were given not less than 10 injections and who attended our leprosy clinic for not less than two months. A 3 per cent. solution of Alepol was made; this was well sterilized and carbolized (0.5 per cent.). It was changed every fortnight. Injections were given twice weekly beginning with 1 c.cm. The dose was gradually increased by 1 c.cm. each week. The dose was not increased beyond 8 c.cm. No reaction was marked in any of the cases till the dose was 5 c.cm. and when the dose was increased beyond 5 c.cm. in some cases a slight reaction, vague pain in the joints and slight fever, was noticed, but this reaction subsided the next day. Besides giving Alepol injections, the nodules and leprotic patches were occasionally painted with trichloracetic acid 1:3 and chaulmoogra oil was given every week for local application to the body. Other minor ailments were attended to symptomatically. Cases whose Wassermann reaction was positive were also given antisyphilitic treatment.

Cases 1 and 8 who have had about 50 injections up till now and are under treatment for the last 9 months showed marked improvement. The superficial anæsthetic patches have all cleared up and the skin has assumed a 'crushed-tissue-paper' appearance, owing to the resolution of the leprosy infiltration. Cases 3 and 4 are cousins; perforating ulcers in their feet have all cleared up though no special treatment was given for the ulcers. Deep anæsthetic patches are also clearing up and the superficial anæsthetic patches have all cleared up. Case 15 has had only 10 injections up till now. He had only one anæsthetic patch which is now much reduced in size. In case 19 the erythema of the face has much subsided and the anæsthetic patches are also better. In short the general condition of all the above cases is improving and they are looking much better; the anæsthesia, nodules, and erythema are clearing up and the general debility has much subsided.

Conclusion.—Alepol is well tolerated by patients, it is less irritating than and does not cause as much reaction as E.C.C.O. and other preparations of hydnocarpus oil, and it is comparatively cheaper.

My thanks are due to Major J. B. Vaidya, I.M.S., Civil Surgeon, Gorakhpur, for his kind permission to publish this note.

OBSERVATIONS ON A PLASMODIUM INFECTION WHICH CAUSES HÆMOGLOBINURIA IN CERTAIN SPECIES OF MONKEY

By L. EVERARD NAPIER, M.R.C.S., L.R.C.P.
Officer-in-Charge
and
H. G. M. CAMPBELL, I.M.D.
Kala-azar Enquiry, Indian Research Fund Association
(From the Calcutta School of Tropical Medicine)

WHILE carrying out routine blood examination on monkeys, as a preliminary measure to certain hæmatological experiments in connection with our kala-azar research work, one of us (H. G. M. C.) encountered a protozoal parasite in a red blood corpuscle. On further investigation we found that in the peripheral blood of this monkey, subsequently identified as *Cercopithecus pygerythrus,* there were a number of different forms of a plasmodium which had many of the characters of *Plasmodium kochi.* (We have made no further study of the parasite from the protozoal aspect as this is being done by other workers in the School.) The monkey did not seem to be in any way affected by this infection which was never intense and which was not always patent, even when a careful and prolonged examination of the blood was made.

About two cubic centimetres of the blood of this monkey was taken from a vein added to an equal quantity of citrate saline and inoculated intravenously into three monkeys, two *Cercopithecus pygerythrus* and one *Macacus rhesus:* the former two became infected on the 14th and 15th days, respectively, taking a mild infection, and in the other, the *rhesus* monkey, parasites were first found on the 9th day; by the 12th day he was found to be suffering from a most intense infection, with at least 60 per cent. of the red cells infected, and to this he rapidly succumbed.

Just before his death blood was taken—less than one cubic centimetre; this was diluted with citrate saline and injected subcutaneously into four more *M. rhesus* monkeys.

Subsequently, the plasmodial infection was passaged through a series of monkeys of both these species in order to study the cytological changes which occurred in the blood during the development and resolution of this infection, in connection with our kala-azar investigations. No special plan was followed in passaging this strain; some monkeys were given small doses of quinine to prevent the infection killing them, others were left untreated. Our primary consideration was to avoid losing the strain and such monkeys as were available for passage were used.

We found that by giving the monkeys one grain of quinine sulphate a day by the mouth for about a week, and then a single dose of one or two grains now and then, the infection was kept in check.

