells. In others, the infection was continuously severe up to the 27th day when the blood showed 80 per cent cell infection. Such severe infections have not been observed by us previously.

It is possible that the high protein diet compensates the increased metabolism induced by STH and enables the animal to resist relatively even exceptionally severe infections.

These results pose a question as to how an exclusive diet of proteins would affect the infection in mice. Our experiments have proved that the effect of such

a diet is very appreciable. The prepatent period in animals on such diet is prolonged and the survival period is increased by five to eight days in comparison to the control animals. Sometimes the infection is distinctly reduced at the terminal period.

In conclusion, PAS, contrary to what is observed in *P. gallinaceum* infection, does not exercise any action on *P. berghei* infections. Administration of anti-anæmic agents (folic acid, vitamin B_{12}) compensates the anæmia due to infection but otherwise as inefficacious as an exclusive milk diet or one in which milk is enriched by vitamin complex (B_1, B, nicotinamide, calcium pantothenate). On the contrary STH, and a diet exclusively of proteins, exercise a retarding and distinctly neutralizing effect on *P. berghei* infections in mice without, however, being able to save the animals from death.

ATTEMPTS WITH PLASMODICIDAL DRUGS.

Different authors have pointed out the curative action of antimalarials in *P. berghei* infections. Vincke and van den Bulcke (1949) were the first to point out the curative effect of Aralen (Nivaquine) which clears rapidly the parasites from the peripheral blood. The majority of treated mice had latent infections for long periods but some appear to have been definitely cured. The number of such cured animals increased proportionately to the number of animals in which the relapses were treated.

Other authors, particularly Baldi and Della Rocca (1951), have confirmed these findings. These two authors reinoculated treated mice two to four months old after the primary inoculation and found that the re-infection was severe. The first infection was thus found to have had no effect on the second.

We have investigated whether repeated treatment of relapses with small doses of nivaquine, thus prolonging the infection, would produce any premunition. The basis of treatment was determined by the observed daily parasitæmia as well as the general condition of the animal, i.e. colouration of skin, appearance of hair, and general behaviour.

We examined blood films daily and studied the hæmatology both during periods of patent parasitæmia and when the parasitæmia was not patent. We shall not enter into the details of the experiments. They were carried out on 40 mice. Only a dozen of these had a certain number of relapses which we have been able to follow sufficiently long. With the object of determining the presence of any resistance to reinfection in the mice treated for several relapses, we have re-inoculated them with the homologous strain from 20 to 200 days after treatment. The conclusions of the investigations are as follows:

Repeated treatment with nivaquine of successive relapses in mice infected with *P. berghei* establishes a progressive resistance to the parasite as indicated by the progressive prolongation of the inter-relapse periods proportionately to the increased doses of nivaquine. In other words, the hæmatological modifications (with particular reference to leucocytes) are less pronounced in the course of different relapses, and remain constant. This resistance is variable in individual mice. It is sometimes found to be absent altogether as the mice are re-infected easily even after one or more relapses having been treated.

In some cases, the resistance is of a low order. The re-inoculated mice exhibit a prolonged prepatent period as compared to controls.

In other cases, the resistance is high and it is not possible to re-infect the mice. In one case the period of 11 days that elapsed between re-inoculation and death of the animal, cannot indisputably be taken to indicate prolongation of the prepatent period. Nevertheless, we feel that a high degree of resistance to infection was shown by the animal.

A remarkable observation is in the case of a mouse which has acquired sterilizing immunity. This mouse exhibited a mild parasitic relapse after 45 days of latency. The relapse regressed and disappeared spontaneously without any treatment. Normally untreated relapses in mice terminate fatally. Thus it would appear that some resistance to infection can develop in certain mice as a result of repeated treatment. The resistance would appear to vary in different mice from being completely absent in some which are easily re-infected, while in others is sufficiently high to prevent re-infection. This immunity in mice appears, however, to be short lived as after sometime they can be re-infected.

CONCLUSIONS.

In terms of investigations discussed above, it appears that non-plasmodicidal substances are rarely capable of modifying favourably the course of *P. berghei* infection in mice. An interesting instance is that of pituitary STH which exercises a retarding and distinctly neutralizing action on *P. berghei* infection. It appears that this action of the hormone can be re-inforced by a diet rich in proteins. The proteins probably act by compensating the increased metabolism induced by the hormone and spare the infected mouse a longer period of survival. It must also be remembered that a similar effect is encountered when the mice are fed on a diet rich in proteins even in the absence of any further treatment.

Plasmodicidal drugs used to treat succesive relapses are capable of producing a progressive resistance to parasite in the host. The degree of such resistance, however, varies in different mice. It differs from a complete absence in some mice to a sufficiently high degree of a sterilizing immunity to prevent re-infection totally.

We have intentionally confined ourselves to the results of our investigations on mice. It appears to us that attempts to produce resistance to re-infection with *P. berghei* to be significant, must be made in mice in which the infection is uniformly severe.

With regard to the rat, results are more difficult to interpret since it is known among them that there exists an immunity which is greater in older animals. We have already had occasion in our previous publications to emphasize this fundamental difference between mouse and rat, and the preference to the former for investigations to study the diet and medication for a protective effect against *P. berghei* infection.

SUMMARY.

Immunity is difficult to study in rats, which recover spontaneously. Whilst in mice the course of infection by *Plasmodium berghei* is always fatal, the slightest favourable effect interfering with this course is conspicuous. Different methods were used.

1. *Biological.*—Injection of serum of rats having recovered from their infection, or serum of infected mice. No favourable result was obtained. Besides, mice are very susceptible to heterogeneous proteins and do not support necessary doses to build a passive immunity. No results were obtained with injections of infected blood partly digested in the mid-gut of *Rhodnius prolixus*.

2. *Non-specific substances.*—Neither para-aminosalicylic acid which inhibits infections by *Plasmodium gallinaceum* had any effect on the course of *P. berghei* in mice nor a vitamin complex (B_1, B_2, nicotinic acid, calcium pantothenate). In order to prevent anæmia, heavy doses of vitamin B_{12} and folic acid were given preventively as well as curatively. The treated mice died one to three days after controls. Milk diet was absolutely ineffective.

Somatotrophic hormone (STH) has a definite inhibiting effect on *P. berghei* if it is injected before and during infection. The results are variable. In all cases, the prepatent period is prolonged (eight days ; two days in controls). Treated mice died much later than controls with few infected red cells (5 to 15 per cent on the 24th day; 60 per cent on 8th day in controls). To neutralize anabolizing effect of STH we added to the diet total milk protein extract. Death was considerably delayed (28th day) and occurred with a very slight (5 per cent) or heavy (80 per cent) infection. Protein extract, used exclusively, has also some inhibiting effect on the infection.

3. *Plasmodicidal drugs.*—We tried to obtain immunity in mice by way of repeated treatment of each recurrence of the infection with small doses of nivaquine. A definite resistance develops and the duration of the latent phase increases after each relapse followed by treatment. A phase of true immunity occurs, more or less soon, during which the animal is able to control, without treatment, a parasitic relapse. This phase is short. It is followed by entire clinical recovery and abolition of parasitism. Animals become again susceptible and the result of reinoculation is exactly the same as in untreated controls.

REFERENCES.

