

STUDIES ON THE LIFE-CYCLES OF MURINE MALARIA.

PARASITES FROM NIGERIA

by

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A thesis submitted for the degree of
Master of Philosophy of the University
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December 1969.

ABSTRACT

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An historical review is given of the discovery of murine malaria parasites in 4 localities in Africa. The topography and climate of the Nigerian locality are described, and methods of trapping and examining sylvatic rodents are given. The re-discovery of a Plasmodium vinckei-like parasite of Nigerian rodents is reported, with the new finding of a P. berghei - like parasite of thicket-rats in the same place.

The sporogony of the Nigerian P. vinckei is described in 3 species of mosquitoes, and unsuccessful attempts to demonstrate the exoerythrocytic schizogony are reported. The first description of blood stages of P. vinckei ssp. in a naturally infected rodent is given, and a comparison is made with the morphology in mice. The blood stages of P. vinckei ssp. from Nigerian Thamnomys were found to resemble those of a P. vinckei - like parasite of Pracomys found in the same locality in 1954; it is concluded the parasites are the same.

The full life-cycle of the Nigerian P. berghei is described. Oocysts resembled those of P. b. killicki, but the sporozoites were significantly longer than those of P. b. berghei, P. b. yoelii and P. b. killicki. Sporogony of the Nigerian parasite was adversely affected

by incubation at 28°C, but sporozoites retained some degree of infectivity. At 24°C, as few as 80 sporozoites always infected mice.

A statistical study was made of sporozoites of all murine malaria parasites. The classification is discussed, and it concluded that the berghei-group consists of two subspecies; one, P. b. berghei, is restricted to Katanga, while the other, P. b. yoelii, is represented by the P. berghei-like parasites of Nigeria, the Central African Republic and Brazzaville. No similar conclusion was reached with the less well studied vinckei-group.

In an appendix, a check-list of the blood-inhabiting Sporozoa of rodents is given with an index of hosts.

ACKNOWLEDGEMENTS

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This work was begun in the Department of Parasitology, London School of Tropical Medicine (August 1967 - January 1969) and was completed in the Department of Zoology and Applied Entomology, Imperial College, Ascot (February - December 1969). The field work in Nigeria was carried out while I was acting as a Consultant for the World Health Organisation.

I wish to offer warm thanks to Professor P.C.C. Garnham F.R.S. for giving me guidance and encouragement throughout the work. It has been a great privilege to have been able to work on malaria under his supervision.

In the planning of the field work Professor L.J. Bruce-Chwatt and Dr. Irène Landau took part in discussions leading to the method of approach. I thank them both, and especially acknowledge the kindness of Professor Bruce-Chwatt in making the trip to Nigeria possible.

In Lagos, I was ably assisted by Mr. G.T. Shute and Mr. A.O. Lambo. It is true to say the field work could not have been accomplished in the short time available without the help of these two colleagues, so thoroughly familiar with the locality.

In London, I benefited much from stimulating discussions with Professor R.S. Bray, Dr. J.R. Baker and Dr. McWilson Warren, all of whom helped me on mass dissections of mosquitoes. Mr. P.E. Nesbitt, Mr. R.L. Jacobsen, Mr. M.W. Guy, Mr. G.S. Gill and Mr. Wan Sai Pan provided valuable

technical assistance. Mr. Gill bred the Anopheles stephensi, used in the transmissions.

Special mention must be made of the support given by Mr. Guy when I was in Africa. He received the newly isolated strains flown from Africa to London, established them in mice and mosquitoes, and stored them in the deep-freeze. Without his help the strains may well have been lost, and the field work would have been in vain.

In Ascot, I wish to acknowledge the facilities provided by Professor F.R.E. Southwood and Dr. Elizabeth Canning in the later stages of the work, and to thank Dr. George Murday for advice on the statistical treatment of the lengths of sporozoites, and Professor J.V. Scorza for kindly preparing a painting from which Plate 11 was prepared.

It is a pleasure to acknowledge permission given by Mr. J.P. Adam, Professor L.J. Bruce-Chwatt, Mr. F.D. Gibson Dr. R. Lainson, Dr. I. Landau and Miss W. Tadros to include their unpublished records in the check-list.

Finally, I thank Mr. D.R. Rosevear of the Mammal Room British Museum (Natural History) for help in identifying some of the rodents caught in Nigeria, and for guiding me along the tortuous paths of rodent taxonomy

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Les resultats obtenus dans un laboratoire
sont très imparfaits à côté de ceux que
peut réaliser la nature.

Alphonse Laveran, 1914.

The most numerous, widespread and diverse of all the orders of living mammals are the rodents. Of the 932 genera listed by Simpson (1945), 344 are rodents grouped into 30 families. These highly adaptable animals have spread to every continent of the world exploiting widely differing ecological niches. A great variety of forms capable of thriving in deserts, tropical forests, Arctic tundras or temperate meadows has arisen. Most are terrestrial, but some are truly arboreal having evolved wing-like structures enabling them to glide from tree to tree high in the tropical forests. A few are aquatic, but no forms have arisen which never come to land, and none is a truly marine animal.

In spite of this abundance and wide distribution, little is known of many of the protozoa parasitizing the blood cells of rodents. An exception is the group of true malaria parasites (Plasmodium) of the murine rodents of Africa, some of which have been extensively studied since their discovery a little more than 20 years ago. As soon as they were found, their value as research tools was immediately recognised, and man's preoccupation with the control of his own diseases at once stimulated great interest in Plasmodium of rodents, strains of which were so easily maintained and studied in the laboratory.

The first reports of two malaria parasites of rodents in Katanga (Vincke and Lips, 1948; Rodhain, 1952) led to the

assumption that such infections were restricted to very limited areas of the tropics, where the parasites only just managed to survive in forest rodents and delicate, little-known sylvatic mosquitoes. Then, in 1965 and 1966, a new school of French protozoologists led by Professor Alain Chabaud and Dr. Irène Landau found that in at least two new localities, the Central African Republic and the Republic of Congo (Brazzaville), malaria was common in one particular semi-arboreal rodent (Landau and Chabaud, 1965; Adam et al., 1966).

The work of the French investigators gave rise to the idea that there were probably still undiscovered places in Africa, and possibly in other parts of the Old World tropics, where malaria parasites would be found to be equally widespread and firmly established in rodents. This led to the writer visiting Nigeria in 1967 in an attempt to re-discover an apparently rare Plasmodium first found by Bruce-Chwatt and Gibson (1955), 13 years earlier, in one of thousands of rodents captured in the western part of the coastal forest belt. The search was successful; both Bruce-Chwatt's parasite and another were found, and strains were established in the laboratory (Killick-Kendrick et al., 1968b).

In the present work, accounts are given of (i) the discovery of malaria parasites of African murines, (ii) the Nigerian locality and the field work which led to the isolation of the West African strains, and (iii) the life-

cycles and morphology of the new parasites in laboratory-bred rodents and mosquitoes. The immunology and pathology of the infections were not studied.

The distribution and morphology of the murine species and subspecies of Plasmodium are compared and contrasted, and a revision of the berghei-group is suggested. Finally, a check-list of the Sporozoa of the blood-cells of rodents is given as an appendix, with an index of the hosts.

THE HISTORY OF MALARIA PARASITES OF

MURINE RODENTS

(1) Democratic Republic of the Congo (Kinshasa)

(a) *P. berghei berghei* Vincke and Lips, 1948.

Malaria parasites of rodents were discovered by Vincke and d'Ursel in 1943 (van den Ber^ghe, 1954). They were found as sporozoites in the salivary glands of Anopheles durenii millescampsii collected in forest galleries in the highlands of Katanga. The first step towards identifying the parasite was to discover which vertebrates were fed upon by the sylvatic mosquito. Unlike A.d. durenii, A.d. millescampsii rarely feeds on humans (see Gilles and de Meillon, 1968), and it was quickly realised that the sporozoites were not those of a Plasmodium of man. Red blood cells in the stomachs of freshly fed wild-caught mosquitoes were never nucleated and it was known, therefore, that the source of food was a mammal, not a bird, amphibian or lizard. Precipitin tests performed on the blood meals of A. d. millescampsii collected in the gallery forests showed that the mosquito did not feed upon horses, cattle, sheep, dogs, cats, antelope or primates. Attention was then focused upon the only other numerous mammals inhabiting the forest, the rodents and insectivores, and eventually 2 thick-knee rats (Thamnomys surdaster) out of 358 rodents were discovered with infections of a new malaria parasite (Vincke, 1954). With this as a guide, sporozoites from wild-caught A. d. millescampsii were inoculated into laboratory rats, and it was shown that the infection in the

mosquitoes was of a Plasmodium of sylvatic rodents. This concluded a most elegant piece of field work which had occupied the Belgian workers for five years. During the investigation, other less commonly infected rodents, Praomys jacksoni and Leggada bella, were discovered (Vincke, 1954).

The new parasite was named P. berghei by Vincke and Lips in 1948, and 18 years later, when P. berghei yoelii was recognised as a subspecies, the Katangan parasite became the nominate subspecies, P. berghei berghei.

By the generosity of the Belgian workers a number of strains isolated from wild-caught mosquitoes were distributed to laboratories all over the world, and even today one strain (Keyburg 173 - known also as N strain) still forms the basis of many studies requiring a syringe-passaged strain of high virulence. More recently Yoeli et al. (1964) and Bafort et al. (1968) have collected new strains from A. d. millecampsii in Katanga which are more suitable for studies on cyclically transmitted infections.

(b) P. vinckei vinckei Rodhain, 1952.

When Vincke and his team were isolating strains of P. b. berghei from A. d. millecampsii, they found that rats were the most susceptible of their laboratory rodents (Vincke and Lips, 1950). Mice were, however, occasionally used, and in one mouse inoculated with sporozoites from a naturally infected mosquito, an infection arose which was at once recognised as different from P. b. berghei. The strain was sent to Antwerp where, after a close study of the blood stages,

Rodhain confirmed that the field workers had indeed stumbled on a second species of malaria parasite in the sylvatic mosquito. The second parasite was named P. vinckei, and later became the nominate subspecies, P. vinckei vinckei, when P. chabaudi was placed as a subspecies (Bafort, 1968).

In spite of hundreds of isolations of malaria parasites from the salivary glands of A. d. millecampsi in Katanga during the late 1940s and early 1950s, no other strains of P. v. vinckei came to light. Fifteen years after the discovery of the second parasite, Bafort (1967), on his second expedition to Katanga to re-discover P. vinckei, was at last successful, and he took back to Antwerp a freshly isolated strain obtained, once again, from a mosquito. Working with the new strain, which had been obtained in difficult circumstances at a time of the civil war in the Congo, Bafort (1967, 1968) was able to reveal the sporogony and tissue schizogony of P. v. vinckei for the first time.

No infections of P. v. vinckei have been seen in wild-caught rodents, nor have infections of this parasite arisen in white mice inoculated with the blood of Thamnomys caught in localities where A. d. millecampsi carries P. b. berghei (Vinke and van den Bult^cke, 1949; Bafort, 1967). But since Thamnomys is the principal vertebrate host of P. b. berghei, thought to be transmitted solely by A. d. millecampsi, it is assumed that the natural vertebrate host of the other Plasmodium carried by the mosquito is also Thamnomys.

The view that P. vinckei was a mutant of P. berghei (Garnham, 1966) was an attractive explanation for the apparent rareness of the lesser known parasite. However, the recent records from other parts of Africa, where infections of subspecies of P. vinckei greatly outnumber those of P. berghei (Landau and Chabaud, 1965; Adam et al., 1966; Killick-Kendrick et al., 1968b), suggest this idea is no longer tenable (Garnham, 1968). Possibly the Katangan subspecies has still undiscovered hosts other than A. d. millecampsi and Thammomys. It is difficult otherwise to understand how the parasite survives at such low levels of infection.

(ii) Nigeria

(a) P. vinckei subspecies

Spurred on by the exciting findings in Katanga, Bruce-Chwatt and Gibson, working in Nigeria, began systematically to examine large numbers of sylvatic rodents by inoculating pooled samples of blood into white mice. Inevitably a backlog of unexamined slides from the mice formed, especially since this work had to take second place to studies on the epidemiology and treatment of malaria in man. One mouse which had been inoculated with blood from a Praomys tullbergi was found to have had an infection of a P. vinckei-like parasite, but by the time it was discovered, the infection was no longer patent (Bruce-Chwatt and Gibson, 1955). Attempts to recover the strain from the mouse were unsuccessful, and the parasite was lost. These determined workers then embarked upon a search which was abandoned only

after they had fruitlessly examined a total of two and a half thousand rodents from two different localities in Western Nigeria (Bruce-Chwatt pers. comm.). Possibly because their parasite was not available for laboratory studies, Bruce-Chwatt and Gibson's report of a second locality was largely unnoticed.

In 1967, thirteen years after the discovery of the Nigerian parasite, Killick-Kendrick et al. (1968b) collected and examined one species of rodent which was not in the early collections. This was the semi-arboreal thicket rat Thamnomys rutilans, which, in the interval, had been found commonly to harbour malaria parasites in the Central African Republic (Landau and Chabaud, 1965) and the Republic of Congo (Brazzaville) (Adam et al., 1966). This fresh approach, soundly based on new information and well supported by workers in Nigeria familiar with the locality where the first discovery was made, quickly led to the isolation of new strains of Bruce-Chwatt's parasite which was found in a third of the Thamnomys examined. It was then realised that the single infected Praomys must have been an accidental host of a parasite of Thamnomys, or possibly that the infected animal was a Thamnomys wrongly identified as a Praomys.

The invertebrate host of the Nigerian P. vinckei is not yet known. Nine species of anophelines are recorded in the area (Bruce-Chwatt et al., 1955), and it has been suggested that the mosquito transmitting the infection in

nature is A. cinctus, A. coustani or A. obscurus (Killick-Kendrick et. al., 1968b).

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A full account of the field work which led to the re-discovery of Bruce-Chwatt's Plasmodium and the first isolation of P. berghei in the same locality forms part of the present work.

(b) P. berghei subspecies.

During the recent investigation of the Nigerian locality, a single strain of P. berghei was isolated from a Thamnomys rutilans which was also infected with P. vinckei (Killick-Kendrick et al., 1968b). The P. berghei was separated from the other parasite during cyclical passage through A. stephensi, and a description of its life-cycle is given elsewhere in the present work, together with a discussion on the identity of the Nigerian strain and its relationship to the three named subspecies of P. berghei. As with the P. vinckei from the same place, the mosquito vector is at present unknown.

(iii) Central African Republic (C.A.R.)

(a) P. vinckei chabaudi Landau, 1965.

The discovery of P. v. chabaudi in a Thamnomys rutilans from the C.A.R. in 1964 was a turning point in understanding the murine malaria parasites. For nearly 20 years it had been assumed that this was a rare group of parasites of the Katangan highlands. The surprising finding that every adult Thamnomys collected from villages near La Maboké, 150 k.m. from Bangui, was infected with P. v. chabaudi

(Landau and Chabaud, 1965) stimulated new field work in the two other known foci of infection, Nigeria and Katanga, and led to new discoveries in the Congo (Brazzaville).

The parasite was first found by Dr. Irène Landau in Paris, in a Thamnomys which was one of a number of wild rodents collected by the eminent French mammalogist Mr. F. Petter. In 1965 the blood stages were described, and the parasite named P. chabaudi. Later, the discovery of a new subspecies of P. berghei in the same locality suggested that P. chabaudi was perhaps better considered as a subspecies of P. vinckei (Landau and Killick-Kendrick, 1966b). After Bafort (1968) had studied the complete life-cycle of P. vinckei vinckei from Katanga, he placed the parasite from the C.A.R. in the lower taxon.

No infections of P.v. chabaudi have been reported in rodents other than Thamnomys. Much remains to be done before a full comparison of the epizootiology of the parasites of the C.A.R. can be made with that of the Katangan parasites. The invertebrate host of P. v. chabaudi is undiscovered, although it has been suggested that the mosquito most likely to be the vector in the C.A.R. is A. nili, A. cinctus, A. vinckei or A. rhodesiensis (Vincke, 1967).

(b) P. berghei yoelii Landau and Killick-Kendrick, 1968

During field work on the incidence of P. v.

chabaudi in the C.A.R., about half the Thamnomys were found to be harbouring a second Plasmodium which, from the blood stages, was indistinguishable from the Katangan P. berghei (Landau and Chabaud, 1965). A close study of the sporogonic and tissue stages in laboratory-bred mosquitoes and white rats at once revealed differences (Landau and Killick-Kendrick 1966 a,b) which were sufficiently great for this Plasmodium to be recognised as a subspecies of P. berghei, and it was named P. b. yoelii in honour of Professor Meir Yoeli, the discoverer of the tissue schizogony of the murine malaria parasites.

No other species of rodents were found infected with P. b. yoelii and, as with all murine malaria parasites except those from Katanga, the natural mosquito host is not yet known.

(iv) Republic of Congo (Brazzaville)

(a) P. vinckeï subspecies

Stimulated by their findings in the C.A.R., the French workers turned their attention to another part of Africa, and in 1966, with their colleague M. Adam, they reported a second unexpected enzootic in a forest gallery near N'ganga Lingolo, 15 km. from Brazzaville in the Republic of Congo (Adam et al., 1966) Once again two parasites, one of

the vinckei - group and the other of the berghei - group, were found in Thamnomys rutilans. At first the specific identify of the rodents was uncertain, but it was later found from an examination of the skulls that all the Thamnomys examined were T. rutilans (Landau et al., in press). Ten out of 24 of these rodents were found to have patent infections of P. vinckei. Strains were established in the laboratory and the life-cycle was studied in Paris and London. After some difficulty, preparations of all stages were obtained, enabling the investigators to make a thorough comparison with P. v. vinckei and P. v. chabaudi; the parasite from Brazzaville is now being named as the third subspecies of P. vinckei (Landau et al., in press).