The animals did not exhibit any regular pyrexia. Occasionally their temperature rose to 105°F. from their normal which is between 102°F. and 103°F., but the fever bore no relationship to the parasite count; the only characteristic feature was the rapid drop to well below normal when the infection rose to above a million parasites per cubic millimetre.

About the same time blood was taken from the original monkey by Dr. B. M. Das Gupta who wished to study the parasite from the protozoological aspect, and the infection was

passed through a second series of monkeys of a number of different species and, as a therapeutic measure, to three human patients.

Amongst the monkeys of the first series (inoculated in the kala-azar laboratory) a number were found to be suffering from hæmoglobinuria; this complication was not at first noted amongst the monkeys of Dr. Das Gupta's series, but at a later date some of these also developed hæmoglobinuria.

It was noticed that hæmoglobinuria occurred only in *M. rhesus,* but it was not clear why some did and others did not develop this complication. By way of investigating this point we decided to trace the course of the plasmodial strain in the various monkeys through which we had passaged it. We made tables summarizing the experiments and drew a 'geneological tree' showing the source of the morbid material with which each monkey had been infected, the length of the incubation period, and the fate of the infected monkeys. A few facts can be noted from the chart and the tables.

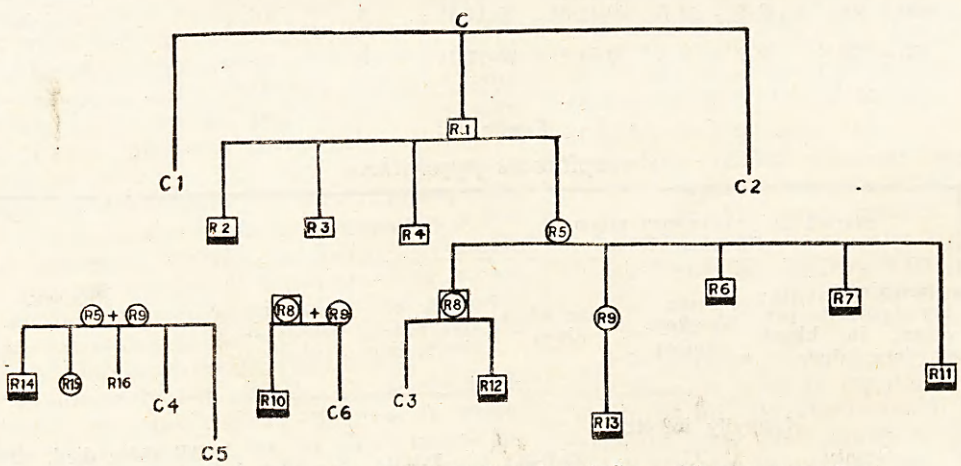
was not noted; it was noted in only one of the three untreated monkeys of the second passage; but in the third and fourth passages 7 out of 8 *M. rhesus* monkeys died with hæmoglobinuria.

(*d*) The incubation periods in days are shown in the table below :—

	Days before appearance of parasites in peripheral blood	Mean number of days
1st passage ..	9	9
2nd passages ..	8, 8, 9, 9	8.5
3rd „ ..	4, 5, 5, 7, 13	6.8
4th „ ..	5, 5, 5, 6, 7, 9	6.3

There would, therefore, appear to be a tendency towards diminution in the incubation period in the later passages. The same diminution in the survival period in the untreated monkeys will be observed; in the first two passages the mean was 12.25 days, and in the latter passages 9.6 days, if the monkey which showed some 'resistance' to infection and survived be excluded.

CHART



C = Original naturally-infected *Cercopithecus pygerythrus.*
C 1 to C 6 = *Cercopithecus pygerythrus.*
R 1 to R 16 = *Macacus rhesus.*
○ indicates treated. □ indicates died.

▬ indicates died with hæmoglobinuria. ◎ indicates died despite treatment.

The lengths of the vertical lines are proportionate to the incubation period.

(*a*) In *Cercopithecus pygerythrus* the infection runs a mild course; excluding the monkey in which the original strain was found, six were infected and none of these acquired a fatal infection although no treatment was given; in this species the mean incubation period was 10.5 days.

(*b*) In *Macacus rhesus* the infection runs a virulent course; out of 16 inoculated, 4 were given quinine and of these 3 survived; 12 were not treated, and of these 11 died.