ALIBAUX-FERNET, M. (1952) ... ... *Presse Medicale*, **60**, p. 1621.
BALDI, A. (1952) ... ... *Riv. Malariol.*, **31**, p. 41.
BALDI, A. and DELLA ROCCA (1951) ... *Ibid.*, **30**, p. 173.
DESCHIENS, R. and PICK, F. (1949) ... *C. R. Soc. Biol.*, **144**, p. 662.
EVANS, and LONG, (1921) ... *Anat. Rec.*, **21**, p. 61.
GALLIARD, H., BUTTNER, A. and BOURGART, N. (1953) ... *C. R. Soc. Biol.*, **140**, p. 1695.
GALLIARD, H. and LAPIERRE, J. (1950) ... *Bull. Soc. Path. Exot.*, **43**, p. 317.
Idem (1951) ... ... *Ibid.*, **44**, p. 185.
GALLIARD, H., LAPIERRE, J. and MURARD, J. (1945) ... *Ann. Parasit. Hum. Comp.*, **29**, No. 3
GOODWIN, L. G. (1949) ... ... *Nature*, Dec. 31, p. 1153.
HILL, J. (1950) ... ... *Ann. Trop. Med. Parasit.*, **44**, p. 291.
LABORIT, H., FAVRE, A., DUCHESNE, G., DECHEN, J. and BASTIT, G. (1953) ... ... *Presse Medicale*, **61**, p. 1085.
LAPIERRE, J. (1954) ... ... *Bull. Soc. Path. Exot.*, **47**, p. 380.
MUDROW REICHENOW, L. (1951) ... *Zeitschrift Tropenmed Parasit.*, **2**, p. 471.
SCHNEIDER, J. and MONTEZIN, G. (1950) ... *Bull. Soc. Path. Exot.*, **43**, p. 44.
SELYE, H. (1952) ... ... *Amer. Physiol. Soc. 64th Annual Meeting*, New York, p. 14. Also in *Fed. Proc.*, **2**, p. 144.
THURSTON, J. P. (1950) ... ... *Brit. J. Pharm. Chem.*, **5**, p. 409.
VINCKE, J. H. and VAN DEN BULCKE, M. A. (1949) ... *Ann. Soc. Belge Med. Trop.*, **29**, p. 526.

Indian Journal of Malariology, **8**, 4, December 1954.

THE ABSENCE OF CROSS IMMUNITY BETWEEN *PLASMODIUM BERGHEI* (VINCKE AND LIPS) AND *PLASMODIUM VINCKEI* (RODHAIN)*.

BY

J. RODHAIN.

(*Prince Leopold Institute of Tropical Medicine, Antwerp.*)

(August 13, 1954.)

THE Plasmodia of small rodents discovered at Katanga, Belgian Congo, considered to be morphologically and biologically different from one another, have nevertheless more than one character in common.

First, they both have the interesting characteristic of being transmissible to white mice although their pathogenicity to mice are of different degrees. Another common character is that the wild mosquito *Anopheles dureni* (Edwards) is the vector for both. It was considered of interest to examine whether there is any cross immunity between the infections due to the two parasites. The existence of cross-immunity should in a way bring the two species close to one another. On the contrary its non-existence should favour their separation into two species. With this object in view, we carried out a number of experiments with mice and rats.

It is necessary to recall briefly the respective pathogenic characteristics of the two parasites with regard to the animals used in the experiments, and briefly review what is known about the immunity which manifests itself in the rodents after their infections have been cured.

It has been established that *P. berghei* infections in mice are always fatal ; this excludes experiments on immunity in these animals during the course of uninterrupted infection in them. It is different in the rats. The proportion of rats inoculated with *P. berghei* that survive, varies with age. The young animals die in greater numbers than adults. In the latter, almost 50 per cent are cured. The animals which survive show resistance to re-inoculation. They acquire

*Thanks are due to Dr. D. Metselaar, M.B., D.T.M. & H. (England), Chief of the Malaria Division of Public Health Service, Netherlands, New Guinea, for translating the original paper written in French into English, during his visit on W.H.O. fellowship.—*Editor.*

immunity the peculiarities of which have been the object of numerous investigations. To cite only those of Corradetti (1950) in Italy and of H. Galliard and Lapierre (1951) in France, have been of great interest.

With regard to *P. vinckei*, in the course of experiments 80 per cent of the infected mice survived. For this reason it was possible to investigate if these animals had an initial advantage when exposed subsequently to *P. berghei* infection. The results of such experiments are given later.

The results of an opposite experiment which consisted of re-infecting with *P. vinckei* rats naturally cured of their *P. berghei* infections seemed to us less convincing. As a matter of fact, the first adult rat inoculated with parasitized mouse blood did not contract the infection. Vincke (1953) himself had similar negative results. Besides, of the three splenectomized rats inoculated with *P. vinckei* one did not acquire the infection, and the two others had a mild parasitæmia. Finally, in young rats the weight of which varied between 25 and 30 g., we found 90 per cent of the inoculated animals had a fatal course. The fatal course diminished proportionately when animals with higher weight were used. But the infections were always accompanied with abundant parasitæmia.

Working with young rats cured of *P. berghei* infection, it became possible to observe that they were still susceptible to *P. vinckei* infection. In different series of experiments, the animals were inoculated always by the intraperitoneal route with three drops of blood containing abundant parasites. The blood was taken from the tail of mice, rats and hamsters. We shall first describe the experiments on mice cured of *P. vinckei* infections and subsequently inoculated with *P. berghei*. After that we shall describe the results obtained in young rats cured of *P. berghei* infections and later exposed to *P. vincke*.

I. MICE CURED OF *P. VINCKEI* INFECTIONS AND INOCULATED WITH *P. BERGHEI*.

Our first series was carried out on three mice which were naturally cured of their infection. Table I gives the details :—

TABLE I.

The course of P. berghei *infection in mice cured of* P. vinckei *infection.*

Number of the mouse	Date of inoculation	Result of blood examination	Date of *P. berghei* inoculation	Results
1	April 8, 1952	25·4 : +++ 30·4 : 0 Cured	July 8, 1952. Hamster blood +++, Mukata strain I.	11·7 : +++ 22·7 : fatal
9	May 10, 1952	Infection type 29·5 : 0 Cured	July 8, 1952. Hamster blood +++, Mukata strain I.	11·7 : + 22·7 : fatal
44	August 8, 1952	Infection was noticeable till August 30, after which it was cured.	September 2, 1952. Mice blood, Mukata strain II.	12·9 : fatal

In each experiment, control mice were inoculated which died in the same period as Mice 1, 9 and 44. In a second series of experiments, we used mice cured once of a first infection of *P. vinckei* and re-inoculated for a second time with *P. vinckei*. Table II describes the details.

TABLE II.

Mice having survived two inoculations of P. vinckei, *were later on inoculated with* P. berghei.

Number of the mouse	Date of first inoculation	Result of blood examination	Date of second inoculation	Result of blood examination	Date of *P. berghei* inoculation	Results
29	July 2, 1952.	Positive July 2, 1952. Cured July 16, 1952.	Aug. 29, 1952.	Positive Sept. 1, 1952. Cured Sept. 8, 1952.	Sept. 8, 1952. *Praomys* strain	Positive on Sept. 10, 1952. Died Sept. 16, 1952.
34	July 22, 1952.	Positive July 24, 1952. Blood containing very few parasites.	Aug. 19, 1952.	Very few parasites seen till Sept. 8, 1952.	Sept. 8, 1952. Mukata strain I.	Positive on Sept. 10, 1952. Died Sept. 20, 1952.
36	July 20, 1952.	Positive July 24, 1952. On August 19, 1952, very few plasmodiums.	Aug. 19. 1952.	Very few parasites on Aug. 2, 1952. Few parasites on Aug. 23, 1952.	Sept. 2, 1952. Mukata strain II.	Positive on Sept. 5, 1952. Died Sept. 9, 1952.
42	July 30, 1952.	Positive Aug. 4, 1952. Few parasites on Aug. 18, 1952.	Aug. 19, 1952.	Positive Aug. 30, 1952. Few parasites on Sept. 9, 1952.	Sept. 8, 1952. *Berghei* strain IV.	Positive on Sept. 12, 1952. Died Sept. 26, 1952.