The biotope has not been extensively investigated and only few rodents other than Thamnomys have been examined without, so far, other vertebrate hosts being discovered. In the salivary glands of one A. cinctus collected in the N'ganga Lingolo forest, however, Adam (pers. comm.) found very long sporozoites typical of those seen in A. stephensi experimentally infected with P. vinckei from Brazzaville. It seems likely, therefore, that A. cinctus is the vector of the murine malaria parasites of this most recently discovered locality.

(b) P. berghei killicki Landau, Michael and Adam, 1968. /

Two strains of a P. berghei - like parasite were

encountered in Thamnomys rutilans from Brazzaville (Landau et al., 1968). The life-cycle was easily obtained in A. stephensi and laboratory rodents, and the parasite was recognised as the third subspecies of P. berghei.

The natural invertebrate host of this parasite is not known, but A. cinctus must be suspected because of the strong suggestion that this is the mosquito transmitting the P. vinckei - like parasite in the same locality.

THE NIGERIAN ENZOOTIC OF MURINE MALARIA

Introduction and previous work

The Nigerian enzootic was discovered by Bruce-Chwatt and Gibson (1955), who briefly described a P. vinckei - like parasite isolated from a Praomys tullbergi collected near Ilobi in Western Nigeria. Full details of this work were not published, but, by kind permission of Professor L.J. Bruce-Chwatt, a list of all the murine rodents examined is given in table 1.

Rodents were trapped in two places, Agege and Ilobi. No infections of malaria were found in 1,007 murines from Agege, and only one, in the Praomys mentioned above, in 1,573 from Ilobi, 1038 of which were P. tullbergi.

The routine method of examination was to inoculate white mice intraperitoneally with pooled samples of blood from the wild rodents; blood films from the mice were then regularly examined. In addition, stained blood films from many of the wild rodents were examined directly for parasites. The ability of the microscopists to recognise infected red-cells is well illustrated by the high incidence of babesiasis detected, especially in Praomys from Agege, 13 per cent of which were found to harbour an unnamed species of Babesia

(Bruce-Chwatt and Gibson, unpublished). The inoculation of the mice, however, would be expected to be an even better method of diagnosing malaria, since it would reveal subpatent infections such as Vincke (1954) described in Thamnomys surdaster in Katanga. In spite of tremendous efforts, with reliable methods of diagnosis in the hands of experienced malariologists, the puzzle of the astonishingly low incidence of malaria remained unsolved.

In December, 1966, while reviewing the distribution and differential characters of murine malaria parasites, Professor P.C.C. Garnham and the writer were struck by the possibility that Bruce-Chwatt's Plasmodium was perhaps primarily a parasite of thicket rats, Thamnomys rutilans, none of which was in the very large collections at Ilobi and Agege (see table 1). We suspected that the apparent absence of Thamnomys could be explained by the positioning of the traps, which had always been placed on the ground. In Brazzaville, Mme. Landau (pers. comm.) had noticed that although Thamnomys were often taken in traps set up in bushes, they seldom entered traps set at ground level. Suspicions that thicket-rats were likely to be infected in Nigeria were based on the accounts, which had just been published, of extraordinarily high incidences of infection in T. rutilans from the Central African Republic, and the Republic of Congo

(Brazzaville) (Landon and Chabaud, 1965; Adam et al., 1966).

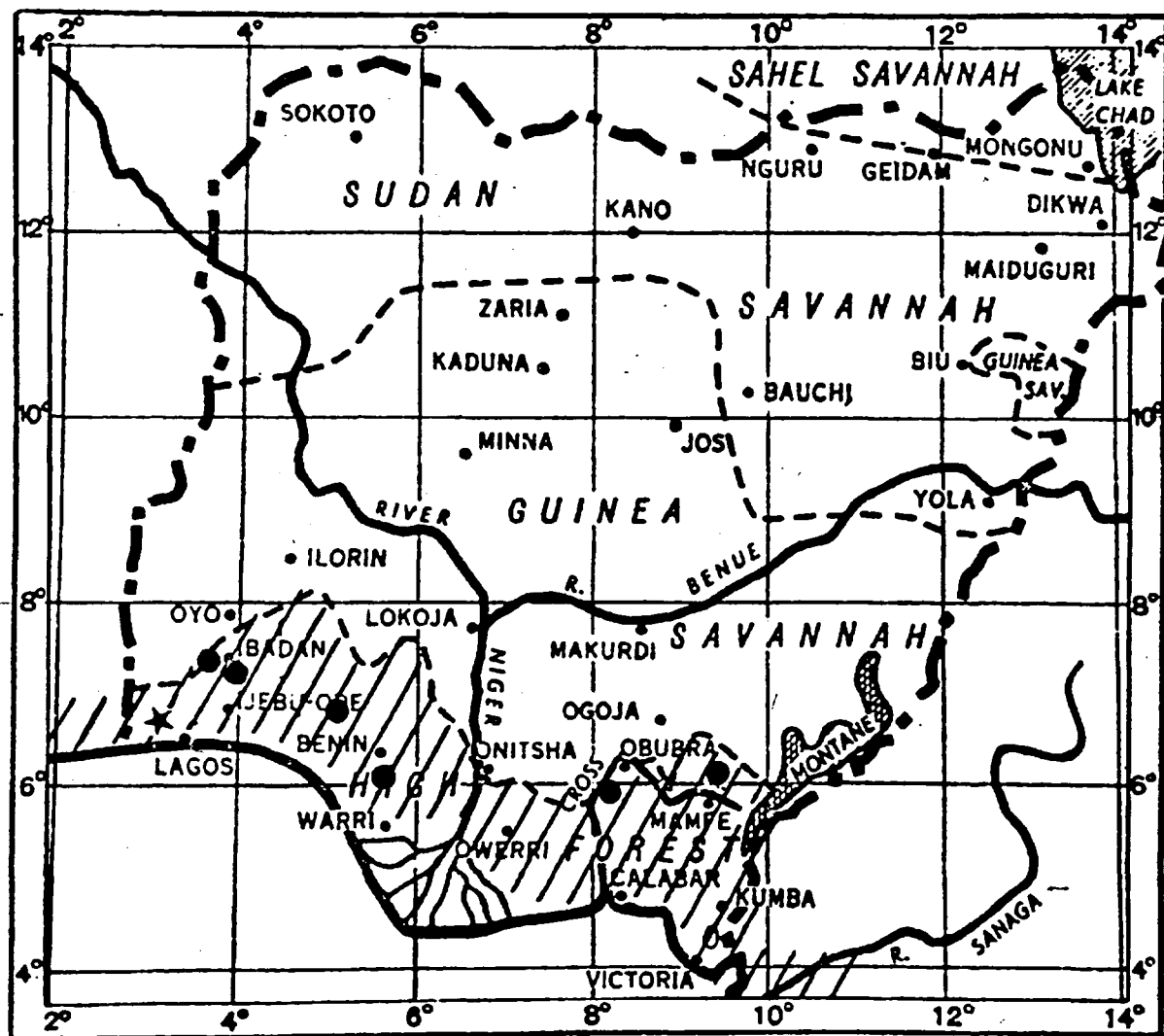
We then sought information on the distribution of Thamnomys in Nigeria. Rosevear (pers. comm.) confirmed that T. rutilans had been collected in the Gambara Forest Reserve south of Ibadan and, in his view, the range of this species in Nigeria would certainly include Ilobi. In a check-list and atlas of Nigerian mammals, Rosevear (1953) had earlier assumed that Thamnomys occurred throughout the coastal forest belt of Nigeria (see map, fig. 1). In August, 1967, Bruce-Chwatt's locality was therefore visited with the express purpose of collecting and examining Thamnomys in the expectation, later shown to be correct, that this rodent would be found to be the principal vertebrate host of the parasite discovered at Ilobi thirteen years earlier in only one of more than 1,000 Praomys.

FIGURE 1

Map showing the distribution of Thamnomys rutilans in Nigeria.

Black dots represent localities where specimens of Thamnomys in the British Museum were collected; the star N.W. of Lagos indicates Ilobi; and the shaded area indicates the probable distribution of Thamnomys in the High Forest Zone (after Rosevear, 1953).

Shining Thicket Rat



Thamnomys rutilans rutilans

Topography and Climate of the Locality

The village of Ilobi lies near the Ilaro Forest Reserve at longitude $3^{\circ}03''\text{E}$, latitude $6^{\circ}45''\text{N}$, in the old Egbado Division of Abeokuta Province in the Western State of Nigeria. . Physically the Egbado Division differs little from most of the Nigerian coastal forest belt. The altitude is 70-130 m. The country is undulating, with no important heights and is best described as well forested and patchily cultivated (Bruce-Chwatt et al., 1955). Small rivers with shallow beds, lying in wide valleys, drain the land southwards to the sea.

Western Nigeria is one of the most heavily populated parts of Africa south of the Sahara, and the vegetation has been much affected by the activities of man. There is virtually no truly virgin forest left (Rosevear, 1953), and at one time or another most of the land has been cultivated. The method of farming is one of shifting cultivation, so common in Africa; land is cleared, crops are grown, and after one, two or at the most three years, the plot is left fallow for a variable time before it is farmed again.

Growth of vegetation is so rapid that within the first fallow year herbs and grasses abound, and there is soon little resemblance to a farm. Shrubs, bushes and saplings spring up, and in a few years the fallow land becomes secondary forest with an open canopy, above which tower

emergent trees, spared when the land was cleared. If the land is abandoned and left undisturbed, the saplings become trees, creepers grow and the canopy becomes closed. Denied light, the grasses and herbs on the forest floor no longer flourish, and the lower levels of the forest become progressively more and more open.

All stages of this regeneration of forest are seen at Ilobi. Close to the north-eastern side of the village lies the Ilaro Forest Reserve (see plate 1) a patch of old, mixed deciduous forest comprised mainly of Sterculiaceae Leguminosae, Moraceae and Combretaceae (Bruce-Chwatt et al., 1955). It has a closed canopy 30 m. high, and it is about 62 sq. km. (24 square miles) in area. This wooded land, which extends in patches throughout much of Western Nigeria (Rosevear, 1953), lies in the High Forest Zone at the western edge of the southern Nigerian region of secondary forest (see map, fig. 2). This, though much degraded, forms the most westerly part of the great Congo Forest, separated from the Guinea Forest by the Dahomey Gap.

Within the Ilaro Forest, wood-gathering and hunting are strictly prohibited, and an active Forestry Department ensures it remains undisturbed. This kind of forest provides a poor habitat for arboreal murine rodents, which prefer bushes and thickets, and no trapping was done in



PLATE 1

Path at the edge of Ilaro Forest Reserve, W.
Nigeria. *Thamnomys rutilans* was trapped in the
secondary forest on the right.

FIGURE 2

Map of the south-western corner
of Western State, Nigeria, showing
the position of Ilaro Forest Reserve.

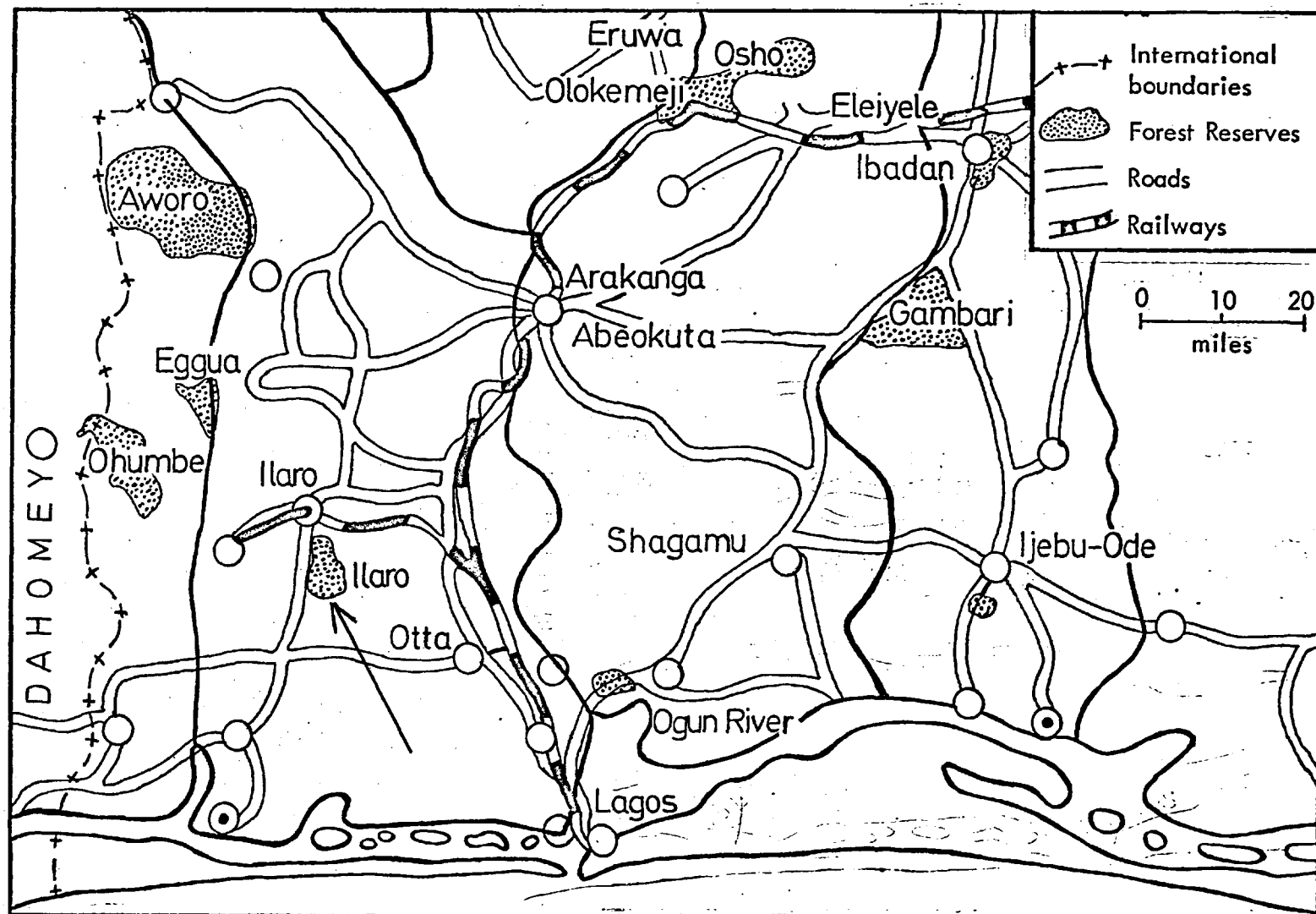


Fig. 2. MAP SHOWING POSITION OF ILARO FOREST RESERVE

the reserve itself. Along the southern edge of the reserve lie small plots of cocoa and banana trees among fallow farmland and secondary forest (plates 1 and 2]. Trapping was carried out almost entirely in the secondary forest which had an open canopy 6 m. high, interspersed with emergent trees left standing during earlier periods of cultivation. Remnant cocoa bushes and banana trees, still cropped by their owners, were dotted throughout the secondary growth.

The climate at Ilobi is common to much of the West African coastal belt. During the summer rains, the prevailing wind is south-west, but in the cooler winter months it is from the north, bringing fine dust from the Sahara; This northerly wind is known, in West Africa, as the Harmattan. Rainfall^{is} heavy; in 1949-1953, the mean annual rainfall at Ilaro was 150 cm. (59 ins) (Bruce-Chwatt et. al., 1955), with most precipitation occurring during 90 days of the year. The rains begin in April, and become progressively heavy reaching a first peak in June and July. There is then a lull during August, and a second smaller peak in September. In October the rains fall off, and from the beginning of December until the end of February, there is very little.

Temperatures within the biotope at Ilobi are not known, but at Abeokuta, 35 km. (22 miles) from Ilaro (see map, fig. 2), Bruce - Chwatt et. al. (1955) recorded a mean

daily minimum temperature of 22°C (72°F), and a mean daily maximum of 31°C (87°F). It is unlikely that the higher temperature is reached in shaded secondary forest and farm-bush. The relative humidity at Abeokuta was given as 80 per cent throughout the year except during the cool dry season in January and February, when it may fall below 50 per cent for short periods.



Fig.(i) Ilobi, Western Nigeria; cleared farmland
and secondary forest on fallow farmland.



Fig.(ii) Ilobi, Western Nigeria; cocoa plantation

Collection of rodents

In August, 1967, the civil war in Nigeria made field work difficult. As military road-blocks controlled all traffic on roads out of Lagos, permission to work at Ilobi was at first withheld. To gain experience of trapping a visit was therefore made to Akessan, a small village a few miles east of Agege.

The farmers at Akessan recognised Thamnomys, and said it was a common rodent of the fallow farmland around the village. Because of its arboreal habits, it was known to them as the "up-rat"; its Yoruba name is "Iwo". The villagers knew its nest, and after a short search one was found in a banana tree 2.5 m. from the ground (plate 3). A rodent was caught with the hands in the nest, and was immediately identified as a young adult Thamnomys. This was a most encouraging start, since the presence of thicket-rats in a habitat which stretched continuously to Ilobi was now confirmed.