(*c*) Hæmoglobinuria was noted in 8 of the 11 *M. rhesus* monkeys that died. In the *M. rhesus* of the first passage, hæmoglobinuria

There can be no question about the conclusion regarding the susceptibility of the two species but the other two observations require some comment.

There was no association between the occurrence of hæmoglobinuria and the giving of quinine; it appeared before quinine was given and in no instance in which there was not already evidence of hæmoglobinuria did it appear after quinine was given. In some instances the hæmoglobinuria was apparently associated with suppression of urine, as in these cases no urine was passed and on post-mortem examination only a small quantity of dark re-

TABLE I

Macacus rhesus

Serial number of monkey	Source of inoculated blood			Infection		Death		Remarks
	Approximate number of parasites per c.mm. in inoculated blood	From monkey number	Date when given	Date of first positive finding	Incubation period in days	Date	Days from first inoculation	
R 1	Scanty	C	23-7-31	1-8-31	9	4-8-31	12	
R 2	2,000,000	R 1	4-8-31	12-8-31	8	15-8-31	11	Hæmoglobinuria.
R 3	2,000,000	R 1	4-8-31	12-8-31	8	18-8-31	14	
R 4	2,000,000	R 1	4-8-31	13-8-31	9	16-8-31	12	
R 5	2,000,000	R 1	4-8-31	13-8-31	9	Alive	..	Quinine gr. i daily, given from 13-8-31 for one week.
R 6	1,200	R 5	15-9-31	19-9-31	4	24-9-31	9	Hæmoglobinuria.
R 7	1,200	R 5	15-9-31	20-9-31	5	21-9-31	6	Hæmoglobinuria.
R 8	2,000	R 5	21-9-31	26-9-31	5	12-11-31	..	Quinine gr. i from 28-9-31.
R 9	2,000	R 5	21-9-31	28-9-31	7	Alive	..	Quinine gr. i from 28-9-31.
R 10	Scanty+300	R 8 + R 9	19-10-31	26-10-31	7	28-10-31	9	Hæmoglobinuria.
R 11	400	R 5	8-11-31	21-11-31	13	23-11-31	15	Hæmoglobinuria.
R 12	300	R 8	8-11-31	14-11-31	6	19-11-31	11	Hæmoglobinuria.
R 13	1,200	R 9	8-11-31	17-11-31	9	18-11-31	10	Hæmoglobinuria.
R 14	300 + 600	R 9 + R 5	20-11-31	25-11-31	5	27-11-31	7	Hæmoglobinuria.
R 15	300 + 600	R 9 + R 5	20-11-31	25-11-31	5	Alive	..	Quinine grs. ii from 28-11-31.
R 16	300 + 600	R 9 + R 5	20-11-31	25-11-31	5	Alive	..	

TABLE II

Cercopithecus pygerythrus

Serial number of monkey	Source of inoculated blood			Infection		Remarks
	Approximate number of parasites per c.mm. in blood inoculated.	From monkey number	Date when given	Date of first positive finding	Incubation period in days	
C	Naturally infected.					
C 1	Scanty	C	23-7-31	7-8-31	15	All three died, obviously of some intercurrent infection, a few weeks later.
C 2	Scanty	C	23-7-31	6-8-31	14	
C 3	400	R 8	28-10-31	4-11-31	7	
C 4	Scanty + 30	R 5 + R 9	19-10-31	26-10-31	7	Alive.
C 5	500 + 300	R 5 + R 9	13-11-31	25-11-31	12	
C 6	400 + 300	R 8 + R 9	28-10-31	5-11-31	8	

urine was found in the bladder; in others the monkey passed red urine, and after death a distended bladder full of hæmoglobinized urine was found.

When passed the urine was bright red, later turning to a dark brown colour. Urine recovered from the bladder of a monkey that had died over-night was almost completely black. Chemically it gave the usual hæmoglobin reactions and the presence of hæmoglobin was confirmed spectroscopically. No unhæmolysed red blood cells were observed in the specimen.

The hæmoglobinuria was always associated with very heavy parasite counts in the peripheral blood.

In the second series of monkey passages carried out by Dr. B. M. Das Gupta hæmoglobinuria was not noted; this could probably be accounted for by the fact that at first he passaged mostly from Cercopithecus monkeys. In his series the only monkeys that showed hæmoglobinuria were a few *M. rhesus* after passage of the virus through other *M. rhesus* monkeys.