Examination of the above table makes one conclude that there is complete absence of resistance with regard to *P. berghei* in the four mice which had been exposed to two infections of *P. vinckei*. Furthermore, let us also say that, in the course of these experiments we have used four different Strains of *P. berghei* which enhances the value of these experiments. The *berghei* Strain IV originates from nearby Elisabethville, while the three other strains have been isolated in the north, the Mukata strains I and II by inoculation of sporozoites from *A. dureni* in white rats, the *Praomys* strain by inoculation of blood from a *Praomys* found naturally infected.

We must record some more peculiarities observed in mice which have been submitted to a second inoculation of *P. vinckei*. The blood of Mouse 29 was free of parasites during 34 days when it was inoculated for the second time. A few parasities, schizonts and gametocytes, reappeared in the peripheral blood for only five days. The behaviour of Mouse 34 was slightly different. The infection was serious in her as a result of the first inoculation. There were still a few parasites in her blood when she was re-inoculated. The parasites increased during six days, to diminish from the eighth day onwards. But they were still present 19 days after

the second inoculation. In the meantime, the general appearance of the animal maintained well which allowed us to inoculate her with *P. berghei*. The malaria which resulted from the last infection of *P. berghei*, ended in death in ten days.

Mice 36 and 42 behaved almost like Mouse 29. The second inoculation of *P. vinckei* resulted in the reappearance of a few parasites in the blood during five days. We describe the details because they show that first infection of *P. vinckei* in the mice develops a degree of resistance with regard to re-infection. Re-infection, nevertheless is followed by parasitæmia of low intensity, is maintained more than 15 days, the animals remaining in a state of premunition. During this period schizonts and gametocytes may persist in the blood and we have observed macrogametocytes which did not show the faintest sign of a vaccuole.

II. YOUNG RATS CURED OF AN INFECTION OF *P. BERGHEI* AND RE-INOCULATED WITH *P. VINCKEI*.

We have done our experiments with two different series of animals. In the first series, the two animals cured of *P. berghei* had become semi-adults before being inoculated with *P. vinckei*. Their first malaria had progressed with an average intensity and the parasitæmia in the one lasted for nine days and in the other for 24 days. Their blood remained free of parasites from September 30, 1953. They were inoculated with *P. vinckei* on February 18, 1954, by means of heparinized blood from a young rat (Number 72) in which the infection was of the 22nd passage in young rats. The weight of the two animals was above 120 g. Both became infected. Parasites appeared first on February 21. The parasitæmia lasted for seven days in one and for 11 days in the other. The parasites were never abundant. This completely corresponds with what is known to happen in clean semi-adult rats.

In the second series, the interval between inoculation with *P. berghei* and *P. vinckei* was shorter in order to observe the results in relatively younger animals. *P. vinckei* used in this series had passed 43 and 44 passages in young rats. Four animals were used in the experiment, their weights being, respectively, Rats 11 and 12 of 35 g., Rat 13 of 50 g., and Rat 14 of 45 g. They were inoculated intraperitoneally on May 3, 1954, with blood of Mouse 24 containing abundant parasites of Kisanga strain.

Parasites appeared in the blood in three rats on May 6, and in the fourth on May 10. The parasitæmia was heavy in two and moderate in the other two. Duration of parasitæmia in Rats 11 and 13 was 22 days, not more than 19 days in Rat 12, and 15 days in Rat 40. The blood of the animals was free of parasites on May 29, and showed reticulocytosis which we know accompanies a cure. Rats 12 and 14 were inoculated with *P. vinckei* on May 29; Rats 11 and 13 on June 1. Both received intraperitoneally heavily parasitized blood obtained from the tail of young rats (Numbers 112 and 113) in which *P. vinckei* was of 43rd and 44th passage in rats. The four animals became infected. The first parasites appeared in the blood on the third day of inoculation. The parasitæmia developed normally with regard to their weights which had increased despite their previous infection with *P. berghei*. Their weights were respectively 52, 50, 62 and 55 g.

The presence of parasites in the peripheral blood persisted during ten days in Rat 14, 14 days in Rat 11, 25 days in Rat 12 and 27 days in Rat 13.

Also the morphology of the parasites was typical of *P. vinckei*. Regarding the long persistence of parasites in Rat 13, we inoculated 0·25 c.c. of blood obtained from the heart of the latter into two mice with the purpose to exclude the possibility of a relapse of *P. berghei*. These two mice developed a typical fatal infection of *P. vinckei*.

CONCLUSION.

Mice which survived *P. vinckei* malaria, remained susceptible to *P. berghei* of which they died in the same time as clean control mice.

Young rats surviving *P. berghei* infection, remained susceptible to *P. vinckei*.

These experiments show that there is no cross-immunity in mice and rats as a result of the two infections. The absolute absence of cross-immunity between the two parasites proves their specificity.

Note.—Since the beginning of the year 1952 in which we started in our laboratory the maintenance of a strain of *P. vinckei* in mice, the parasite has considerably increased its pathogenicity for these animals. Besides, the serial passage in young rats seemed to have also increased the pathogenicity of the infection. The pathogenicity of *P. vinckei* has, in this way, increased to that of *P. berghei*. Morphologically, however, there has been no visible alteration.

REFERENCES.

Corradetti, A. (1950)	*Riv. Parassitol.*, **11**, pp. 201-223.
Fabiani, G., Vargues, R., Grellet, F. and Clausse, J. (1951)	*C. R. Soc. Biol.*, **145**, pp. 1131-1134.
Galliard, H. and Lapierre (1951) ...	*Bull. Soc. Path. Exot.*, **44**, pp. 185-194.
Rodhain, J. (1953)	*Ann. Inst. Pasteur.*, **84**, p. 572.
Idem (1954)	*Ann. Soc. Belge Med. Trop.*, **34**, (In press).
Vincke, I., Peeters, E. and Frankie, G. (1953)	*Bull. Inst. Roy. Col. Belge*, **24**, p. 1364.

Indian Journal of Malariology, **8**, 4, December 1954.

SOME OBSERVATIONS ABOUT IMMUNITY TO *PLASMODIUM BERGHEI.*

BY

R. M. DE SMET

AND

G. FRANKIE.

(*S.E.R.A.M., B.P.* 1190, *Elisabethville.*)

(August 14, 1954.)

THE following experiments were undertaken in an attempt to establish whether immunity or premunition to *P. berghei* exists or not.

For this study, the white mouse was unsuitable ; the course of infection being always fatal in this animal.

We experimented on three species of rodents (*Cricetomys ansorgei*, *Rattus norvegicus* var. *albinos* and *Thamnomys surdaster surdaster*) which usually showed an infection with relapses and final recovery.

These three species showed a definite resistance to re-inoculation but their reactions being slightly different, we will describe them separately for each species.

I. *CRICETOMYS ANSORGEI.*

OBSERVATION 1.

Cricetomys 6086.—

December 20, 1952 : Inoculated with blood from the tail of a positive white rat.

December 24, 1952, to January 26, 1953 : Positive.

January 27 to August 12, 1953 : Negative : four subinoculations to the white mouse were negative (July 29, 30 and 31, 1953 : Treatment with daraprim).

August 13, 1953 : Inoculated with one c.c. blood from the heart of an infected mouse.

August 14 to September 4, 1953 : Negative.

September 5, 1953: Injected with 0·5 c.c. blood from the heart of an infected mouse.