At Akessan and, later, at Ilobi the presence of nests, most of which were old and abandoned, was always found to be a certain guide to the presence of Thamnomys. At the time of the field work the nests had not been well described



PLATE 3

Nest of *Thamnomys rutilans* 2.5 m from the ground in a
banana tree. Akessan, W. Nigeria. An adult *Thamnomys*
was caught with the hand in this nest.

and since they may be confused with birds nests, careful notes were made of their appearance (plates 3, 4 and 5). They were generally oval, but their shape was largely determined by the site. The outer layer consisted of untidy woven dead leaves which varied according to the tree or bush in which the nest was built (fig.(1) plate 5). Within this layer the centre of the nest was made of very fine grass with no obvious entrance or resting-place (fig. (ii) plate 5). This description does not agree with that of Rosevear (1969) who examined nests of T. rutilans collected in the Central African Republic and sent to him in London. He makes no mention of the leafy outer layer, and records the size as 6 x 4 ins. The nests seen in Nigeria were a little larger than this (7 x 5 ins), and it seems possible that Rosevear's specimens had lost the outer layer on collection.

All the nests seen at Ilobi were from 1.5 - 2.5 m. from the ground. Local farmers, however, asserted that they sometimes came across nests very much higher than this, occasionally as high as 10 m.

On the day after the visit to Akessan, tension eased and work began at Ilobi. Because of road-blocks, it was not possible to set traps at dusk and then return to Lagos, as had been planned, and local help was therefore sought. A man who had assisted Bruce-Chwatt's trapper in



Fig. (i)

Fig. (ii)



PLATE 4

Nests of *Thamnomys rutilans* in cocoa trees at Ilobi,
Western Nigeria.



Fig. (i) Showing outer
layer of cocoa
leaves.

Fig. (ii) Outer layer
of cocoa leaves
removed; showing
inner layer of
grasses and vines.



PLATE 5

Nest of *Thamnomys rutilans* (shown *in situ* in fig. (i), plate 4), removed from cocoa tree; Ilobi, Western Nigeria.

the 1950s was employed and shown how to set the traps. A 45 trapping area was selected from local knowledge of the distribution of Thamnomys, and the ease with which nests were found.

The traps used were a model selected by a French worker to trap galagos in the Republic of Congo (Brazzaville). It had been found that Thamnomys were often taken when the traps were set in bushes. In setting the traps at Ilobi, the methods used in Brazzaville were closely followed. The trapper was shown how to select suitable bushes or banana trees, and how to place the open traps at least 1.5 m. above the ground. With his earlier experience, there was initially some difficulty in persuading him to site the traps correctly, and at the beginning, he often put them too low, or on fallen logs. He clearly found it difficult to ignore his early training when Bruce-Chwatt and Gibson had collected terrestrial rodents.

The traps were obtained from "Manufrance", St. Etienne, France (described in the distributor's catalogue as "16-7870 Ratier"). They were made of galvanised wire, spaced at intervals of 1 cm., forming a square tunnel 40 cm. long, 12 cm. wide and 14 cm. high (Plate 6). There were two doors, one at each end, which were spring-loaded and released by the movement of a vertical locking-wire bearing the bait.

In later work in Sierra Leone, the American-made

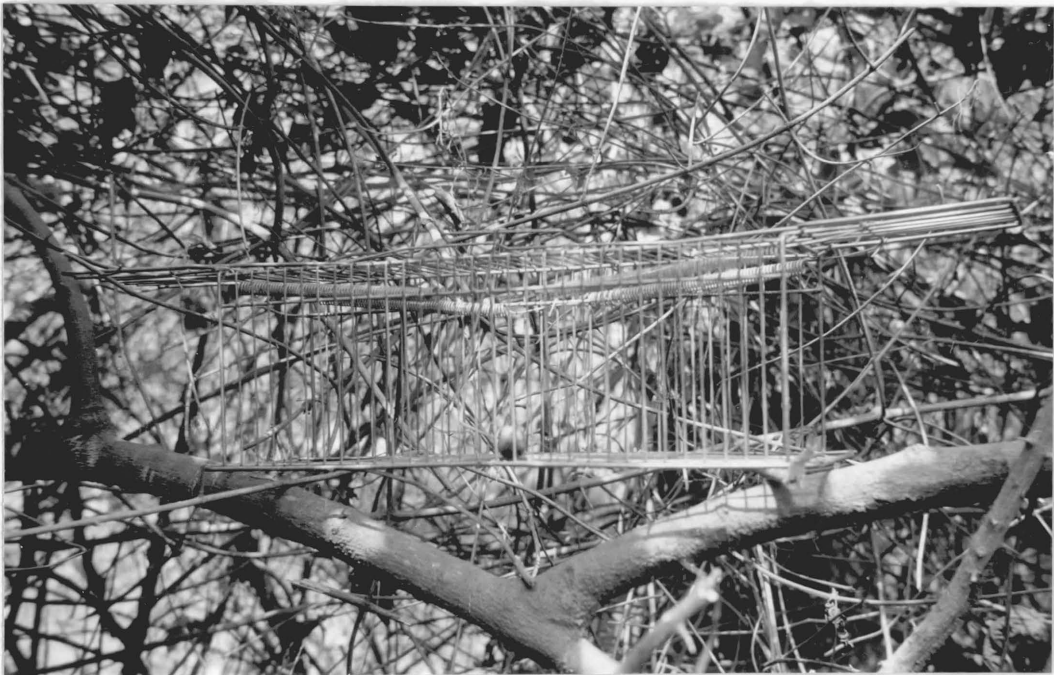


PLATE 6

Spring-operated rat-trap with double doors
set in a bush at Ilobi, Western Nigeria.

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"Havahart" trap was used, but it was found to be unsuitable for setting in bushes because its gravity-activated mechanism was fired by the slightest movement. The only problem with the "Manufrance" trap was interference from galagos (which could take the bait by reaching through the wires) and from giant centipedes (which, when banana was used as bait, ate the pulp leaving a ring of peel); because of their size, the centipedes easily fired finely set traps.

Since Thamnomys is frugivorous, and is known to be fond of apple in captivity, this was the first bait used. The supply was irregular, however, and locally obtainable fruits, banana and palm-nut, were tried; the former was as attractive as apple, but the latter was ignored. It was later found that, unlike Praomys, captured Thamnomys did not eat palm-nuts.

Because of the civil unrest, trapping at Ilobi was limited to only 8 days, from the 16th - 23rd of August, 1967, in the lull in the rains, the same time of year Bruce-Chwatt and Gibson had caught the single infected Praomys. The trapping site was visited each morning and the catch transferred to cages packed with hay ready to take to the laboratory in Lagos for examination. The catch was augmented by buying a few terrestrial rodents from boys in the village. Peri-domestic rats and mice were not bought, and the number

caught in the boys' ingeniously made devices formed but a 48
small part of the total.

The tails of all the rodents stripped easily, and the skin of many, especially young Thamnomys, often tore if they were caught by the scruff of the neck. They were most conveniently handled by tipping the contents of the cages into a dust-bin, and catching the rodents with a cloth.

Identification of rodents

Preliminary identifications were made on living rodents using Rosevear's (1950) excellent key, for the non-specialist, of the Nigerian murine rodents based solely on external characters. In Nigeria, Thamnomys rutilans is the only rat with fine but obvious hairs on its tail, most numerous at the distal end and ending in a tiny tuft like a very small paintbrush. The identification of this species presented no special problem (Plates 7 and 8). (In some other parts of Africa, however, the appearance of the tail cannot be used as the sole criterion for field identifications of Thamnomys.)

Specimens of each of the rodents examined were eviscerated and fixed in 4 % aqueous Formaldehyde. They were then sealed in plastic bags containing swabs of cotton-wool soaked in the fixative. Skulls and skins of the specimens were kindly prepared by Mr. Louis Bellier in the Ivory Coast, and the skulls were later given to the British Museum (Natural



PLATE 7

The four species of murine rodents trapped at
Ilobi in 1967.

From left to right: Thamnomys rutilans,
Praomys tullbergi, Stochomys longicaudatus,
and Malacomys edwardsi.



PLATE 8

Adult Thamnomys rutilans (No. N48) caught at
Ilobi in August, 1967.

This rodent had a patent infection of
P. vinckei showing all stages of development.

History), where Mr. D.R. Rosevear identified each specimen. 51
The field identifications were all found to be correct.

Since the work at Ilobi, Rosevear (1969) has published a superb memoir on the rodents of West Africa which should enable workers to identify most of their catch. Immature animals present special difficulties, however, and this, together with the possibility of coming across new races, makes the specialist indispensable.

Examination for malaria parasites.

At first, attempts were made with five rodents caught at Akessan, to collect blood from the orbital plexi or tails with heparinised capillary tubes. It was difficult to get enough blood, and the method was abandoned. Heart puncture under ether anaesthesia, using a 21 gauge hypodermic needle for small animals and a 17 gauge for large, proved to be more satisfactory. The heart was most easily entered close to the left of the sternum, slightly above a point halfway between the clavicle and bottom of the rib-cage. Heparin (0.5 i.u./ml.) was first drawn into the syringe to prevent coagulation. The volume of samples varied from 0.6 - 1.0 ml., and almost all the rodents survived.

Blood samples were equally divided between two or three small white mice, which were inoculated intraperitoneally. Thin blood films from the mice were examined only once or

twice between the 5th and 9th days after inoculation, as 52
there was insufficient time to keep them under observation
for a longer period.

Thick and thin blood films from the wild
rodents were prepared and examined directly for malaria
parasites. Since it was feared that the heparin in the
blood from the heart might adversely affect staining, the
films were made with blood obtained by snipping the end of
the tail. Thin films were fixed with absolute methanol and
were stained with Giemsa's stain. Thick films were dried
in a desiccator overnight and were then simultaneously lysed
and stained in the same stain at a weaker dilution.

When stained by conventional methods (see
Shute and Maryon, 1966), thick films from rodents are almost
impossible to lyse satisfactorily. To overcome this problem,
Mr. G.T. Shute recommended a modified staining method which
he had found ideal for the diagnosis of malaria in man by
the examination of thick films. Shute's method differs from
others by the higher salt concentration in the buffered water
used to dilute the stain. To avoid making two solutions
one for thick and the other for thin films, a single stock
solution of buffered water is prepared and diluted differently
depending upon which kind of film is being stained.

The technique is as follows:-

- (i) Prepare a stock solution of buffered water

p^H 7.1 - 7.2

Na₂HPO₄ (anhydrous) 5.0 g.

KH₂PO₄ 2.1 g.

Distilled water to 1,000 ml.

- (ii) For thick films, dilute 2 parts of the stock solution up to 5 parts with distilled water. Add Giemsa's stain to 2.5 - 3 per cent., and stain films upside down for 30 mins. Rinse briefly with dilute buffer and allow to drain and dry.
- (iii) For thin films dilute 1 part of the stock solution up to 5 parts with distilled water. Add Giemsa's stain to 8-10 per cent. Fix the films by flooding momentarily with absolute methanol and, without rinsing, put in the stain for 30-40 mins. Rinse very rapidly in dilute buffer solution and allow to drain and dry.

This method almost completely solved the problem of staining thick films of rodent blood (Plate 9), except for a few individual animals, the blood of which perhaps contained a higher than normal proportion of immature red cells which leave strongly staining envelopes after lysis.

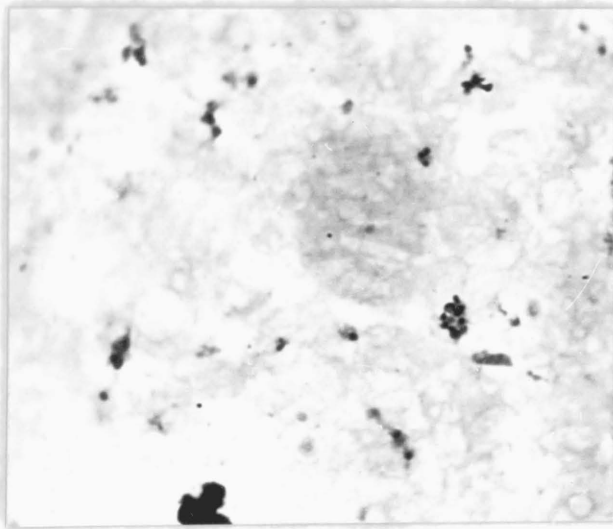


PLATE 9

Rings, trophozoites and a schizont of
Nigerian P. vinckei in a thick-blood film
from a naturally infected Thamnomys rutilans
(N48). (x1,500)

At least 200 fields of each thick film were searched with a X100 objective and X6 oculars. Thin films were used to study the morphology of the parasites.

The first infected rodent found was one of the few which died as the heart puncture was performed. It was immediately opened and blood was collected from the heart before it stopped beating. The spleen and, to a lesser degree the liver, was seen to be heavily pigmented, and the diagnosis of malaria was made even before the blood films were stained. This suggests that a rapid means of roughly making a malaria survey of rodents is simply to kill the animal and look at its spleen. Spleens of all infected rodents were never much larger than those of uninfected ones, but pigmentation was always obvious.

RESULTS OF SURVEYS OF NIGERIAN RODENTS

The results of the survey carried out in August 1967, are shown in Table 1. Of 41 rodents collected at Ilohi, 6 out of 18 Thamnomys rutilans were found with patent infections of P. vinckei ssp. indet.; one of these animals was also infected with P. berghei ssp. indet. No subpatent infections were detected in 12 Thamnomys, 2 Stochomys, 20 Praomys, and 1 Malacomys, but the period of observation of white mice inoculated with blood from the wild rodents was too short (9 days) to be certain that there were no very low-grade infections.

In Table 1, the unpublished results of the extensive survey of Bruce-Chwatt and Gibson are combined with the results obtained in 1967. Out of a total of 2,621 murine rodents from Western Nigeria (1,614 from Ilohi) only 7 have been found with malaria. Of 1,607 Praomys tullbergi (1,058 from Ilohi), one was found in 1954 harbouring P. vinckei (Bruce-Chwatt and Gibson, 1955), and the 6 infected Thamnomys mentioned above were found 13 years later (Killick-Kendrick et al., 1968b).

These combined results clearly show that Thamnomys is the principal vertebrate host of murine malaria parasites at Ilohi. Since the 1967 survey, Lambo (personal communication) has found that approximately 50 per cent. of

the Thamnomys are infected, and, on reflection, it seems possible that Bruce-Chwatt's single infected Praomys may have been a misidentified Thamnomys. Even if the identification were correct, Praomys appears to be an unimportant and probably accidental host.

Whatever the explanation, the almost complete restriction to one murine rodent is interesting, especially since sporozoites of at least one of the Nigerian parasites, the P. berghei ssp., are known to be infective to Thamnomys surdaster, Praomys natalensis, Rattus norvegicus, Mus musculus and Cricetus cricetus. Possibly the as yet undiscovered vector feeds almost exclusively on thicket rats, but until the vector is discovered further speculation is useless. But the possibility that the mosquito is attracted to only one of the sylvatic murines, strongly suggests that, in attempts to investigate the problem, insect traps should be baited with Thamnomys.

In Katanga, Leggada (=Mus) bella is occasionally infected with P. b. berghei (Vincke, 1954). The related pigmy mouse L. musculoides almost certainly occurs at Ilobi (see Rosevear, 1953), but, presumably because of the large traps used, it has not yet been collected. Nevertheless, because of the findings in Katanga, it must be considered as a possible host in Western Nigeria.

TABLE 1. Murine rodents from two localities in Western
State, Nigeria, examined for malaria parasites

Rodents	Agege 1950s (1)	Ilobi 1950s (1)	Ilobi 1967 (2)	Totals
<u>Dasymys</u> <u>incomptus</u>	0	1	0	1
<u>Arvicanthis</u> <u>niloticus</u>	1	8	0	9
<u>Lemniscomys</u> <u>striatus</u>	37	63	0	100
<u>Hybomys</u> <u>trivirgatus</u>	273	6	0	279
<u>Stochomys</u> <u>longicaudatus</u>	18	51	2	71
<u>Praomys</u> <u>tullbergi</u>	549	1038 ⁽³⁾	20	1607
<u>Thamnomys</u> <u>rutilans</u>	0	0	18 ⁽⁴⁾	18
<u>Malacomys</u> <u>edwardsi</u>	18	21	1	40
<u>Lophuromys</u> <u>sikapusi</u>	108	384	0	492
<u>Cricetomys</u> <u>gambianus</u>	3	1	0	4
TOTALS	1007	1573	41	2621

- (1) Bruce-Chwatt and Gibson (unpublished)
 (2) Killick-Kendrick et al., (1968b)
 (3) One with malaria parasites
 (4) Six with malaria parasites

POSSIBLE VECTORS OF THE NIGERIAN PARASITES

No determined effort has yet been made to identify the vector of the malaria parasites of Thamnomys in Nigeria. It is, nevertheless, possible to suggest which anophelines are most likely to be the invertebrate hosts at Ilobi.

The mosquito host of the murine malaria parasites of Katanga is A. durenii millecampsi, an anopheline with a range restricted to parts of Katanga only (Gilles and de Meillon, 1969). A single record of A. durenii in the Ivory Coast (Hamon et al., 1956) is now regarded as a mistake (Gilles and de Meillon, 1968). Thus there is no doubt that the host of parasites of Nigeria, the R.C.A. and Brazzaville must be a mosquito other than A. durenii.