A suggestion that has been made regarding the variations in incubation time is that they may have varied with the dose of the virus. This was, however, obviously not the only factor; in the first passages a much larger quantity of blood was used than in the later

passages after we had found that a large quantity was unnecessary; as little as 0.1 cubic centimetre was found to be sufficient. In these cases the incubation period bore no relationship to the dose of virus administered.

A reasonable hypothesis is that the virulence of the infection, as evidenced by a shorter incubation period and hæmoglobinuria, was raised by passage through a series of monkeys of a more susceptible species.

Discussion

The presumption is that in nature the plasmodium passes from monkey to monkey of the species in which we first found it, with some mosquito as intermediate host and transmitter, and that in this monkey, which has either a natural or an acquired immunity, the infection runs a benign course but is maintained as a low-grade infection. If by any chance the infection were transmitted to a *rhesus* monkey, the latter would probably die prior to the development of any significant number of gametocytes and that particular strain of plasmodium would die with it.

One cannot resist drawing comparison between this state of affairs and that which is observed in the case of human malaria, where the indigenous population of certain tropical tracts appear to suffer little inconvenience from their malarial attacks but where strangers suffer severe infections frequently ending in blackwater fever.

From our observations it would appear that the virulence of the strain is increased by a few rapid passages through a highly-susceptible host, and one wonders if the same thing occurs in these highly-malarious districts and accounts for the variations in the severity of the clinical manifestations in different individuals.

It is obviously not possible to be dogmatic on these few observations, but, as it would take a very large number of experiments involving the sacrifice of numerous monkeys to prove the theory which our preliminary observations suggest and as this particular strain of plasmodium has now been passaged so frequently that it may have lost its original pathogenic characteristics, we feel that we should record our observations and leave it to others, better qualified to do so, to carry on this investigation.

THE CURATIVE VALUE OF A LOCALLY-PREPARED SAMPLE OF SULPHARSENOBENZENE—THIOSARMINE

Part II

By B. G. MALLYA, F.R.C.S.E.
MAJOR, I.M.S.
Surgeon, Police Hospital, Calcutta
and
B. B. MAITY, L.M.F. (Cal.)

THE present paper is a continuation of our previous paper with the same title. The compound in question is now available in the market under the name of 'Thiosarmine'.

Group 1.—This series includes patients with inoculation lesions. Total number of cases—3 (cases 51 to 53).

Notes on the cases :—

(*a*) Indurated chancre present in all these cases.

(*b*) *Treponema pallidum* was found in the scrapings from the lesions in all cases.

(*c*) At the beginning of the treatment the Wassermann reaction was negative and became positive during course of treatment.

Observations.—The chancre healed up in all cases after 3 or 4 injections. The Wassermann reaction became negative after completion of treatment in 2 cases (cases 51 and 52), and in case 53 the Wassermann reaction remained positive throughout our observations, though the chancre healed up completely and general conditions were markedly improved. This patient is still under observation and taking a second course of treatment.

Group 2.—This series includes patients with a positive Wassermann reaction with inoculation lesions or with old healed chancres with inguinal adenitis but no other outward signs or history of general infection. Total number of cases—13 (cases 54 to 66).

Notes on the cases :—

(*a*) Old healed chancre with suppurating inguinal adenitis in 4 cases (cases 54 to 57) and without suppuration in case 58.

(*b*) Unhealed chancre with suppurating inguinal adenitis in 8 cases (cases 59 to 66).

Observations.—Local conditions healed up quickly, the general condition was markedly improved and the Wassermann reaction became negative after the course of treatment in all cases, except one, case 66. This patient is still under observation.

Group 3.—This series includes patients with a positive Wassermann reaction with outward signs and symptoms of general infection. Total number of cases—25 (cases 67 to 91).

Notes on the cases :—

(*a*) Cases with syphilitic arthritis of ankle and knee joints, rise of temperature, giddiness and general disturbances in 4 cases (cases 37 to 40).

(*b*) Cases with iritis, keratitis, inguinal adenitis, low fever, general weakness, anæmia, chronic pharyngitis, in 6 cases (cases 71 to 76).

(*c*) Cases with inguinal adenitis, general weakness, chronic pharyngitis, anæmia in 5 cases (cases 77 to 81).

(*d*) Cases with skin eruption, inguinal adenitis, anæmia, pain all over the body in 4 cases (cases 82 to 85).