September 6 to September 23, 1953 : Negative.

September 25, 1953 : Injected with one c.c. blood from the heart of an infected mouse.

October 27, 1953 : Injected with three c.c. blood from the heart of an infected white rat.

October 27, 1953 to January 27, 1954 : Negative.

January 28, 1954 : Re-inoculated with blood from the tail of an infected white rat.

January 29 to July 8, 1954 : Remained negative.

July 9, 1954 : Re-inoculated with one c.c. blood from the heart of an infected white rat.

July 10 to July 20, 1954 : Negative.

OBSERVATION 2.

Cricetomys 5199.—

September 20, 1952 : Inoculated with blood from the tail of an infected *Thamnomys.*

October 3 to October 14, 1952 : Positive.

October 15, 1952 to January 23, 1953 : Negative ; 18 sub-inoculations, negative.

January 24, 1953 : Re-inoculated with 15 drops of blood of a white rat.

January 25 to February 5, 1953 : Negative.

February 6, 1953 : Re-inoculated with blood from the tail of a positive *Thamnomys.*

February 7 to March 1, 1953 : Negative.

March 2, 1953 : Re-inoculated with blood from the tail of a positive mouse.

March 3 to March 19, 1953 : Negative.

March 20, 1953 : Inoculated with blood from the tail of an infected white rat.

March 21 to June 26, 1953 : Negative.

June 27, 1953 : Re-inoculated with blood from the heart of a positive mouse.

June 28 to September 29, 1953 : Negative.

September 30, 1953 : Re-inoculated with blood from the heart of a positive mouse.

October 1 to October 26, 1953 : Negative.

October 27, 1953 : Re-inoculated with two c.c. of blood from the heart of a positive white rat.

October 28, 1953 to January 10, 1954 : Negative.

January 11, 1954 : Re-inoculated with blood from the heart of a positive mouse.

January 11 to July 8, 1954 : Negative.

July 9, 1954 : Re-inoculated with one c.c. of blood from the heart of a positive white rat.

July 9 to July 20, 1954 : Negative.

OBSERVATION 3.

Cricetomys 4066. —

July 18, 1952 : Inoculated with 0·75 c.c. of blood from the heart of a positive white mouse.

July 23 to October 25, 1952 : Positive.

February 20, 1953 : Re-inoculated with blood from the tail of a positive white mouse.

February 21 to March 1, 1953 : Negative.

March 2, 1953 : Re-inoculated with blood from the tail of a positive mouse.

March 3 to March 18, 1953 : Negative.

March 19, 1953 : Re-inoculated with blood from the tail of a positive white rat. On the same day, *Cricetomys* 6202 (test proof), inoculated with blood from the tail of a positive white rat, became positive.

March 20 to July 31, 1953 : Negative.

August 1, 1953 : Re-inoculation with one c.c. of blood from the heart of a positive white mouse. On the same day, *Cricetomys* 6636 (test proof), injected with one c.c. of blood from the heart of a positive mouse, became positive.

August 8, 1953 to August 17, 1953 : Negative.

August 18, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive white rat. *Cricetomys* 6005 (test proof), injected with two c.c. of blood from the heart of a positive white rat, became positive.

August 19 to September 4, 1953 : Negative.

September 5, 1953 : Re-inoculated with 1 c.c. of blood from the heart of a positive mouse. *Cricetomys* 4329 (test proof), injected with one c.c. of blood from the heart of a positive rat, became positive.

September 6 to September 23, 1953 : Negative.

September 24, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive mouse. *Cricetomys* 6889 (test proof), injected with 0·75 c.c. mixed blood from the heart of two positive mice, became positive.

September 25, 1953 to January 6, 1954 : Negative.

January 7, 1954 : Re-inoculated with one c.c. of blood from the tail of a positive *Cricetomys*.

January 8 to April 2, 1954 : Negative. Four sub-inoculations negative.

OBSERVATION 4.

Cricetomys 4317.—

September 20, 1952 : Inoculated with blood from the tail of a positive *Cricetomys*.

September 24 to November 7, 1952 : Positive.

November 12 to November 18, 1952 : Negative.

November 19 to December 3, 1952 : Positive.

December 5, 1952 to February 19, 1953 : Negative; four sub-inoculations, negative.

February 20, 1953 : Re-inoculated with blood from the tail of a positive white rat.

February 21 to March 1, 1953 : Negative.

March 2, 1953 : Re-inoculated with blood from the tail of a positive mouse.

March 2 to March 18, 1953 : Negative.

March 19, 1953 : Re-inoculated with blood from the tail of a positive white rat.

March 19 to November 1, 1953 : Negative.

OBSERVATION 5.

Cricetomys 5137.—

September 9, 1952 : Inoculated with blood from the tail of a positive *Thamnomys*.

September 30 to October 1, 1952 : Positive.

October 2 to October 20, 1952 : Negative.

October 21, 1952 : Re-inoculated with blood from the tail of a positive white rat.

October 22 to November 13, 1952 : Negative.

November 14, 1952 : Re-inoculated with blood from the tail of a positive *Thamnomys*.

November 15 to December 17, 1952 : Negative.

December 18, 1952 : Re-inoculated with blood from the tail of a positive *Thamnomys*.

December 19 to December 28, 1952 : Negative.

December 29, 1952 : Re-inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

December 30, 1952, to July 23, 1953 : Negative.

OBSERVATION 6.

Cricetomys 5183.—

July 18, 1952 : Inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

July 23 to September 15, 1952 : Positive.

September 17 to September 30, 1952 : Negative.

October 3 to October 11, 1952 : Positive.

October 13, 1952 to February 2, 1953 : Negative ; two sub-inoculations, positive ; two sub-inoculations, negative.

February 17, 1953 : Inoculated with blood from the tail of a positive white rat.

February 18, to February 23, 1953 : Negative.

February 24, 1953 : Re-inoculated with blood from the tail of a positive mouse.

February 24 to March 10, 1953 : Negative.

March 11, 1953 : Re-inoculated with blood from the tail of a positive white rat. *Cricetomys* 6240 (test proof), injected with blood from the tail of a positive white rat, became positive.

March 11 to July 7, 1953 : Negative.

OBSERVATION 7.

Cricetomys 6202.—

March 19, 1953 : Inoculated with blood from the tail of a positive white rat.

March 25 to April 14, 1953 : Positive.

April 15 to May 18, 1953 : Negative ; one sub-inoculation, positive.

May 20 to May 30, 1953 : Positive.

June 10 to August 12, 1953 : Negative ; seven sub-inoculations, negative.

August 13, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive white mouse. *Cricetomys* 6655 (test proof), injected with one c.c. of blood from the heart of a positive mouse, became positive.

August 14 to September 20, 1953 : Negative.

September 21, 1953 : Re-inoculated with one cc. of blood from the heart of a positive mouse. *Cricetomys* 6655 (test proof), injected with one c.c. of blood from the heart of a positive mouse, became positive.

September 21, 1953, to January 6, 1954 : Negative.

January 7, 1954 : Re-inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

January 8 to February 14, 1954 : Negative.

February 15, 1954 : Re-inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

February 16 to July 8, 1954 : Negative.

July 9, 1954: Re-inoculated with one c.c. of blood from the heart of a positive white rat. *Cricetomys* 35 (test proof), injected with one c.c. of blood from the heart of a positive white rat, became positive. *Cricetomys* 36, injected with one c.c. of blood from the heart of a positive white rat, became positive.

July 9 to July 20, 1954 : Negative.

Observation 8.

Cricetomys 5996.—

December 24, 1953 : Inoculated with eight positive salivary glands of *Anopheles dureni*.