Four sylvatic anophelines are common to the localities other than Katanga. These are: A. cinctus, A. coustani, A. obscurus and A. nili. (Gilles and de Meillon 1968; Adam, pers. comm.) Two of these, A. cinctus, and A. obscurus, are thought to be rare in Nigeria (Hamon et. al. 1956; Gilles and de Meillon, 1968), but relatively little work has been done on the sylvatic anophelines of West Africa, and collections using man as bait are of little help in revealing the true prevalence of species which are not anthropophilic.

Of the four species known in the forests of Nigeria, the C.A.R. and Brazzaville, there is a suspicion that A. cinctus is the most likely vector of murine malaria. Adam (pers. comm.) has found very long sporozoites typical of subspecies of P. berghei and P. vinckei of Brazzaville in the salivary glands of an A. cinctus caught in the N'ganga Lingolo forest, where about half the Thamnomys harbour malaria parasites. This mosquito is therefore the probable vector of P. b. killicki and the Brazzaville P. vinckei and may well be the host of the parasites in Nigeria and the C.A.R.

Support for this idea comes from the close relationship between A. cinctus and A. durenii, which are both grouped in the ardensis section of series Neomyzomyia in the subgenus Cellia (Gilles and de Meillon, 1968). A. nili is also included in this section, and as one of its resting places is the holes of rodents (Hamon et.al., 1956), it must be viewed as a possible host of their malaria parasites.

The importance of discovering the vectors of the parasites in Nigeria, the C.A.R. and Brazzaville as an aid to understanding the apparent speciation of murine Plasmodium has been stressed by Garnham (1968).

The life-cycles of the Nigerian parasites were studied in the laboratory in an attempt to reveal their relationships to the murine malaria parasites of other parts of Africa. In the accounts of their life-cycles, the new parasites are referred to as the Nigerian P. berghei and the Nigerian P. vinckei to distinguish them from the named subspecies from other places.

Materials and Methods

The methods used were basically those described by Landau and Killick-Kendrick (1966b) in their study of the sporogony and tissue schizogony of P. v. chabaudi and P. b. yoelii, and later adopted and modified by Wéry (1968).

(i) Mosquitoes

Anopheles stephensi was bred at 27°C (relative Humidity 70-80 per cent) by the methods described by Shute and Maryon (1966). This mosquito is the best laboratory host of murine malaria parasites yet discovered (Yoeli et al., 1965b; Landau and Killick-Kendrick, 1966b; Vincke et al., 1966; Wéry, 1968). The strain bred for the work was an Indian one originally established in the U.K. by Shute in 1947 (Shute and Maryon, 1966). This is the same strain as that used previously by many workers in various studies on the

sporogony of P. b. berghei, P. b. yoelii, P. b. killicki, 62
P. v. chabaudi and P. v. vinckei.

To avoid handling adult mosquitoes with suction tubes, a procedure which markedly shortens their life and makes it difficult to obtain the complete sporogony of any malaria parasite, pupae were collected from larval pans and were then placed in mosquito cages (12"x12"x12") to permit adults to emerge undamaged.

(ii) Rodents

All mice used were from a commercially available outbred line of "Theiler's Original" strain. Since inbred strains vary widely in their suitability as hosts of murine malaria parasites (e.g. Oxbrow, 1969), there are obvious advantages in using genetically varied animals when studying an unknown parasite.

Thamnomys surdaster were obtained from a colony at the National Institute for Medical Research by the kindness of Dr. F. Hawking. This colony was originally established with animals from the laboratory of Professor Meir Yoeli in New York (see Yoeli et al., 1963).

Praomys natalensis were bought from commercial suppliers. Colonies in the U.K. were first established in the early 1950s when they were described as Mastomys coucha; the classification is confused (see Davis 1963, 1965).

(iii) Infective feeds of mosquitoes

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On emergence, mosquitoes were maintained for 5-10 days on glucose solution before being given an infective feed. Mice were strapped in a supine position on a cork board and the abdominal hair was clipped short. They were then put inside the cage of mosquitoes. Blood meals were readily taken when the board bearing the mouse was held close to the top of the cage on a metal tripod stand.

All mosquitoes which took a blood meal had normally completed their feed within 3 hours. The mouse was then removed, and unfed female mosquitoes and surviving males were caught and killed in a test-tube containing chloroform on cotton-wool. When feeding large numbers of mosquitoes, two or three mice with similar gametocytaemias were offered. The ratio of mosquitoes to mice was an estimated 150 females to each rodent; if more fed on a small mouse, it usually died.

After an infective feed, mosquitoes were maintained at 24° - 26°C in an insectary. The relative Humidity was kept between 70 and 85 per cent (if it falls below 60 per cent, A. stephensi soon dies). Twenty-four hours later, an egg bowl containing water and a filter paper, and a pot of glucose solution were provided, and throughout the period of sporogony, no blood meals were offered. The growth of oocysts and the viability of sporozoites of P. b. yoelii is the same in A. stephensi fed on glucose as it is in

mosquitoes. maintained on blood (Wéry, 1968), and from the 64 point of view of standardisation, the former technique is the better of the two.

Potential carriers of gametocytes were infected by intraperitoneal inoculations of 1-2 drops of blood from a rodent with, whenever possible, a rising primary parasitaemia. The best time to feed mosquitoes was chosen on the basis of Wéry's (1968) observations on P. b. berghei, P. b. yoelii and P. v. chabaudi. With the Nigerian P. berghei this was 4-6 days after inoculation, when the first mature gametocytes were abundant. With the Nigerian P. vinckei, the best time was less easily seen since similar numbers of oocysts were found on the midguts of mosquitoes fed early in the infection or, as Wéry suggests for the related P. v. chabaudi, fed after the crisis in parasitaemia.

As gametocytes of murine malaria parasites rapidly lose their vitality when strains are maintained by even a comparatively short series of passages by transfer of blood (Wéry, 1968), care was taken to ensure that blood-passages of the Nigerian strains were as few as possible. When transmissions were not being done with mosquitoes, strains were stored at -70°C or -193°C with glycerol as a protective agent at a final concentration of 7.5 per cent. (Molinari, 1961).

(iv) Examination of sporogonic stages

Midguts and salivary glands of mosquitoes were dissected out by the well-known methods described by Shute and Marston (1966) and Garnham (1966).

The growth of oocysts was observed on freshly dissected midguts in normal saline gently flattened under a cover-slip. Phase-contrast illumination was helpful in deciding whether or not oocysts were dead; with this illumination unhealthy oocysts were yellowish in colour. Measurements were made with an ocular scale calibrated against a stage micrometer. Drawings of pigment patterns were made with the aid of a "Wild" drawing-tube and X2.5 tube magnifier fitted to a "Wild" M20 microscope.

As with infections of other murine parasites in laboratory-bred mosquitoes, sporozoites were often few in salivary glands, and no glands were recorded as negative without squashing them under a cover-slip. Examinations of salivary glands were made with phase-contrast optics (X20 and X40 objectives), since in earlier work it was found that sporozoites in very lightly infected glands were sometimes not noticed under bright-field illumination. When both midguts and glands were examined towards the end of sporogony, the greater efficiency of phase-contrast illumination required special care to ensure that sporozoites from mature oocysts were not carried over to

salivary glands on soiled needles.

Sporozoites were approximately measured in fresh preparations with a calibrated ocular scale. As this stage of the parasite is slightly curved, such measurements are not accurate, and more precise measurements were made by drawing midlines of sporozoites at a magnification of X3,800 with the same microscope and equipment used to draw oocysts. The length of the sporozoites was then obtained using a pair of dividers calibrated to 1 μ against a scale drawn from a stage micrometer/ (Bruce et al., 1909) When drawing midlines, no selection other than the rejection of obviously damaged or obscured parasites was made, and the series was of consecutively encountered sporozoites.

(v) Harvesting and inoculating sporozoites

To obtain tissue schizonts of the parasites studied, sporozoites were harvested from the salivary glands of experimentally infected mosquitoes. The mosquitoes were stunned in a test-tube, their legs and wings were removed and the glands were dissected into cold tissue culture medium 199 (Morgan et al., 1950) on a perspex slide. Batches of five pairs of glands were picked up with a Pasteur pipette and collected in a tube kept cool with crushed ice. An even suspension of sporozoites was then prepared by gently

breaking the glands in a "Teflon" tube homogeniser, size S2267
(Camlab (Glass) Ltd., Milton Road, Cambridge).

In earlier work, before the "Teflon" grinder was used, lobes of glands with sporozoites oozing from them were often encountered in the counting chamber, and accurate counts were impossible. Its use led to the adoption of the counting technique as a routine.

An improved Neubauer counting chamber was loaded with the suspension and allowed to stand in a damp chamber for 10 minutes. The sporozoites sank slowly, and unless time was allowed for them all to sink, the count through the depth of 0.1 mm was difficult and inaccurate. Sporozoites lying in the four outer blocks of 16 squares were counted at a magnification of X300 (X20 objective, X15 oculars). The Wild microscope used was fitted with a phase-contrast condenser with a working length sufficiently great for good phase-contrast illumination to be obtained on the upper surface of the thick counting chamber. The volume of the suspension was measured, and the number of sporozoites in 0.1 ml was calculated.

The total time from the beginning of a dissection until the inoculation of sporozoites was almost always less than 1 hour; throughout this time suspensions of glands or sporozoites were kept cool with crushed ice ($+4^{\circ}\text{C}$).

Sporozoites were inoculated intravenously into

the caudal veins of white rats and mice with a 1 or 2 ml disposable syringe fitted with a No. 21 hypodermic needle; 68 the veins were first dilated in warm water. Since the caudal veins of Thamnomys and Praomys are obscured by pigmented skin, sporozoites were usually given intraperitoneally to these animals.

In the infectivity experiment with the P. berghei ssp., diluted suspensions of sporozoites were prepared with pipettes in Kahn tubes kept cool by standing them in iced water. Inoculations were done beginning with the most dilute suspension using a standard volume of 0.1ml throughout the series.

(vi) Timing of exoerythrocytic schizogony

Blood was taken under deep ether anaesthesia from the hearts of white mice at timed intervals from single intravenous inoculations of sporozoites. The blood was inoculated intraperitoneally into other mice, blood films from the tails of which were examined daily for ten days..

(vii) Haematological histological techniques

Thin blood films were stained in Giemsa's stain at pH 7.1 - 7.2 by the method given in detail in the techniques used in the field work.

Pieces of liver were fixed in Carnoy's fluid

(absolute ethanol 60 parts; chloroform 30 parts; glacial 69
acetic acid 10 parts), dehydrated in a graded ethanol series,
cleared in clove-oil and imbedded in paraffin wax (M.P. 56°C).
Sections were cut at 4 μ , and were stained by Wolbach's (1911)
Giemsa-colophonium method (Bray and Garnham, 1962).

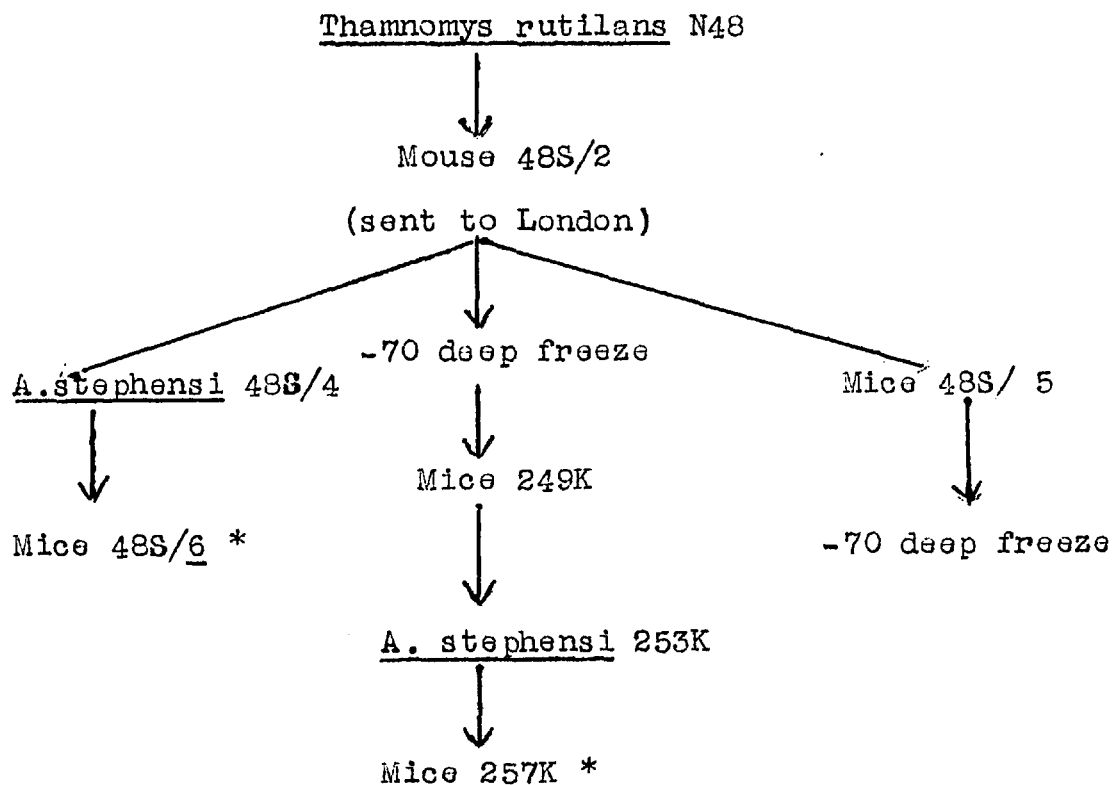
The Life Cycle of the Nigerian *P. vinckei*

All infected *Thamnomys rutilans* trapped at Ilobi had patent infections of *P. vinckei* which were easily seen in thick blood films (plate 9). Four of the six naturally infected rodents had parasitaemias sufficiently high for parasites to be found easily in thin blood films (e.g. plate 12).

Two isolations were made in white mice from *Thamnomys* N48, and the mice were flown to London on separate occasions. One of these lines (N) caused the death mice within a few days after inoculation, often before parasitaemias were detectable. It was assumed that the line was contaminated with a virus lethal to mice, and it was therefore abandoned. The second line (S) was more easily handled, and the sporogony was obtained in *A. stephensi*; sporozoites were, however, not infective to laboratory mice. The history of line S of strain N48 is shown in Fig.3.

A second strain was isolated from *Thamnomys* N54, which was taken to London and used as a source of material on several occasions. The first line, established in white mice by inoculating them with heart blood from the *Thamnomys* on August 22nd, 1967, was mixed with *P. berghei*. Lines derived from caudal blood on January 12th, 1968, and February 21st, 1968 (when the animal was sacrificed), were only of *P. vinckei* and the rodent had apparently lost the infection of *P. berghei*.

History of N48 strain (line S) of
Nigerian *P. vinckei*



* no infection

by spontaneous cure. The history of strain N54 of the Nigerian P. vinckei, excluding the line mixed with P. berghei, is shown in Fig. 4. Sporogony was obtained in three species of anophelines, but, as with strain N48, the sporozoites were never infective.

A third strain of Nigerian P. vinckei (designated 1/69) has recently been isolated from an infected Thamnomys rutilans collected at Ilobi by Mr. A.O. Lambo, and flown to London. Blood from mice infected from the wild rodent is stored at -179°C , and new attempts will be made to obtain infective sporozoites of this strain in laboratory mosquitoes. Until this is done, and primary exoerythrocytic schizonts are seen, it is not possible to assign the Nigerian parasite to a subspecies.

The sporogony of the Nigerian P. vinckei

Attempts to transmit the N54 strain of Nigerian P. vinckei are summarised in the scheme of experiments shown in Fig. 4. Although oocysts grew on the stomachs of A. stephensi, A. quadrimaculatus and A. labranchiae atroparvus, and sporozoites reached the salivary glands of all three mosquitoes, mice or laboratory-bred Thamnomys inoculated with suspensions of crushed infected glands never became infected.

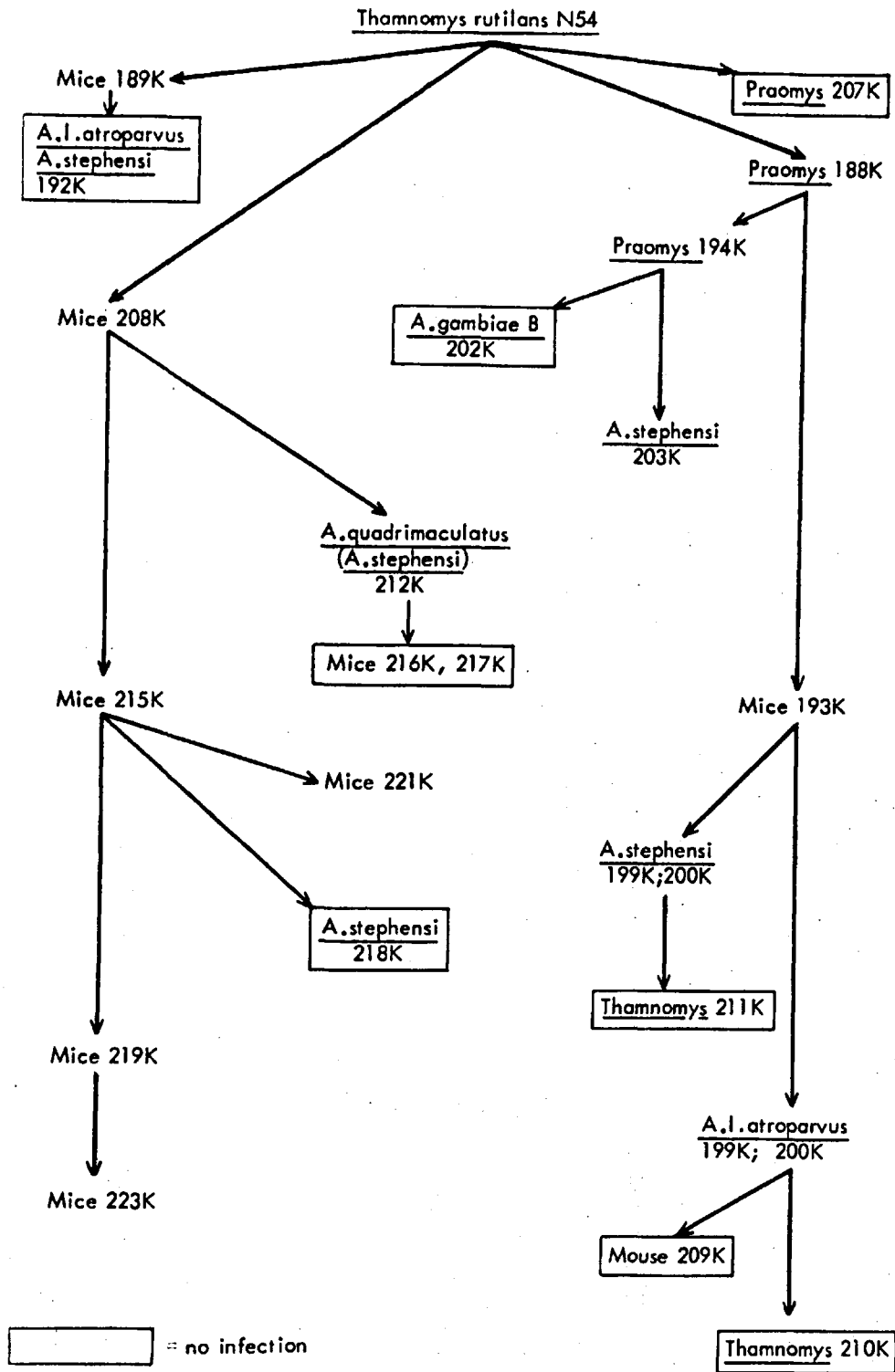
The temperature at which the mosquitoes were incubated was nearly always $24-26^{\circ}\text{C}$. This was chosen because

FIGURE 4

Scheme of attempts to transmit
the Nigerian P. vinckei cyclically
through mosquitoes.

Scheme of attempts to transmit the Nigerian P.vinckei (strain N54)
cyclically through mosquitoes

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(1) it is the best temperature for the successful sporogony of the murine parasites of the Central African Republic (Wéry, 1968), where the conditions are similar to those of the Nigerian locality, and (ii) it was an ideal temperature for the production of infective sporozoites of the Nigerian strain of P. berghei isolated from the same place as the strains of P. vinckei studied in the present work. The unusually low temperature (19-21°C) required for the successful sporogony of most, if not all, strains of P. b. berghei from Katanga (Vanderberg and Yoeli, 1965) was thought most unlikely to be the optimum^{at} for parasites from the much warmer climate of Nigera.

A description is given below of the sporogony of the Nigerian P. vinckei in A. stephensi. Accounts of the experiments using other mosquitoes are then given. In these experiments, A. stephensi were added to the cages of mosquitoes and permitted to feed upon the same gametocyte carrier to check the viability of the gametocytes.

Nigerian P. vinckei in A. stephensi

The sporogony was studied in more than a dozen batches of A. stephensi in which the oocyst rate ranged from 66-90%. There were no detectable differences in the sporogony of strains N48 and N54. At 24-26°C, sporozoites were first seen in the salivary glands of the mosquitoes 12

days after the infective feed.

The youngest oocysts were seen in fresh preparations of midguts on day 3. Their shape, which was always markedly oval, suggested they were soft, and were being squashed by the cells of the midgut. Later, presumably after the basement membrane of the midgut had ruptured (Garnham et.al., 1969a), the oocysts assumed a spherical shape. At 3 days, the abundant pigment was a striking feature; it lay in loose masses and short lines which were sometimes arranged as a fan (plate 10, fig. (1)). The mean diameter of 10 3-day oocysts, calculated by taking the average of the longest and shortest diameters of the oval shapes, was 9.5μ ($10.8 - 8.2 \mu$).

By day 5, the diameter of the oocysts had increased to 16μ ($11-19 \mu$; 20 oocysts). Their shape was now spherical, and the pigment was still visible. Growth continued evenly, with the oocysts measuring 27μ ($20-33 \mu$; 20 oocysts) on day 7, and 32μ ($24-43 \mu$; 20 oocysts) on day 8. By this time, there was still no differentiation of the contents of the oocysts, and even in some of the largest oocysts pigment was often visible lying, not in lines as in younger forms, but in one or more loose masses.

On day 9, the first sign of sporozoite formation became apparent, with many of the larger oocysts showing the growth of finger-like processes; at this point, the growth

PLATE 10

Sporogonic stages of the Nigerian *P. vinckei*
(N54) in *A. stephensi* incubated at 24°C
(X1,500).

Fig. (i) Drawings of living oocysts
showing patterns of pigment;
day 3.

Fig.(ii) Photomicrographs of stained
sporozoites from salivary
glands.

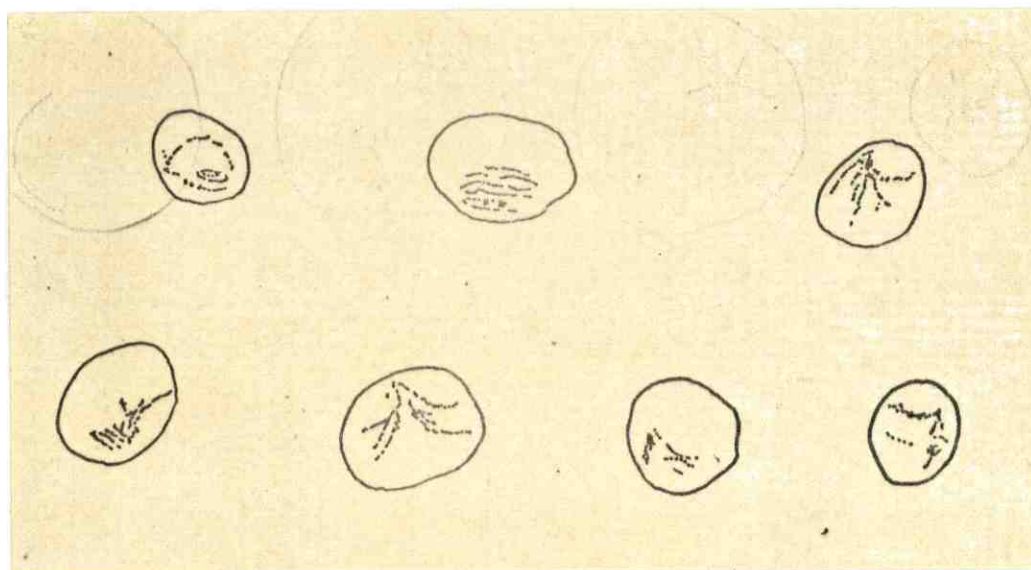


FIG.(1)

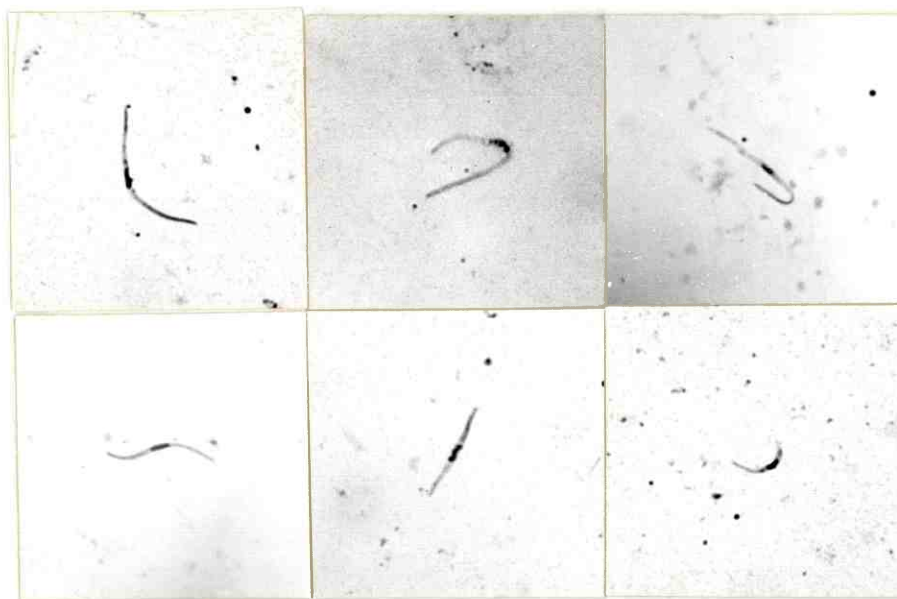


FIG.(ii)

of a number of oocysts stopped, and thereafter apparently 79 dead oocysts measuring about $30\ \mu$ in diameter were seen on all midguts. Under phase-contrast illumination, these oocysts had a yellowish colour, and if the pigment was visible, it was seen in small clumps scattered throughout the parasite with no recognisable pattern. The mean diameter of six oocysts within which sporozoites were beginning to form on day 9 was $47.5\ \mu$ ($40-53\ \mu$). Unlike the oocysts of P. b. berghei and P. b. yoelii, the wheel-like masses of forming sporozoites described by Yoeli (1965) and Wéry (1968) were not seen.

The first fully mature oocysts were found on day 11, but maturity was more usually delayed until a day later. As with oocysts of other species of Plasmodium, the sporozoites lay in randomly placed bundles. The size of the mature oocysts varied widely. The mean diameter of four mature forms on day 12 was $54\ \mu$ ($43-60\ \mu$), but the smallest mature form ever seen was only $40\ \mu$ in diameter, and the largest, $70\ \mu$; these unusual sizes were, however, only occasionally encountered and are considered outside the normal range of $50-60\ \mu$.

Although sporozoites were first found in the salivary glands of mosquitoes on day 12, in the majority of mosquitoes invasion of the glands took place on day 13. In general, the day of invasion seemed to be related to the numbers of oocysts on the midguts, with, surprisingly, the shorter time associated with only few oocysts.

The sporozoite rate in any given batch of A. stephensi was always similar to the oocyst rate; this suggests that as long as oocysts established themselves on the midgut, sporozoites were certain to be produced and to invade the glands. But even when hundreds of oocysts were present, really heavily infected glands with tangled masses of sporozoites pouring from them as soon as a cover-slip was applied - such as are seen, for example, in infections of P. vivax in A. l. atroparvus - were never obtained. In most instances it was necessary to crush the glands before it was possible to see sporozoites. The harvest of sporozoites was therefore low with, in a typical infection, a mean of only 300 sporozoites collected from the glands of each infected mosquito.

In stained smears of crushed salivary glands the sporozoites appeared as long slender bodies with a single nucleus usually lying a little away from the centre of the parasite (plate 10, fig. (ii)). One end of the sporozoites stained a more strongly than the other, and if it is assumed that this is the anterior end with its typical organelles, the nuclei usually lie posterior to the centre. The mean length of 50 sporozoites was 14.7μ (S.E. 0.5μ) with a range of 10.0-24.0 μ .

Experiments in which sporozoites were inoculated into rodents in unsuccessful attempts to demonstrate

the exoerythrocytic schizonts, or to transmit the parasite cyclically, are described below.

Nigerian *P. vinckei* in *A. gambiae* (B)

One experiment with a small number of mosquitoes was performed to test the suitability of *A. gambiae* species B as a host of the Nigerian *P. vinckei*. A control batch of *A. stephensi* was permitted to take a blood meal from the same host at the same time.

The gametocyte-carrier in this experiment was a laboratory-bred *Praomys* (No. 194K/2). The mosquitoes fed on the 12th day after inoculation; unfed mosquitoes were removed and the cage placed in an insectary at 24-26°C.

On day 10 there were no oocysts on the midguts of 5 *A. gambiae*, but 2, 15, 49 and 166 were counted on the stomachs of 4 out of 5 *A. stephensi*. The experiment was not repeated, but the result suggests that *A. gambiae* species B is an unsuitable laboratory host of the Nigerian *P. vinckei*. This is supported by the work of Lambo (1967, personal communication) who found oocysts on the midguts of only 4 out of 120 *A. gambiae* fed on mice infected with a newly isolated strain of Nigerian *P. vinckei*,

Nigerian *P. vinckei* in *A. quadrimaculatus*.

A cage (No. 212K) of *A. quadrimaculatus* mixed

with A. stephensi was permitted to feed upon mouse 208K/1. 8 days after the mouse had been given an intraperitoneal inoculation of blood from a Thamnophis rutilans with a natural infection. Unfed mosquitoes were later removed, and the cage was placed at 24°C.

From guts dissected on days 5, 13 and 15, the oocyst rate in the A. quadrimaculatus (66%) was found to be slightly lower than that of the A. stephensi (83%). No differences were noted in the numbers or sizes of the oocysts in the two species of mosquito, but Ross' black spores were found on the midguts of the A. quadrimaculatus on day 15 a phenomenon never observed in any batches of A. stephensi.

Sporozoites were first found in the glands of one out of two A. quadrimaculatus dissected on day 13; in the glands of four A. stephensi with infected midguts, no sporozoites were seen on that day (development was slightly slower compared with the growth of the parasite in A. stephensi reported above because the temperature was 24°C and not 24-26°C).

On day 15, the sporozoites were harvested from the 35 surviving A. quadrimaculatus of which, from the oocyst rate, 23 were assumed to be infected. The total number of sporozoites collected was only 11,700, i.e. 500 per infected mosquito. Since none of the control A. stephensi was living on day 15, no comparison of the numbers of

sporozoites was possible, but the number per infected A. quadrimaculatus is similar to that found in A. stephensi in other experiments.

Two mice each inoculated intravenously with 5,850 sporozoites from the A. quadrimaculatus did not become infected.

Although only one attempt was made to produce infective sporozoites in this species of mosquito, it is concluded A. quadrimaculatus is an unsuitable host. Ross' black spores suggest a hostile environment, and the small number of sporozoites reaching the glands supports this view. In addition the sporozoites were not infective to mice. Possibly they may have infected a Thamnomys rutilans, the natural vertebrate host, but since P. vinckei from other parts of Africa grows well in the livers of mice (Landau and Killick-Kendrick, 1966b; Bafort, 1968; Landau et al., in press) the most likely explanation is that sporozoites from A. quadrimaculatus suffer so severely from an unsuitable habitat that they lose the ability to establish themselves in a vertebrate host.

Nigerian P. vinckei in A. l. atroparvus.

Two experiments, each with control batches of A. stephensi, were performed with A. l. atroparvus. Mixed mosquitoes were permitted to take blood meals from mice (193K)

on the 8th day after the inoculation of infected blood (batch 199K), and on the 9th day (200K). Unfed mosquitoes were removed, and the cages put at 24-26°C.

In batch 199K, the oocyst rate in the A. l. atroparvus was 25%, compared with 63% in the control A. stephensi. The numbers of oocysts on the midguts of the A. l. atroparvus were never more than 20, whereas from 23 to 300 were counted on the guts of the A. stephensi. In batch 200K the oocyst rate was again lower in the A. l. atroparvus (50%) than in the A. stephensi (82%). In the former mosquito the largest number of oocysts counted on any midgut was only 32, but in the latter the maximum number was more than 200.

Small numbers of sporozoites invaded the glands of the two species in both batches, and on day 15 (199K) and day 14 (200K - fed a day after batch 199K) sporozoites were harvested from both groups of A. l. atroparvus and pooled. Similarly, sporozoites were collected from the salivary glands of equal numbers of A. stephensi from each cage. Doses of 10,500 and 14,000 sporozoites from the A. l. atroparvus were inoculated intraperitoneally into two Thamnomys surdaster, and a third dose of 7,000 sporozoites was given intravenously to a white mouse. A single dose of 6,500 sporozoites from the control A. stephensi was inoculated intravenously into a second white mouse. None of the rodents became infected.

From these experiments it is concluded that

A. l. atroparvus is an even less suitable host than A. stephensi. Although sporozoites invaded the glands of the A. l. atroparvus, the lower oocyst rate and smaller number of oocysts on the guts of this mosquito, in comparison with the control batches of A. stephensi, suggest that the ookinete finds greater difficulty in penetrating the gut wall of A. l. atroparvus and establishing itself as an oocyst than it does in A. stephensi.

The Tissue Schizogony of the Nigerian *P. vinckei*

The tissue stages of the Nigerian *P. vinckei* have not been demonstrated. Attempts were made to transmit the parasite cyclically through laboratory-bred mosquitoes to discover the maturation time of the schizonts in the livers of laboratory rodents. Eleven rodents were given sporozoites of strains N48 and N54 from 3 species of anophelines (figs. 3 & 4). One laboratory bred *Thamnomys surdaster* and 4 mice received sporozoites from *A. stephensi*; 2 *Thamnomys* and 1 mouse received sporozoites from *A. l. atroparvus*; and 3 mice received sporozoites from *A. quadrimaculatus*. No infections arose in the blood of any of these rodents.