January 22 to February 17, 1953 : Positive.

February 18 to February 23, 1953 : Negative with one negative and four positive sub-inoculations.

February 24 to March 9, 1953 : Positive.

March 16 to July 30, 1953 : Negative : 13 negative sub-inoculations.

July 31, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive mouse.

August 6 to August 12, 1953 : Positive.

August 13 to August 27, 1953 : Negative ; two negative sub-inoculations.

August 28, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive mouse.

August 29 to November 17, 1953 : Negative : five negative sub-inoculations.

November 18, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive white rat.

November 19, 1953 to January 31, 1954 : Negative.

February 1, 1954 : Re-inoculated with one c.c. of blood from the heart of a positive white rat.

February 2 to July 8, 1954 : Negative.

July 9, 1954 : Re-inoculated with one c.c. of blood from the heart of a positive mouse.

Test proof : *Cricetomys* 36, injected with one c.c. of blood from the heart of a positive white rat, became positive.

Cricetomys 35, injected with one c.c. of blood from the heart of a positive white rat, became positive.

OBSERVATION 9.

Cricetomys 5358.—

August 5, 1952 : Inoculated with blood from the tail of a positive *Thamnomys*.

August 21 to October 5, 1952 : Positive.

October 7, 1952 to January 23, 1953 : Negative ; 16 negative sub-inoculations.

January 24, 1953 : Re-inoculated with blood from the tail of a positive mouse.

January 24 to February 5, 1953 : Negative.

February 6, 1953 : Re-inoculated with blood from the tail of a positive *Thamnomys*.

February 7 to February 23, 1953 : Negative.

February 24, 1953 : Re-inoculated with blood from the tail of a positive mouse.

February 24 to March 17, 1953 : Negative.

March 18, 1953 : Re-inoculated with blood from the tail of a positive mouse.

March 19 to July 31, 1953 : Negative.

August 1, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive mouse.

Test proof : *Cricetomys* 6636, injected with one c.c. of blood from the heart of a positive mouse, became positive.

August 2 to August 17, 1953 : Negative.

August 18, 1953 : Re-inoculated with 1·5 c.c. of blood from the heart of a positive white rat.

Test proof : *Cricetomys* 6605, injected with two c.c. of blood from the heart of a positive white rat, became positive.

August 19 to October 2, 1953 : Negative.

October 3, 1953 : Re-inoculated with one c.c. of blood from the heart of a positive *Cricetomys*.

Test proof : *Cricetomys* 4, injected with one c.c. of blood from the tail of a positive *Cricetomys*, became positive.

January 14 to July 8, 1954 : Negative.

July 7, 1954 : Re-inoculated with one c.c. of blood from the heart of a positive mouse.

Test proof : *Cricetomys* 36, injected with one c.c. of blood from the heart of a positive white rat, became positive.
Cricetomys 35, injected with one c.c. of blood from the heart of a positive white rat, became positive.

OBSERVATION 10.

Cricetomys 4885.—

July 4, 1952 : Inoculated with 0·75 c.c. of blood from the heart of a positive white rat.

July 9 to August 9, 1952 : Positive.

August 11 to August 19, 1952 : Negative.

August 20 to August 26, 1952 : Positive.

August 30 to September 10, 1952 : Negative.

September 15, 1952 : Positive.

September 17, 1952 : Negative.

September 26 to October 3, 1952 : Positive.

October 5, 1952, to February 19, 1953 : Negative ; 28 negative sub-inoculations.

February 20, 1953 : Re-inoculated with blood from the tail of a positive house.

February 21 to March 1, 1953 : Negative.

March 2, 1953 : Re-inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

March 3, 1953 to January 27, 1954 : Negative ; nine negative sub-inoculations.

January 28, 1954 : Re-inoculated with one c.c. of blood from the heart of a positive white rat.

January 28 to July 8, 1954 : Negative.

July 9, 1954 : Inoculated with one c.c. of blood from the heart of a positive white rat.

Test proofs : *Cricetomys* 36, injected with one c.c. of blood from the heart of a positive white rat, became positive.
Cricetomys 35, injected with one c.c. of blood from the heart of a positive white rat, became positive.

WHITE RATS.

OBSERVATION 1.

White rat 888.—

March 2, 1953 : Injected with blood from the tail of a positive white rat.

March 7 to March 13, 1953 : Positive.

March 18 to March 25, 1953 : Negative.

April 2, 1953 : Positive.

April 8 to August 15, 1953 : Negative.

April 28 and 29, 1953 : Two positive sub-inoculations.

May 22 to August 15, 1953 : Nine negative sub-inoculations (two after treatment with daraprim).

July 29, 30 and 31, 1953 : Treated with daraprim.

August 16, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

Test proof : Rat 1375, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive.

August 17 to September 2, 1953 : Negative.

September 3, 1953 : Injected with 0·50 c.c. of blood from the heart of a positive mouse.

September 9, 1953 : Positive.

September 10 to September 23, 1953 : Negative.

September 24, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

Test proof : Rat 1410, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive.

September 25 to October 2, 1953 : Negative.

October 3, 1953 : Injected with 0·75 c.c. mixed blood from the heart of three positive mice.

Test proof : Rat 1440, injected with 0·75 c.c. blood, became positive.

October 4, 1953, to January 8, 1954 : Negative.

January 9, 1954 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

Test proof : Rat 1614, injected with 0·75 c.c. of blood, became positive.

January 10 to May 5, 1954 : Negative.

OBSERVATION 2.

White rat 897.—

March 3, 1953 : Inoculated with blood from the tail of a positive white rat.

March 7 to March 18, 1953 : Positive.

March 20, 1953 : One positive sub-inoculation.

March 25 to April 2, 1953 : Negative.

April 8, 1953 : Positive.

April 15 to August 15, 1953 : Negative with four sub-inoculations (two after treatment with daraprim).

August 16, 1953 : Inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

Test proofs : White Rat 1,376, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive. White rat

1375, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive.

August 17 to September 2, 1953 : Negative.

September 3, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

September 4, 1953 to March 28, 1954 : Negative.

OBSERVATION 3.

White rat 898.—

March 3, 1953 - Injected with blood from the tail of a positive white rat.

April 8, 1953 : Positive.

March 3 to August 15, 1953 : Negative with four negative sub-inoculations (two after treatment with daraprim).

August 16, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

Test proof : White Rat 1,375, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive.

August 16 to September 9, 1953 : Negative.

September 3, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

September 3 to December 4, 1953 : Negative.

OBSERVATION 4.

White rat 833.—

January 27, 1953 : Injected with blood from the tail of a positive mouse.

January 31 to February 13, 1953 : Positive.

February 16 to April 7, 1953 : Negative.

April 10 and 11, 1953 : Positive.

April 14 to April 29, 1953 : Negative but one positive sub-inoculation.

May 4, 1953 : Positive.

May 7 to August 11, 1953 : Negative with nine negative sub-inoculations (two after treatment with daraprim).

August 12, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

September 16 and 18, 1953 : Positive.

September 22 to December 3, 1953: Negative.

January 28, 1954 : Injected with blood from the tail of a positive mouse.

Test proof : White Rat 1,679, injected with blood from the tail of a mouse, became positive.

January 28, to March 5, 1954 : Negative.

OBSERVATION 5.

White rat 876.—

February 21, 1953 : Injected with blood from the tail of a positive *Praomys.*

February 25 to March 18, 1953 : Positive.

March 25 to August 11, 1953 : Negative with three negative sub-inoculations (two after treatment with daraprim).

August 12 to December 3, 1953 : Negative.