In studies on the tissue schizogony of *P. v. chabaudi*, Landau and Killick-Kendrick (1966a, b) found that although schizonts grew well in the livers of laboratory rats, no infection became established in the blood of this abnormal host. Similarly Wéry, Killick-Kendrick and Bray (1968), showed that exoerythrocytic schizonts of *P. b. yoelii* grew in the livers of the guinea-pig and the rabbit, but the parasite failed to infect the blood of either animal. In the attempts to infect rodents with sporozoites of the Nigerian *P. vinckei* the livers were not examined, and it would appear possible that tissue stages could have arisen

but, as in the examples given above, that no infections became established in the blood. This is, however, unlikely, since transfer of infected blood from one mouse to another always gives rise to infections, whereas rats are normally insusceptible to blood forms of P. v. chabaudi, as are guinea-pigs and rabbits to P. b. yoelii.

In future attempts to obtain infective sporozoites of the Nigerian P. vinckei in order to obtain tissue forms in laboratory rodents, two fresh approaches are suggested by recent studies of the life-cycle of the new subspecies of P. vinckei from Brazzaville (Landau et al.; in press). Firstly, it was found that strains differ in the ease with which sporogony may be obtained in A. stephensi and further work should be carried out with a series of new strains of the Nigerian parasite. Secondly, sporogony of the subspecies from Brazzaville was most easily obtained when the gametocytes were provided by an experimentally infected Hybomys univittatus which had been splenectomised. In earlier studies on P. v. vinckei, Bafort (1968) had found that splenectomy of mice used as gametocyte-carriers greatly assisted in obtaining a successful sporogonic cycle with the production of infective sporozoites. In the unsuccessful work with the Nigerian P. vinckei mice and multimammate rats provided gametocytes; in future work other laboratory-bred rodents should be used, and the effect of splenectomy investigated.

NATURALLY INFECTED THAMNOMYS (Plates 11 and 12)

On capture, Thamnomys rutilans N48 was found to have a patent parasitaemia of P. vinckei with 1-3 infected cells in each field of a thin blood film (X100 obj.). All stages were present, making it possible to describe the morphology of P. vinckei in a naturally infected host for the first time. Patent parasitaemias of P. v. chabaudi and the Brazzaville P. vinckei have been reported previously, but the infections were lighter than that seen in the Nigerian Thamnomys, and the reports were unaccompanied by descriptions (Landau and Chabaud, 1965; Adam et al., 1966)

No special affinity for immature red cells was seen, and infected erythrocytes were neither stippled nor, with few exceptions, enlarged.

The nucleus of the smallest rings, with a diameter of 1.5μ , was round/ and stained deeply (plate 11, fig.i; plate 12, fig.vi). The nuclei of the ring forms were commonly divided into two equal masses (plate 11, fig.ii; plate 12, fig.i) or, more rarely, into three with one mass noticeably smaller than the other two. The cytoplasm was a pale blue wisp of material lying close to the nucleus. Double infections were occasionally seen, and a few cells were

PLATE 11

Nigerian *P. vinckei* in the blood of a
naturally infected *Thamnomys rutilans* (N48)
(from a painting by Prof. J.V. Scorza)

Top row, figs. (i), (ii), (iii)

Young ring; ring with double nucleus;
3 young trophozoites in an enlarged cell.

Second row, figs. (iv), (v), (vi)

Compact trophozoite; late asexual
trophozoite or young macrogametocyte;
binucleate schizont.

Third row, figs. (vii), (viii), (ix)

Developing schizonts.

Bottom row, figs. (x), (xi), (xii)

Mature schizont free from host cell;
female gametocyte; male gametocyte.



PLATE 11

Nigerian *P. vinckei* in the blood of a
naturally infected *Thamnomys rutilans* (N48).

found with three parasites (plate 12, fig.3). Erythrocytes with multiple infections were usually larger than normal which, on first sight, suggested that the rodent was also infected with P. berghei. Subinoculations of blood, however, revealed only P. vinckei, and it seems probable either that the cells with several parasites were immature erythrocytes, which are larger than normocytes, or that they were enlarged by the polyparasitism.

As the parasite grew, a vacuole appeared, and the parasite then adopted the typical signet-ring shape of the malaria parasite. The nucleus of the young trophozoite often lay within the vacuole which sometimes persisted until the parasite reached 3.5 μ in diameter, i.e. half the diameter of the cell. At this point of growth, very fine pigment, often lying at the periphery, first became visible. Parasites were never markedly amoeboid, and further growth was accompanied by a progressive reduction in the size of the vacuole until it disappeared completely, leaving the late trophozoite as a round compact body (plate 11, fig.iv; plate 12, fig.iv). The twin nuclear masses, seen in some rings, persisted in the trophozoite simulating an early schizont. The nucleus of the growing form, which often lay in a vacuole (plate 12, fig.iv), was oval, bar - or bean - shaped, but seldom round. Occasionally, a trophozoite lay

in a band across the cell, reminiscent of P. malariae and P. v. chabaudi. 92

Trophozoites were by far the most numerous forms present, whereas schizonts were very few in number. This suggests either that the infection was exhibiting a periodicity, or that schizogony in the blood of the natural host takes place in the deep vessels of the organs.

Nuclear division of the schizonts did not begin until the diameter of the parasite equalled at least half that of the host cells (plate 11, fig.vi). As a result, late trophozoites were indistinguishable from immature gametocytes (plate 11, fig.v).

In later work, when four-hourly slides were prepared from experimentally infected Praomys natalensis, trophozoites ~~resembling~~ immature gametocytes were seen to develop into schizonts, and it is therefore assumed that most of the large parasites with single nuclei seen in the blood of the naturally infected Thamnomys were asexual stages.

The smallest schizont seen had two nuclei, and was a round compact body with a few cytoplasmic extrusions; it occupied a little more than half the host-cell (plate 11, fig.vi). As the parasites grew, the nuclei continued to divide until a maximum of 12 ^{was} ~~were~~ produced (plate 11, figs. vii-ix; plate 12, figs.v-x). The only mature schizont seen

was free from its host-cell, and was composed of eight irregularly shaped merozoites each measuring approximately 1μ across (plate 11, fig.x; plate 12, fig.x) Pigment in schizonts became progressively more abundant as the parasites grew, and was most frequently seen lying at the periphery (plate 11, fig.viii). It clumped into a single yellow mass before the merozoites were fully formed.

Growth of the parasites was accompanied by damage to the host cell which, by the time six nuclei had formed, was seen as a misshapen and palely staining rim around the parasite (plate 11, fig.ix; plate 12, figs.v,vi and ix). This damage was similar to that seen in infections of P. fragile in monkeys (Dissanaike et al., 1965)

The nuclei of the developing schizonts were round or irregular or bar - shaped, and often had a strongly staining portion resembling an eccentrically placed nucleolus (plate 11, figs.vii and ix). The cytoplasm stained a blue colour, and usually contained one or two small indistinct vacuoles (plate 11, fig.viii; plate 12, figs.v and vi).

Fully grown female gametocytes were spherical, and measured $7-8\mu$ in diameter. The host cell was not visible, and it is assumed either that they completely filled the cell or, possibly, that they were extracellular. The nucleus, which was small and situated peripherally, stained a deep purplish red. The cytoplasm was the blue colour

typical of macrogametocytes of Plasmodium, and contained a variable number of grains of fine yellow pigment (plate 11, fig.xi; plate 12, fig.xi).

Only two male gametocytes were found. They were the same size as the macrogametocytes, but were palely staining pink bodies with a single nucleus about twice the size of that of the female forms. The nucleus was pale pink in colour, and was again situated peripherally. Up to twenty grains of fine yellow pigment were scattered through the cytoplasm (plate 11, fig.xii; plate 12, fig.xii).

The outstanding features of the Nigerian P. vinckei in the blood of its naturally infected vertebrate host may be summarised as follows:-

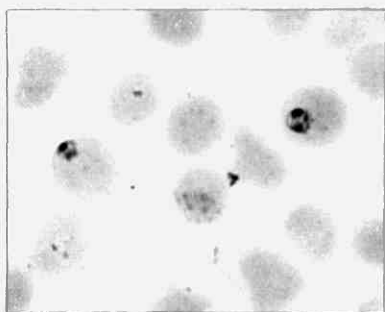
- (i) there is no special predilection for immature erythrocytes,
- (ii) multiple infections of red cells are not uncommon,
- (iii) infected cells are neither stippled nor normally enlarged; they do, however, appear much damaged by the time the parasite has become a young schizont,
- (iv) ring forms commonly have double nuclear masses usually of equal size,
- (v) trophozoites are compact bodies which grow to a comparatively large size before the nucleus

divides and they become schizonts,
(vi) schizonts produce 8-12 merozoites,
(vii) nuclei of gametocytes are situated peripherally
and,
(viii) pigment is a striking yellow colour, and ,
forms a clump before schizonts are fully
mature.

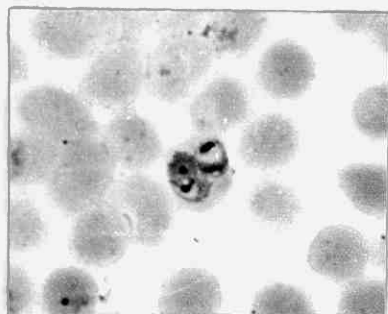
PLATE 12

Nigerian *P. vinckei* in the blood of a naturally
infected *Thamnomys rutilans* (N.48) (x1,500)

- Fig. (i) Ring forms, one with a double nucleus.
- Fig. (ii) Two trophozoites in one cell.
- Fig. (iii) Three trophozoites in an enlarged cell.
- Fig. (iv) Compact trophozoite.
- Fig. (v) Young schizont with 3 nuclei.
- Fig. (vi) Ring form and schizont with 6 nuclei;
note damaged cell.
- Fig. (vii) Two late schizonts.
- Fig. (viii) Schizont with 12 nuclei; pigment
in one mass.
- Fig. (ix) Schizont with 12 nuclei; pigment
in one mass.
- Fig. (x) Mature schizont free from cell;
8 merozoites and one mass of pigment
(see also fig. (x), plate 11)
- Fig. (xi) Female gametocyte
- Fig. (xii) Male gametocyte.



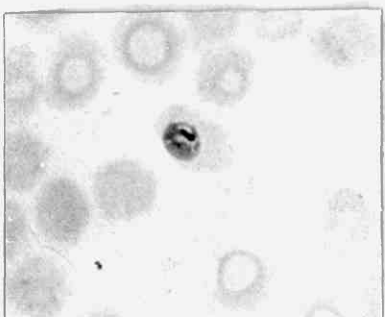
(i)



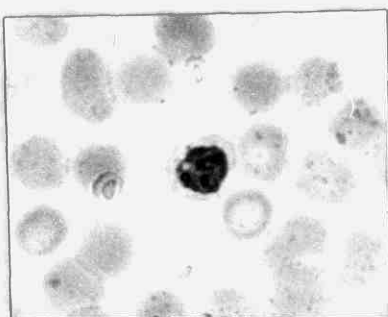
(ii)



(iii)



(iv)



(v)



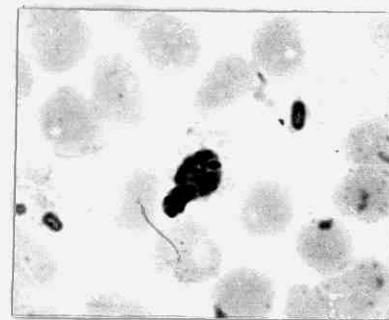
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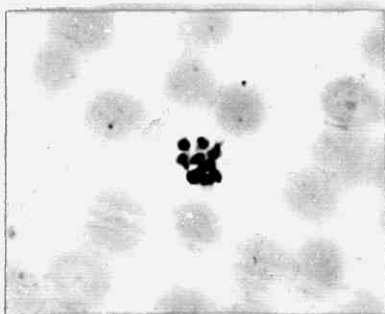
(vii)



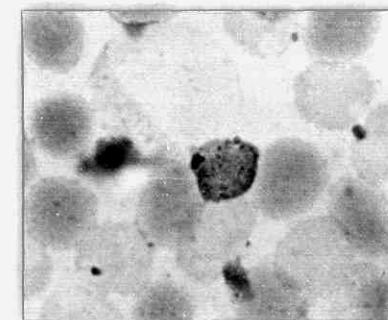
(viii)



(ix)



(x)



(xi)



(xii)

MICE (Plate 13)

Morphologically, the Nigerian P. vinckei in the blood of white mice differed little from that seen in the blood of the naturally infected Thamnomys.

Parasitaemias in mice with blood-induced infections usually became high by the 10th day. Although most mice inoculated with blood from Thamnomys survived the infection - with a spontaneous cure between day 15 and day 25 - after only three consecutive blood-passages the virulence of the strain was enhanced, and mice then seldom lived longer than two weeks. Gametocytes were most numerous as the parasitaemia declined towards a spontaneous cure, as Wéry (1968) noted in infections of P. v. chabaudi, and from the point of view of obtaining infections in mosquitoes, it was important to avoid raising the virulence of the strain by blood-passages.

There were no differences in the morphology of the three strains examined (N48, N54, 1/69). Infected erythrocytes were neither enlarged nor stippled. The reddened host-cell reported with recently isolated strains of P. v. chabaudi and P. vinckei ssp. from Brazzaville (Landau, 1965; Adam et al., 1966) was never seen, although this may be because of differences in methods of staining; according to Mme Landau (1969, pers. comm.) the reddening is demonstrable only when the water used to dilute the Giemsa's stain contains

lithium carbonate.

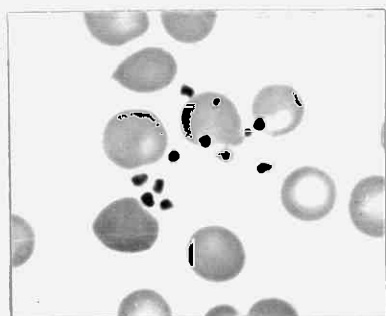
The Nigerian P. vinckei, like the related P. v. vinckei, P. v. chabaudi and P. vinckei from Brazzaville, exhibited no predilection for immature erythrocytes. Multiple infections of red-cells were seen, and were especially common when parasitaemias were high (fig.iii).

Merozoites from mature schizonts ruptured in the preparation of blood films were compact bodies of irregular shape composed of a deeply staining nucleus and small rim of cytoplasm (fig.i); they measured a little less than 1µ across. The youngest intra-erythrocytic forms resembled the free merozoites, but the parasites soon formed the ring stage, with a central vacuole, typical of the genus Plasmodium (fig.ii). As in the Thamnomys, rings with two equal nuclear masses were commonly found (fig.iv). Young trophozoites retained the shape of a ring with the nucleus becoming markedly elongate (fig.v), but as growth continued the central vacuole disappeared, and the parasite then became a compact round form (fig.vi). Pigment in fine yellow grains, usually at the periphery of the parasite, became easily visible in the late trophozoite.

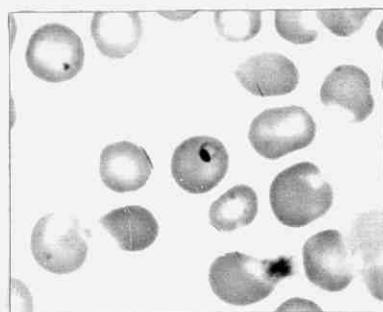
Schizonts were small solid-looking bodies, often causing great distortion of the host-erythrocyte (fig.vii). When mature, they produced 10 or 12 merozoites (figs. ix, x and xi) or, more rarely, as few as 8 or as many as 14 or 16. The

Nigerian *P. vinckei* (strain N54) in the
blood of experimentally infected white mice
(xl, 500)

- Fig. (i) 8 free merozoites and a mass of pigment.
- Fig..(ii) Ring form.
- Fig. (iii) Two trophozoites in one cell.
- Fig. (iv) Two trophozoites with double nuclear
 masses.
- Fig. (v) Two trophozoites with bar-shaped nuclei.
- Fig. (vi) Trophozoite.
- Fig. (vii) Schizont with 4 nuclei in damaged
 erythrocyte.
- Fig. (viii) Schizont with 6 nuclei.
- Fig. (ix) Mature schizont with 12 nuclei and
 clumped pigment.
- Figs.(x) and Mature schizonts with 12 nuclei.
 (xi)
- Fig. (xii) Male (M) and female (F) gametocytes.