January 28, 1953 : Injected with blood from the tail of a positive mouse.

Test proof : White rat 1,679, injected with blood from the tail of a positive mouse, became positive.

January 28 to March 5, 1954 : Negative.

Note.—On August 12, 1953, injected with 0·75 c.c. of blood from the heart of a positive mouse.

OBSERVATION 6.

White rat 852.—

February 14, 1953 : Injected with blood from the tail of a positive mouse.

February 19 to March 4, 1953 : Positive.

March 11 to March 27, 1953 : Negative with one positive sub-inoculation.

April 3 to April 20, 1953 : Negative.

April 22 to April 24, 1953 : Positive.

April 27 to June 15, 1953 : Negative with three positive sub-inoculations.

July 15, 1953 : Positive.

July 23, 1953 : Negative but one positive sub-inoculation.

July 29, 30 and 31, 1953 : Treatment with daraprim, but three positive sub-inoculations (one each on August 4, 10 and 11, 1953).

August 12, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

August 13 to September 23, 1953 : Negative.

September 24, 1953 : Injected with 0·50 c.c. of blood from the heart of a positive mouse.

Test proof : Rat 1410, injected with 0·50 c.c. of blood from the heart of a positive mouse, became positive.

September 25 to December 3, 1953 : Negative but four positive sub-inoculations.

OBSERVATION 7.

White rat 910.—

March 6, 1953 : Injected with blood from the tail of a positive white rat.

March 13 to March 25, 1953 : Positive.

April 2 to April 15, 1953 : Negative with two positive sub-inoculations.

April 21, 1953 : Positive.

April 23 to August 12, 1953 : Negative with 11 negative sub-inoculations (two after treatment with daraprim).

August 13, 1953 : Injected with 0·50 c.c. of blood from the heart of a positive mouse.

Test proof : White rat 1,362, injected with 0·50 c.c. of blood from the heart of a positive mouse, became positive.

August 17 and 18, 1954 : Positive.

August 19 to November 20, 1953 : Negative but two positive sub-inoculations.

January 7, 1954 : Injected with 0·50 c.c. of blood from the heart of a positive mouse.

Test proof : Blood of a positive mouse injected to each of the white rats 1,600, 1,601, 1,602, 1,602, 1,603, 1,604, 1,605, 1,606, 1,607, 1,608 and 1,609 . Each became positive.

January 7 to April 14, 1954 : Negative.

OBSERVATION 8.

White rat 889.—

March 2, 1953 : Inoculated with blood from the tail of a positive white rat.

March 7 to April 2, 1953 : Positive.

April 8, 1954 : Negative but one positive sub-inoculation.

April 15 to April 23, 1953 : Positive.

April 28 to August 15, 1953 : Negative with one positive sub-inoculation (April 29, 1954), and ten negative sub-inoculations (two after treatment with daraprim).

August 16, 1953 : Inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

Test proofs : White rat 1,376, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive.
White rat 1,375, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive.

August 17 to September 12, 1953 : Negative.

September 13, 1953 : Inoculated with 0·75 c.c. of blood from the heart of a positive mouse.

September 15 to September 23, 1953 : Negative.

September 24, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

Test proof : White rat 1,410, injected with 0·75 c.c. of blood from the heart of a positive mouse, became positive.

September 24 to October 2, 1953 : Negative.

October 3, 1953 : Injected with 0·75 c.c. of mixed blood of three positive mice.

Test proof : White rat 1,449, injected with 0·75 c.c. mixed blood from the heart of three positive mice, became positive.

October 3 to November 26, 1953 : Negative.

January 7, 1954 : Injected with 0·50 c.c. of blood from the tail of a positive *Cricetomys*.

Test proofs : White rats 1600 and 1609, each injected with 0·50 c.c. of blood, became positive.

January 7 to June 21, 1954 : Negative.

OBSERVATION 9.

White Rat 879.

February 23, 1953 : Injected with blood from the tail of a positive *Thamnomys*.

February 27 to March 4, 1953 : Positive.

March 11, 1953 : Negative.

March 14, 1953 : One positive sub-inoculation.

March 17, 1953 : Positive.

March 18 to March 27, 1953 : Negative.

March 31 to April 3, 1953 : Positive.

April 7 to August 11, 1953 : Negative.

May 18 to June 2, 1953 : Four negative sub-inoculations.

June 15, 1953 : One positive sub-inoculation.

July 10 to July 18, 1953 : Five negative sub-inoculations.

July 23 to August 11, 1953 : Four positive sub-inoculations (three after treatment with daraprim).

August 12, 1953 : Injected with 0·50 c.c. of blood from the heart of a positive mouse.

August 28 to October 15, 1953 : Negative.

October 16, 1953 : Injected with two c.c. of blood from the heart of a positive white rat.

Test proofs : Rats 1,477 and 1,480, each injected with two c.c. of blood, became positive.

October 16 to November 26, 1953 : Negative.

Note.—August 13 to August 27, 1953 : Negative. On August 28, 1953, injected with 0·50 c.c. of blood from the heart of a positive mouse.

OBSERVATION 10.

White rat 1063.

March 30, 1953 : Injected with 0·50 c.c. of blood from the heart of a positive mouse.

April 3 to April 14, 1953 : Positive.

April 15 to August 11, 1953 : Negative with seven negative sub-inoculations (three after treatment with daraprim).

August 12, 1953 : Injected with 0·75 c.c. of blood from the heart of a positive mouse.

August 17 to August 20, 1953 : Positive.

August 21, 1953 : Died of infection.

III. *THAMNOMYS SURDASTER SURDASTER.*

OBSERVATION 1.

Thamnomys 3302.

August 22, 1951 : Inoculated with blood from the tail of a positive white rat.

August 28 to September 17, 1951 : Positive.

January 29, 1952 : Inoculated with blood from the tail of a positive mouse.

January 29 to March 13, 1952 : Negative.

OBSERVATION 2.

Thamnomys 3442.

August 22, 1951 : Inoculated with blood from the tail of a positive *Thamnomys.*

August 28 to September 10, 1951 : Positive.

September 11 to January 28, 1952 : Negative with one negative sub-inoculation.

January 29, 1952 : Inoculated with blood from the tail of a positive *Æthomys.*

OBSERVATION 3.

Thamnomys 3554.

September 12, 1951 : Inoculated with blood from the tail of a positive *Thamnomys.*

September 17 to September 24, 1951 : Positive.

September 25, 1951 to January 28, 1952 : Negative with one negative sub-inoculation.

January 29, 1952 : Inoculated with blood from the tail of a positive *Thamnomys.*

January 30 to March 27, 1952 : Remained negative.

OBSERVATION 4.

Thamnomys 3738.

October 8, 1951 : Inoculated with blood from the tail of a positive white rat.

October 15 to October 29, 1951 : Positive.

January 29, 1952 : Inoculated with blood from the tail of a positive white rat.

January 29 to February 26, 1952 : Negative.

February 29, 1952 : Died after anæsthesia, accident.

From our experiments on the *Cricetomys ansorgei*, one could easily observe that out of ten animals, five had one attack, four had two, and one had four attacks before we started re-inoculations.

Before proceeding to re-inoculations, we made sure of the complete disappearance of malarial parasites from the peripheral blood, not only by repeated microscopic examinations but also by sub-inoculations to the white mouse (test animal).

Resistance to re-inoculation was noted as early as 34 days after the first inoculation and as late as eight months and 23 days after the first inoculation. It seems probable that in some cases it appears soon after the first attack.

The resistance to *P. berghei* persists as may be proved by the failure of the numerous re-inoculations on the same animal. The number of these successive inoculations varies from three to nine for each *Cricetomys* examined. The shortest interval between two successive injections has been seven days and the longest 10 months 26 days.