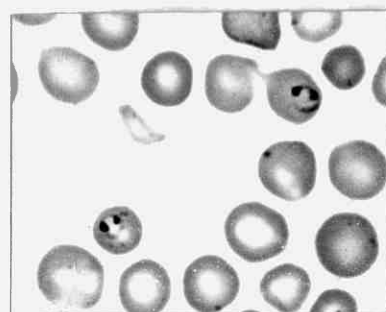
(i)



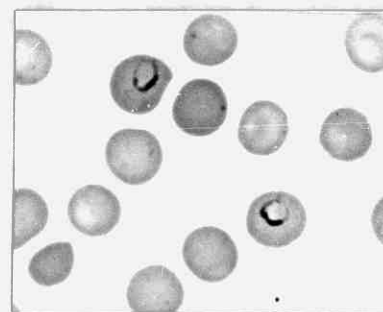
(ii)



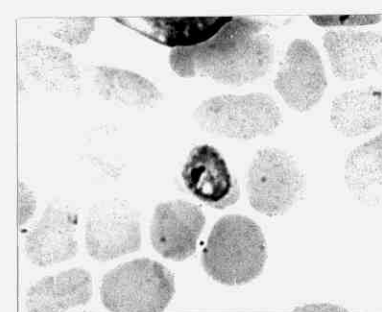
(iii)



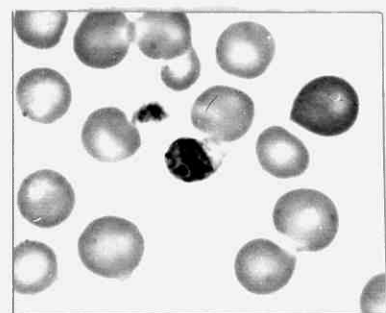
(iv)



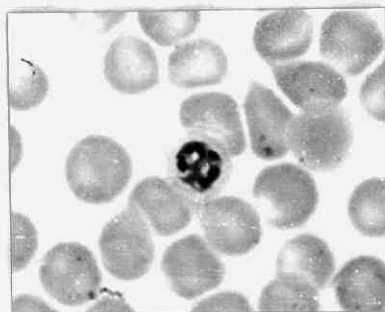
(v)



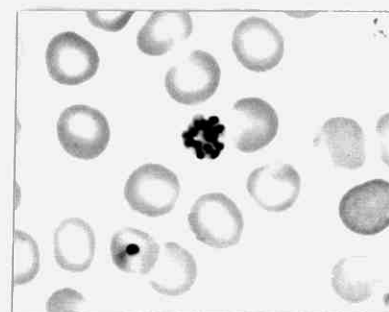
(vi)



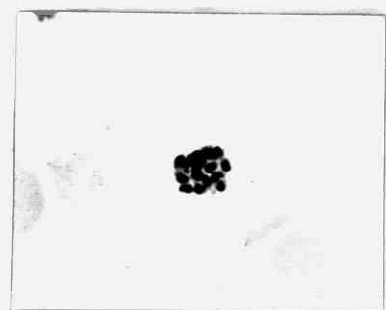
(vii)



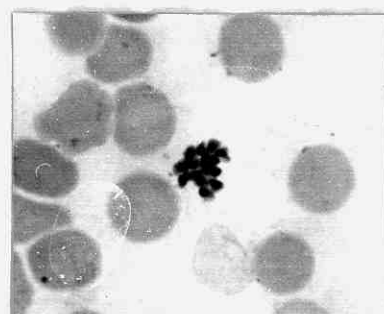
(viii)



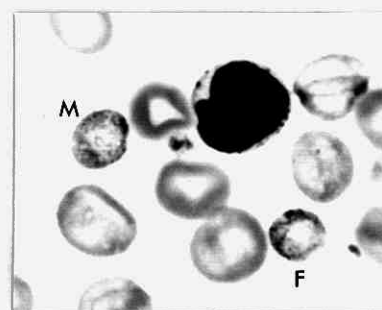
(ix)



(x)



(xi)



(xii)

highest number was perhaps due to two 8-nucleate schizonts in a single cell, and the normal range was therefore 8-14, with 12 by far the commonest number. Just before the schizonts matured, the pigment clumped into one yellow mass.

Gametocytes were most abundant in mice which survived the initial parasitaemia, and which were recovering from the infection. They were round bodies 6-7 μ in diameter completely filling the host-cell, a small rim of which was occasionally visible as a tattered fringe. The usual sexual dimorphism was seen. The nuclei of both male and female gametocytes were always situated peripherally; that of the female (fig.xi) was small and stained a dark red colour, whereas that of the male (fig.xii) was more diffuse and stained pink. The cytoplasm of the macrogametocyte stained a bright blue colour, and contained specks of yellow pigment. That of the male stained a pink colour, but the more abundant pigment tinted the cytoplasm and made it appear the colour of a biscuit.

Surprisingly, there were no differences noted in the morphology of the Nigerian P. vinckei in the naturally infected Thamnomys and in experimentally infected white mice except, perhaps, a tendency for schizonts in mice to produce a few more merozoites. However, very few schizonts were seen in the Thamnomys, and the small number of schizonts in mice producing 14 merozoites, as opposed to 10-12 in Thamnomys, is not considered to represent an important difference

The similarity in morphology was no doubt due to some degree to the fact that the strains in mice were examined shortly after isolation from Thamnomys; prolonged blood-passage in laboratory rodents may well result in changes.

Blood stages of Bruce-Chwatt and Gibson's
Plasmodium of Praomys (plates 14 and 15)

The P. vinckei - like parasite of Nigerian Thamnomys was closely compared with the Plasmodium from Praomys tullbergi discovered in the same locality in 1954. Descriptions of the latter parasite were given by Bruce-Chwatt and Gibson (1955) and Garnham (1966). Fortunately, one blood film from a mouse which had become infected after an inoculation of blood pooled from several Praomys had been preserved in the collection of Prof. P.J.C. Garnham, who very kindly lent the slide for a comparison to be made.

In the smear, the only one now in existence, the infection was heavy, with ring forms predominating. Plate 14 is a photograph of a typical field in which rings are seen closely to resemble those of P. vinckei from Nigerian Thamnomys. No preference was shown for the darkly staining immature erythrocytes, a few cells had double infections, and some rings had twin nuclear masses of equal size.

Young trophozoites retained the vacuole of the ring form (fig. (i), plate 15), but as they grew, they became round compact bodies with the nucleus sometimes still in twin masses (fig.(ii), plate 15).

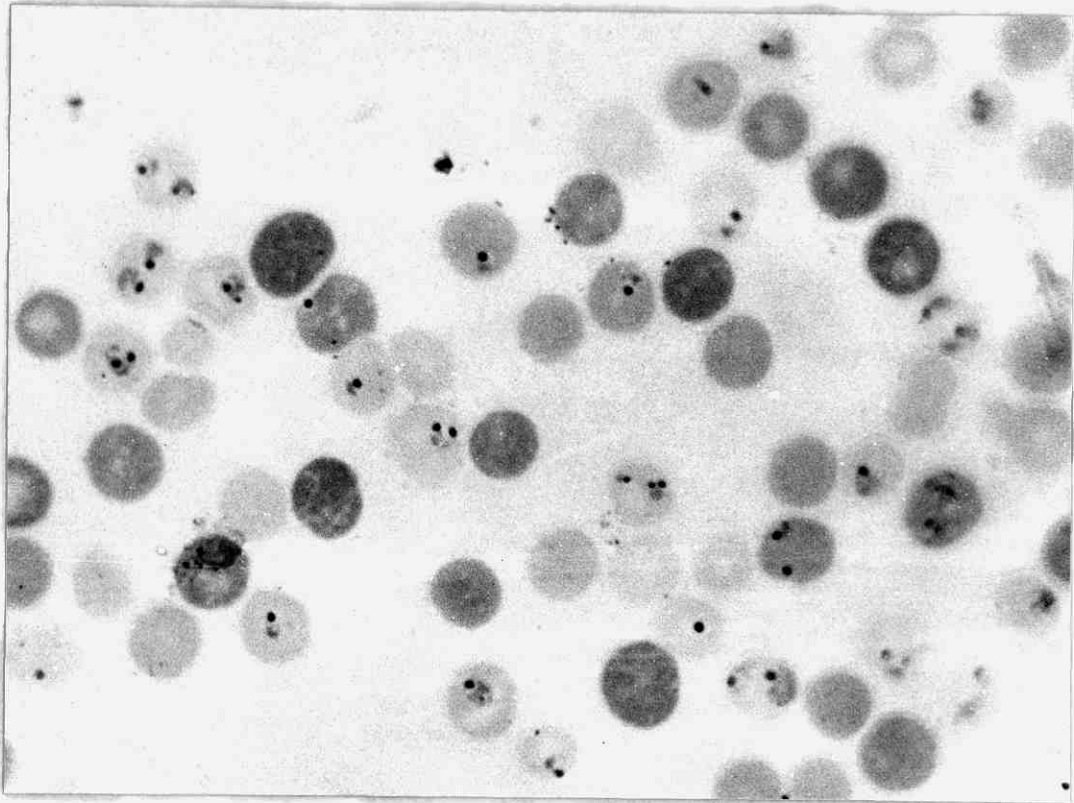


PLATE 14

Plasmodium from Praomys tullbergi
in the blood of a white mouse (xl, 500)
(Bruce-Chwatt and Gibson, 1955)
(from a slide kindly loaned by Prof. P.J.C. Garnham)

Schizonts, which were few in the only smear examined, were small bodies lying in greatly distorted erythrocytes (figs. (iii) - (vi), plate 15). Their nuclei varied in number from 4 to 8, but no fully mature schizonts were seen. In the original report, based on a series of smears, Bruce-Chwatt and Gibson described schizonts with 8-10 nuclei, and Garnham (1966) saw 8-12 merozoites in mature schizonts. As with the strains from Thamnomys, pigment in the schizonts was yellow in colour and usually lay in a single clump.

Gametocytes were rare, and differed from those of strains from Thamnomys by being more vacuolated (figs. (viii) and (ix), plate 15). This was possibly due to senility, although Garnham (1966) commented that the macrogametocyte retained a vacuole during much of its growth. In the first description, pigment in gametocytes was said to be dark brown, whereas in the only smear seen it was better described as light brown or yellow; the colour of pigment, however, varies with the source of light used with a microscope, and this may explain the difference in the observations. Bruce-Chwatt and Gibson noted that gametocytes were most numerous late in the infection.

The P. vinckei - like parasite of the Nigerian Praomys resembles that of Thamnomys in the following ways:-

- (i) infected cells are neither enlarged nor stippled, but become much distorted,
- (ii) there is no predilection for immature erythrocytes,
- (iii) multiple infections are occasionally seen,
- (iv) rings often have the nucleus divided into two equal masses,
- (v) trophozoites grow to be round compact bodies with peripherally situated pigment,
- (vi) schizonts produce 8-12 merozoites,
- (vii): gametocytes measure 6-7 μ , and are most numerous late in the infection, and,
- (viii) the pigment is yellow.

An additional similarity is in the course of infection in mice on primary isolation. The parasitaemia in the mouse infected from the Praomys fell after a week and there was then a spontaneous cure. A similar course of infection is often seen in mice infected from Thamnomys.

One difference is the vacuolation of the macrogametocyte of the parasite from Praomys noted by Garnham (1966). This may, however, be a reflection of ill-health and, with the limited material available, its importance is difficult to assess.

PLATE 15

Plasmodium from Praomys tullbergi in the
blood of a white mouse (xl,500)

(from a slide in the collection of
Prof. P.C.C. Garnham; prepared by Bruce-
Chwatt and Gibson, 1955)

- Fig. (i) Two young trophozoites with double nuclei.
- Fig. (ii) Two trophozoites with double nuclei, and
ring forms.
- Fig. (iii) Two schizonts, one with 4 nuclei, and
the other with 8.
- Fig. (iv) Schizont with 3 nuclei, trophozoites
and ring forms (one cell has a double
infection).
- Fig. (v) Fan-shaped schizont with 7 nuclei.
- Fig. (vi) Schizont with 7 nuclei in a damaged
erythrocyte.
- Fig. (vii) Schizont with 8 nuclei.
- Fig. (viii) Female gametocyte
- Fig. (ix) Male gametocyte



(i)



(ii)



(iii)



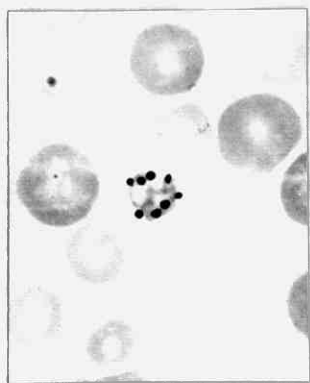
(iv)



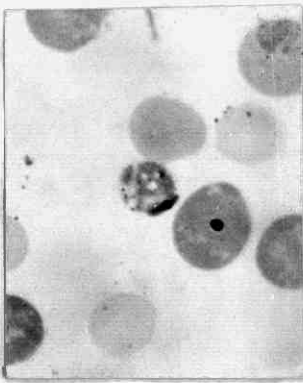
(v)



(vi)



(vii)



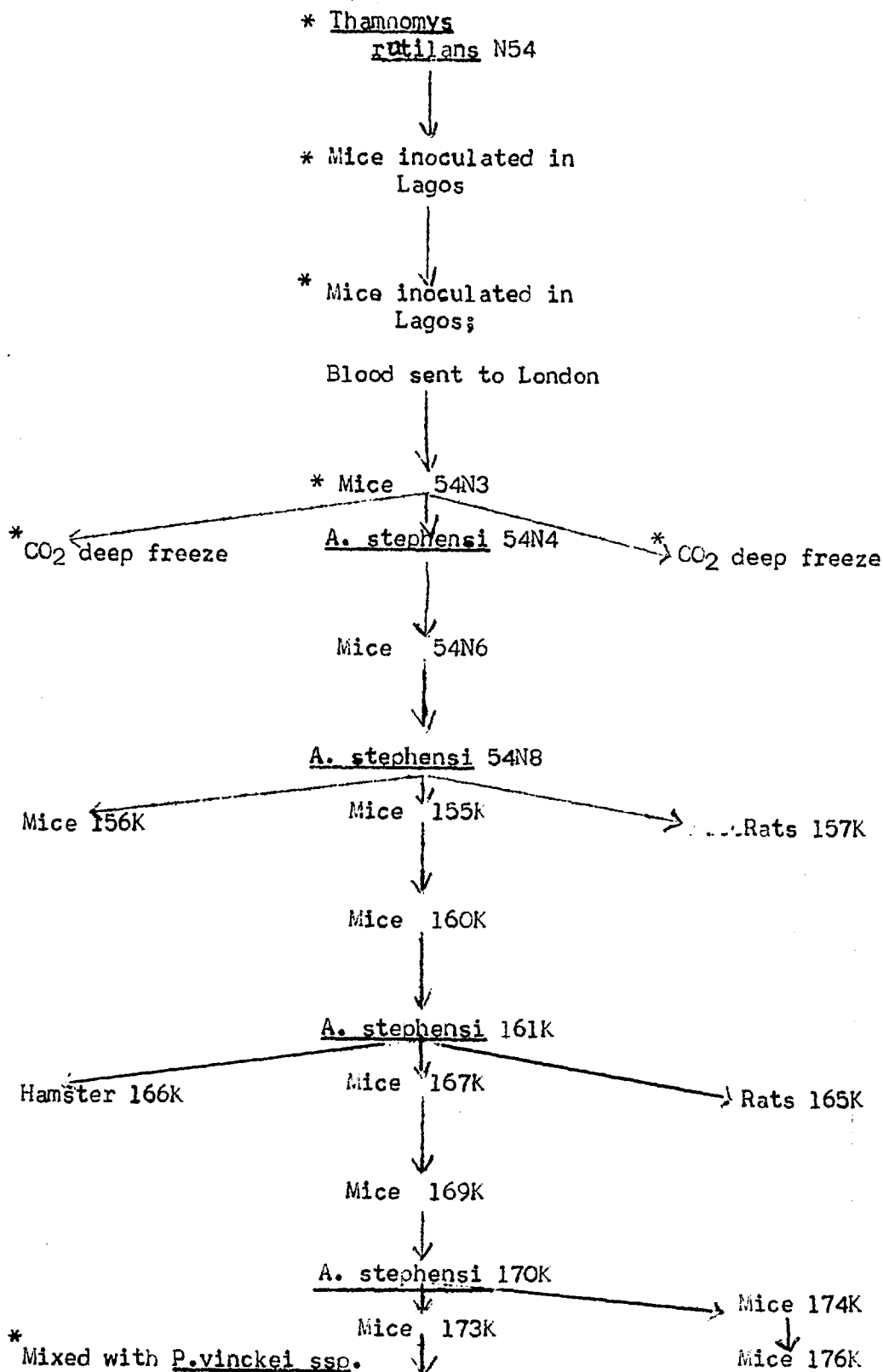
(viii)