The course of infection and the acquisition of immunity is the same whether the *Cricetomys* has been first injected with sporozoites or with infected blood (merozoites). In this last case, it does not matter at all if the donor was a recent or a more ancient passage.

The successive re-inoculations were always done with merozoites and never with sporozoites. The quantity of infected blood injected was ordinarily massive (0·50 c.c. and more), and we may certify that *Cricetomys* injected for the first time (test proofs) with so big a quantity of infected blood, always take the infection.

The resistance was the same whether we used homologous or heterogenous strains for the re-inoculations ; or whether the donor and the receiver were from the same (*Cricetomys*) or from a different species (white mouse, white rat, *Thamnomys*, *Praomys*, *Aethomys*).

From all these experiments, we may conclude that once the *Cricetomys ansorgei* has proved to have no parasites of *P. berghei* in its peripheral blood (microscopic examination and sub-inoculation to the white mouse), all tentatives (even with massive quantity of infected blood) of re-infections fail. So it seems that *Cricetomys ansorgei* acquired a real immunity to *P. berghei*.

The observations on the white rats showed that one had three attacks, two had four, and four had three attacks before we proceeded to re-inoculations.

Out of ten cases studied, eight remained negative in spite of several re-inoculations (two to five per animal). For these, on an average, the resistance appeared five and a half months after the first injection. This delay is superior to the longest interval observed between two successive attacks. As in the case of *Cricetomys ansorgei*, the resistance persists and was not broken by changing the species of rodent used as donor or by injecting merozoites from a recent or a more ancient passage of *P. berghei*, or even by inoculating more and more massive quantities of infected blood.

The test proofs, all of which became positive, were white rats injected with the same quantity of infected blood as the re-inoculated animal.

As we have said above, out of the ten cases studied, two became positive. One of them made only a short and slight infection (Observation 4), the other died of infection (Observation 10).

The resistance does not appear to have the same characteristics as for the *Cricetomys ansorgei*. Not only do we note that one rat (Rat 10) had a fatal attack after the second inoculation (four months after recovery of the first attack) ; but when the animals showed no more parasites on the microscopic examinations of the peripheral blood, sub-inoculations to the white mouse were nevertheless positive in the cases where it was done.

" Premunition " would rather be the proper word in this case.

For the *Thamnomys surdaster surdaster*, each animal showed only one attack before we proceeded to re-inoculations. These were done from three and a half to five months after the first infection. In some cases, re-inoculations were done after the negativation of the peripheral blood had been controlled by negative sub-inoculations to the white mouse.

No re-inoculation succeeded and, here also, no influence, neither of the type of donor nor that of the strain, could be found out.

CONCLUSION.

Considering the observations stated in this work, we may conclude that the *Cricetomys ansorgei*, the white rat and the *Thamnomys surdaster surdaster*, after being once infected with *P. berghei*, develop a resistance to this parasite.

ACKNOWLEDGEMENT.

This work, performed in the S. E. R. A. M. laboratories, was constantly suggested by Dr. I. H. Vincke whom we express our thanks for all benevolent advices that he never failed to give.

REFERENCES.

CORRADETTI, A. (1950) ... ... *Rio. Parasit.*, **11**, pp. 201–209.
FABIANI, G. AND FULCHIRON, G. (1952) ... *C.R. Soc. Biol.*, **146**, pp. 435–437.
FABIANI, G., VARGUES, R., GRELLET, P. AND CLAUSSES, J. (1951) ... ... *Ibid.*, **145**, pp. 1131–1134.
RAMAKRISHNAN, S.P., SATYA PRAKASH AND KRISHNASWAMI, A.K. (1951) ... ... *Ind. J. Mal.*, **5**, pp. 447–454.

Indian Journal of Malariology, **8**, 4, December 1954.

PATHOLOGICAL AND IMMUNOLOGICAL HOST-PARASITE RELATIONSHIPS BETWEEN ALBINO RAT AND *PLASMODIUM BERGHEI*.

BY

AUGUSTO CORRADETTI,

FELICE VEROLINI

AND

GIOVANNI TOSCHI.

(*Superior Institute of Health, Roma, Italy.*)

(December 31, 1954.)

THIS paper summarizes the results of the investigations on the subject carried out from 1949 to 1954 by the senior author in collaboration partially with Verolini (research on the specific cells involved in the erythrocyte series, and on serum proteins) and with Toschi (investigations on serum proteins). The details of the experiments and full description of the techniques can be found in our previous papers published in Italian and referred to in the 'REFERENCES' at the end of this paper.

Our investigations dealt with the following aspects :—

(*a*) Mode of action of *Plasmodium berghei* and changes in the host during the primary attack.

(*b*) Immunological behaviour of the host after the primary attack, effect of superinfection and splenectomy.

(*A*) MODE OF ACTION OF *PLASMODIUM BERGHEI* AND CHANGES IN THE HOST DURING THE PRIMARY ATTACK.

1. *Mode of attack of* Plasmodium berghei *and its relationship to cells of the erythrocyte series during the course of primary attack.*—The blood of the albino rat shows, under physiological conditions, red cells of mature type only (or at least these cells are far the most prevalent in number). Mature red cells have mature acidophilic

hæmoglobin and appear pale-red when stained by Romanowsky or Giemsa stain. Only a moderate degree of anisocytosis is seen. The mature red cell has a diameter ranging from 4·38 to 9·13 microns.

In anæmic conditions, the blood of the albino rat shows a number of red cells characterized by more or less a basophilic cytoplasm (at least non-acidophilic) and by their diameter ranging from 6·3 to 13·3 microns. Some of these basophilic cells occasionally show a reticulum and even nuclear residues. It would appear that maturation of hæmoglobin (from basophilic to acidophilic) is really independent of the disappearance of nuclear and reticular substances. In fact, it is not uncommon to observe markedly basophilic red cells with no nuclear or reticular substances as well as acidophilic red cells still showing both.

In highly anæmic conditions, red cells with non-acidophilic cytoplasm are far the most common in the blood ; basophilic erythroblasts begin to appear in the peripheral blood under such conditions.

We studied the relationship between the parasite and the above described three types of host erythrocytes (acidophilic and basophilic erythrocytes, and basophilic erythroblasts) in nine rats infected with *Plasmodium berghei*. The blood was examined every day beginning from the first appearance of parasites till death of the animal or the end of primary attack occurred. At least 1,000 cells of the erythrocyte series were observed every day in each rat, and classified as belonging to one of the three types. Parasitized cells of each type were enumerated and their percentage to non-parasitized cells of similar type was calculated.

Analysis of the data showed the following facts :—

During the primary attack, there is a progressive decrease of the acidophilic red cells with a corresponding progressive increase of non-acidophilic cells. Basophilic erythroblasts appear during the advanced stages of the infection. This process, in lethal cases, is absolutely progressive until death occurs. In rats which survive, the process is continuously progressive up to a maximum corresponding to the days during which the parasitic crisis occurs. After the crisis, a reversion of the process occurs with a progressive increase of acidophilic cells and a corresponding decrease of non-acidophilic cells and disappearance of basophilic erythroblasts, until finally the hæmatological picture reverts to normal (in six to ten days).

From the beginning of infection, the parasites show an evident tendency to develop inside non-acidophilic red cells. In fact, the percentage of parasitized non-acidophilic erythrocytes to non-parasitized non-acidophilic cells, is very high at any moment of the infection, reaching often almost 100 per cent. On the other hand, the percentage of parasitized to non-parasitized acidophilic cells reaches, at the most, 11 per cent and is generally much below this limit.

At the commencement of the infection, the blood shows none or little departure from the normal physiological picture. The erythrocytes are all of the acidophilic type, and the parasites, still low in number, are found in this type of cells. As the infection becomes more intense in the days following, the destruction of red cells acts as a stimulus for the output of non-acidophilic red cells, and later basophilic erythroblasts. As the non-acidophilic erythrocytes increase in number, the parasites which have a marked affinity to these cells, also increase in number and reach a very high density. The infection at its peak may cease with the death

of the host or with a parasitic crisis. Death occurs generally with a maximum of parasite invasion, with a very low percentage of acidophilic erythrocytes, and with a very high percentage of non-acidophilic erythrocytes and basophilic erythroblasts. The parasitic crisis is characterized by a progressive decrease of the number of parasites till their disappearance in one to three days; during this time the percentage of acidophilic red cells varies around the minimum, and the percentage of non-acidophilic red cells varies around the maximum reached at the peak of the infection. As the parasitic crisis ends, and the parasites disappear, the percentage of acidophilic red cells starts to become continuously higher, with a corresponding diminution of the non-acidophilic red cells. In about six to ten days, the blood shows again its normal picture.

The results showed that the non-acidophilic erythrocytes constitute the preferred pabulum of parasites. The multiplication of parasites appears to be highly favoured by the host response which results in a great number of non-acidophilic cells in the peripheral circulation.

2. *Behaviour of serum proteins during the primary attack.*—We studied the behaviour of serum proteins during the primary attack by electrophoretic (filter paper) method. The experiments were made on 18 rats infected with *Plasmodium berghei* (divided into four groups), and on eight rats inoculated with normal non-infected blood (control group). The rats of each group were examined by electrophoresis before inoculation, and at different moments of the primary attack. Total nitrogen content of the blood was also determined in one group each of the infected and control animals. The course of the infection was followed daily and found to be similar in all the animals.

The variations of the electrophoretic picture of the serum were substantially identical in animals of the four experimental groups. In all of them, an increase of percentage of γ-globulins was observed between the twelfth and sixteenth day from inoculation. This increase was not high but was found to be highly significant on statistical analysis ($P < 0{\cdot}01$). In animals of three out of the four experimental groups, a decrease of α-globulins was found to occur on the same days (in two out of the three experiments the decrease began on the sixth day from inoculation). No significant variations of the total N were observed. The rats of the control group showed no variations. The results of the investigation showed that a significant increase of γ-globulins corresponds to the usual period of parasitic crisis (twelfth to sixteenth day of inoculation).

(*B*) IMMUNOLOGICAL BEHAVIOUR OF THE HOST AFTER THE PRIMARY ATTACK ; EFFECT OF SUPERINFECTION AND SPLENECTOMY.

It was shown in our previous studies that rats which survive the primary attack show a rapid total disappearance of *Plasmodium berghei* from their body. Blood examinations made daily up to a maximum period of 205 days after the end of primary parasitæmia were negative in 20 out of 24 rats. A total of six parasites were detected in the blood films of the four remaining rats, and all of them were found within the first 50 days from the end of primary attack.

The disappearance of parasites is a result of real destruction as demonstrated by both splenectomy and sub-inoculation of the blood of experimental animals. Fifteen rats were splenectomized at different times after the end of primary attack, and none of them showed a relapse. Included in these 15 rats, there were four rats which were splenectomized before the fifteenth day (12, 16, 28 and 45 days) from the end of primary patent parasitæmia. On the other hand, sub-inoculation of blood, from a rat three days after the recovery from the primary attack, into two fresh rats was negative. Consequently, the destruction of the last *Plasmodium berghei* in the body may take place any time from the parasitic crisis to about 50 days thereafter.

Eleven rats which had recovered from infection were not splenectomized. They were re-inoculated with the same strain of parasites between 67 and 205 days after the end of the primary attack. The result was negative in seven rats ; in the other four a few parasites were detectable in the blood for three to five days after re-inoculation, and later on disappeared. This behaviour demonstrates the persistence of immunity to the homologous strain in the absence of parasites ; this immunity was observed to be manifest even when challenged by re-inoculation after 166, 170 and 205 days after the end of primary attack.

Three rats were splenectomized on 79, 109 and 111th day, respectively, after recovery from the primary attack. They did not show a relapse. Twenty-one to twenty-three days after splenectomy, they were re-inoculated with the same strain with positive results. Two of them showed parasites in their blood constantly for a period of 220 and 255 days, respectively. The third rat had a re-infection which lasted for four weeks, the parasites being detected during the following ten weeks. These experiments demonstrate that immunity to re-inoculation is largely due to the spleen, as, in the absence of this organ, the re-inoculated parasites easily multiply in the body of the host, and result in a prolonged chronic infection. Nevertheless, immunity following recovery from the first infection is sufficient to prevent death from re-infection even in the absence of the spleen and sometimes is able to destroy the second infection.

The main result of these experiments is the demonstration that after the recovery from the primary attack of *Plasmodium berghei*, a state of immunity without parasites is established in the rat. Such a state was shown to be sufficient to prevent re-infection for at least 205 days after the primary attack. This immunity has nothing in common with premunition, in which parasites in the body of the host, at least in small numbers, are always present. It is to be considered as true immunity the occurrence of which was practically unknown in protozoal infections.

REFERENCES.

CORRADETTI, A. (1950) ... ... *Riv. Parass.*, **11**, pp. 201-209.

CORRADETTI, A., TOSCHI, G. and VEROLINI, F. (1954) ... ... *Ibid.*, **15**, pp. 141-150.

CORRADETTI, A. and VEROLINI, F. (1951) ... *Ibid.*, **12**, pp. 69-84.

OBITUARY

R. A. Senior White.

It is with profound regret that we have to announce the death on October 30, 1954, of Mr. R. A. Senior White, at St. Augustine, Trinidad.

Mr. Senior White began his career as a malarial entomologist in a tea estate in Ceylon and came to India as an entomologist in the Indian Agricultural Research Institute. He was for three years at the Malaria Institute of India and was later Malariologist, old Bengal-Nagpur Railway. He was also Malaria Adviser to the Indian Railway. After retirement, he became Entomologist in the Malaria Division, Trinidad, which post he held to the last.

While with the Bengal-Nagpur Railway, he controlled malaria in a locality where the construction of the railway was held up for 40 years by mosquitoes unlike Uganda Railway where the delay was due to man-eaters.

He published a number of papers both of agricultural and medical importance. He was the joint author of "*Parasitology of world malaria*". His work on applied research on malaria control during the World War II facilitated the war efforts on the Burma front. He also organized malaria control in Korea Coalfields under the auspices of the Coalmines Welfare Fund. He very usefully served on the committee convened by the Government of India 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.

His pioneer work on the subject under most trying conditions will remain a landmark in the history of malaria research and control in India, and his valuable contributions will serve as a guide and source of inspiration to the future malariologists. His memoir on the bionomics of *Anopheles aquasalis*, a malaria vector of primary importance in Trinidad, is a monumental contribution.

He used to take active part in the deliberations of the Malaria Advisory Committee of the Scientific Advisory Board of the Indian Council of Medical Research.

He was an Honorary Fellow of the National Society of India for Malaria and other Mosquito-borne Diseases. Members of the Society at their general meeting held at Baroda on November 26, 1954, observed two minutes silence in his memory.

We convey our great admiration and deep sympathy to the bereaved family, his colleagues and friends.

J.S.

Indian Journal of Malariology, **8**, 4, *December 1954*.

INDEX OF AUTHORS

INDEX OF SUBJECTS