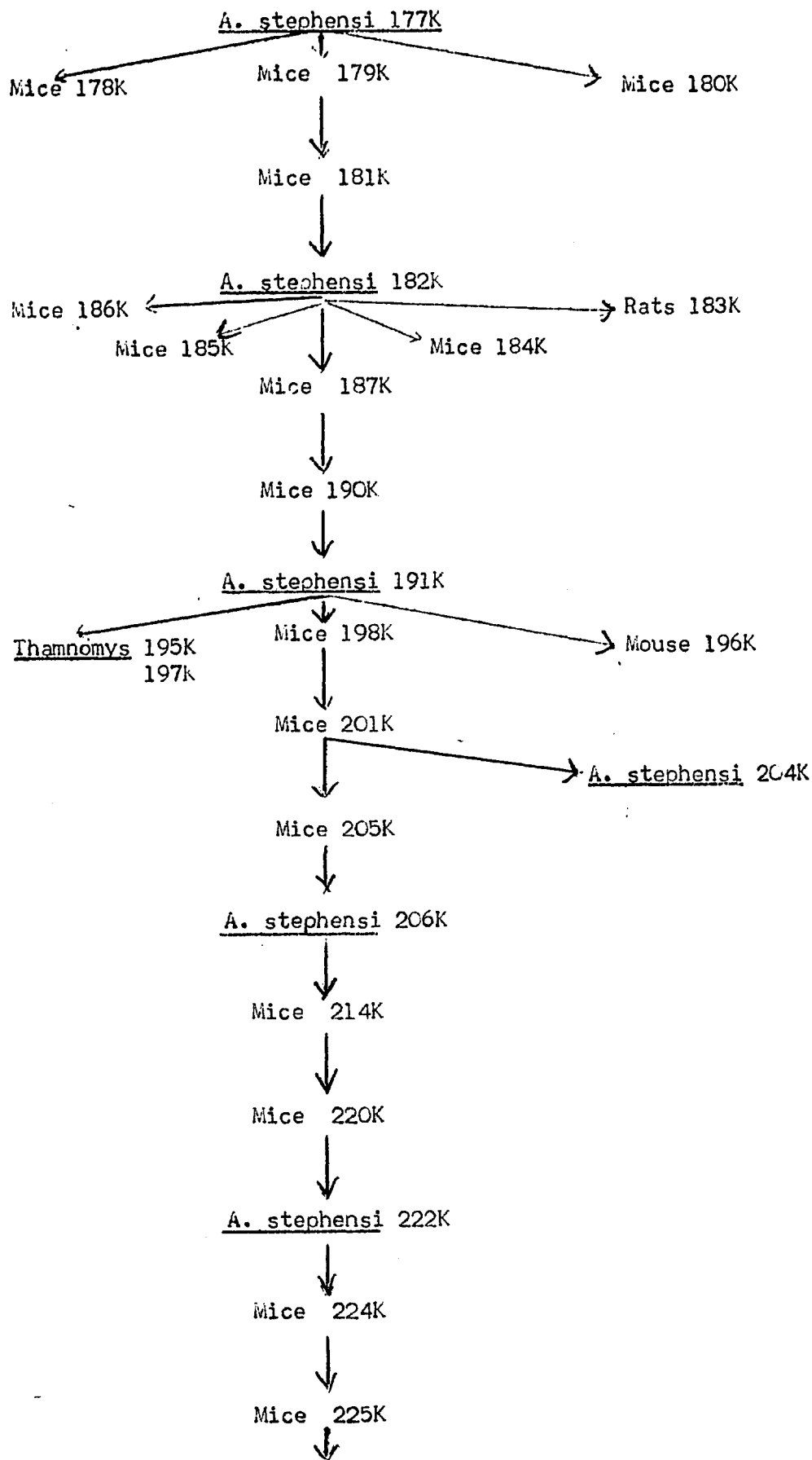


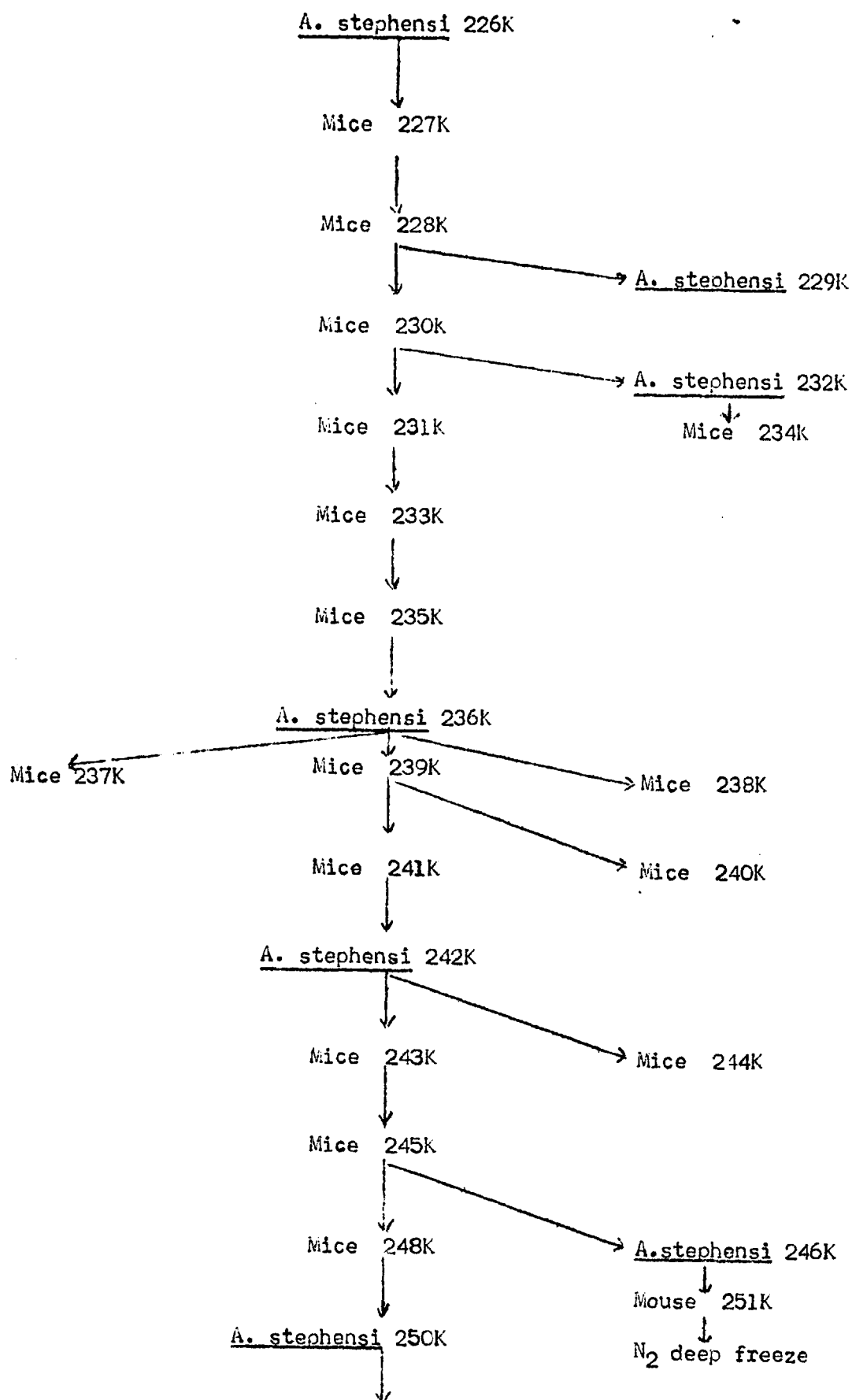
(ix)

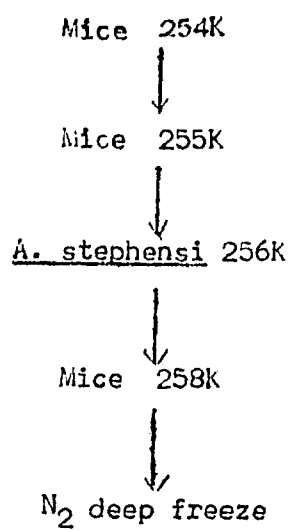
FIGURE 5.

History of the Nigerian P. berghei (N67) in the laboratory.









The Life-Cycle of the Nigerian *P. berghei*

Of the 6 infected thicket-rats collected at Ilobi in August, 1967, one (*Thamnomys* N.54), had a double infection of *P. vinckei* and *P. berghei*. Since infective sporozoites of the *P. vinckei* were unobtainable in *A. stephensi* the *P. berghei* became separated in the first batch of mosquitoes (54N4) fed upon mice (54N3) at the third blood-passage from the natural host (figure 5).

This strain of *P. berghei* is the only one yet found in Nigeria; it was designated strain N67 (from the year of isolation), and the strain of *P. vinckei* from the same rat, No.N54, was given the number of the host. The history of strain N67 is shown in figure 5.

The sporogony of the Nigerian *P. berghei* in *A. stephensi* at 24°C.

From August, 1967 to November, 1968, 18 batches of *A. stephensi* were infected with the Nigerian *P. berghei*. Sporozoite-induced infections were obtained in 26 groups of white mice, 3 groups of white rats, a pair of *Thamnomys* *surdaster*, and a hamster. The strain was remarkable for the ease with which *A. stephensi* became infected, and the constant infectivity of sporozoites for laboratory rodents. Sporogony in *A. stephensi* fed on mice 3-5 days after the

inoculation of infected blood was always successful, with 115 sporozoites invading the salivary glands on day 10 or 11 (at 24°C), and every rodent given 100 or more sporozoites became infected.

On the 4th day after feeding on mice carrying gametocytes, oocysts were easily found on the midguts of A. stephensi. The pigment was clearly visible in the oocysts and tended to lie in one part in short beaded lines, frequently crossing one another (fig.(1), plate 16). The mean diameter of 12 oocysts was 19 μ (15-23 μ).

By day 5, pigment was no longer visible in the largest oocysts, in which faint whorls were now seen (fig.(1), plate 16). The mean diameter had increased to 25 μ (17-32 μ) (10 oocysts). Growth then became slower. On day 6, the mean diameter of 25 oocysts was again 25 μ (16-34 μ), and pigment was still visible in the small oocysts, and whorls in the large.

On day 7, a wide range of sizes was recorded, perhaps because some small oocysts had stopped growing. The mean diameter of the first 20 oocysts seen was 29 μ (21-39 μ) but a few much larger oocysts measuring 47-58 μ were encountered. Even in these very large oocysts, there were still no signs of the formation of sporozoites.

The first finger-like processes of developing sporozoites were seen on day 8, and on the following day a

PLATE 16

Sporogonic stages of the Nigerian
P. berghoi (N67) in *A. stephensi*
incubated at 24°C (X1,500)

- Fig.(i) Drawings of living oocysts showing pigment patterns and, in the larger oocysts, whorls which appear as the pigment becomes obscured; days 4 and 5.
- Fig.(ii) Photomicrograph of stained sporozoites from salivary glands.

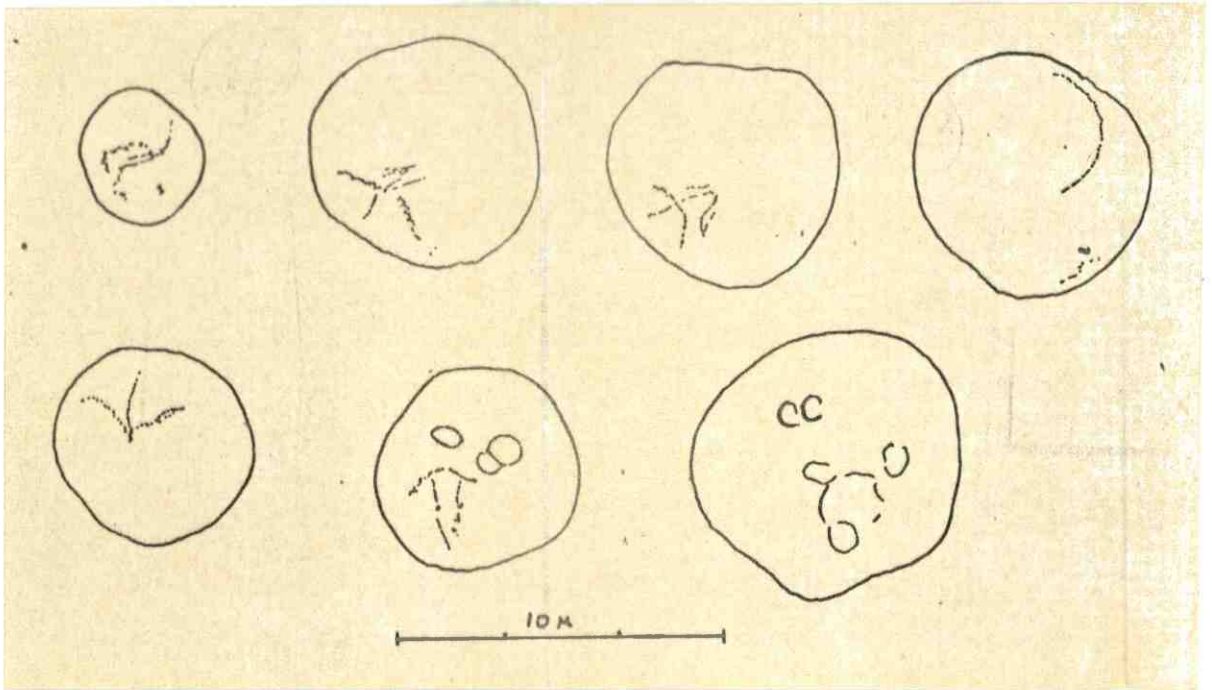
PLATE 16.

Fig. (i)

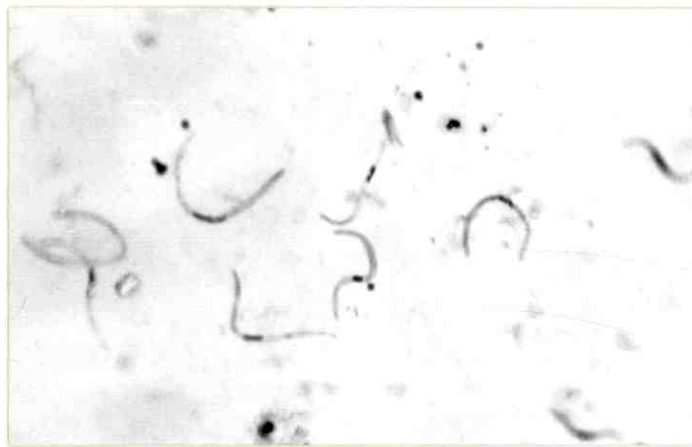


Fig. (ii)

few oocysts were mature. Maturing oocysts often had the appearance of cristate cacti, with the cytoplasm forming convolutions bearing the free ends of developing sporozoites at right angles in regular patterns. Mature oocysts were remarkably consistent in size, and when measured on many occasions on the midguts of mosquitoes incubated at 24°C, their diameter was always approximately 60 μ . No very large oocysts such as were occasionally seen in infections of the Nigerian P. vinckei were seen.

Sporozoites entered the salivary glands of mosquitoes on day 10 or 11. In fresh preparations of crushed glands they were slender, curved immotile bodies. In a stained smear the mean length of 50 sporozoites was 16.72 μ (S.E. 0.22), with a range of lengths of 10.0-19.5 μ . Sporozoites in stained preparations typically lay in curves shaped like the letters C or S (fig. (ii), plate 16). The nucleus was usually in a single elongate mass situated approximately in the centre of the body. One end of the sporozoite tended to stain pink more strongly than the other.

In contrast with infections of the Nigerian P. vinckei, in which the numbers of sporozoites collected were low (no more than 300 per mosquito), from 2,500 to 7,000 sporozoites per mosquito were normally harvested from A. stephensi infected with the Nigerian P. berghei.

Sporogony at 21°C and 28°C is described below, followed by an account of an experiment to determine the infectivity of sporozoites produced at 24°C.

Effect of temperature on the sporogony of
the Nigerian *P. berghei*

Two experiments were performed to investigate the effect of high and low temperatures upon the growth of oocysts and the production of sporozoites of the Nigerian *P. berghei* in *A. stephensi*. The results are summarised in Table 2.

(i) Sporogony at 21°C.

In the first experiment, *A. stephensi* (170K) fed on 3 mice (169K) 3 days after inoculation of blood from another mouse (167K) infected by the inoculation of sporozoites (see figure 5). Unfed female mosquitoes were removed, and the cage was incubated at 24°C for 24 hours. From earlier work it was known that sporogony was successful at this temperature and incubation at 24°C during the formation of ookinetes and their migration into the wall of the gut ensured that oocysts would become established.

After the first 24 hours, the mosquitoes were divided into two groups, one of which was placed at 21°C, while the other was left at 24°C as a control.

From the dissection of small numbers of mosquitoes,

TABLE 2

Sporogony of Nigerian *P. berghei* in *A. stephensi* at
21°C, 24°C and 28°C (1st 24 hrs at 24°C)

Batch No.	Temp. of sporogony	Infections of gut	Diameter of mature oocysts	Day glands first infected	Day glands harvested	Mean number of sporozoites per mosquito	Dose of sporozoites i.v. per mouse*
170K/A	21°C	4/4 -ve >200 oocysts	70 μ	11 or less	12	1,500 (30)	22,000
170K/B	24°C	5/6 +ve >200 oocysts	50 μ	11 or less	12	2,500 (40)	22,000
177K/A	24°C	8/9 -ve 100.250 oocysts	62 μ	10	12	5,650 (74)	14,760
177K/B	28°C	8/8 +ve 1-50 oocysts	60 μ	6	8	194 (70)	13,600

* all mice became infected.

infection rates were found to be high in both batches, and numbers of oocysts were always more than 200 (see table 2). On day 8, sporozoites were forming in the oocysts growing at 24°C, but not in those at 21°C. The oocysts developing at the lower temperature had reached an unusually large size by this day (up to 70 μ in diameter), compared with a maximum of only 50 μ on the guts of mosquitoes at 24°C. By day 11, there were mature oocysts in both batches, measuring the same diameters as on day 8. There were sporozoites in the glands of mosquitoes of both batches on this day.

On day 12, the glands of all surviving mosquitoes were dissected and sporozoites were harvested. The mean number of sporozoites collected from each mosquito incubated at 21°C was 1,500, and from those incubated at 24°C, 2,500. Sporozoites were inoculated intravenously into mice; each of 2 mice were given 22,000 from each batch of mosquitoes. All 4 mice had patent parasitaemias 4 days after inoculation, with 5-10 parasitised cells per field (X100 obj.) of a thin blood film.

It is concluded that the infectivity of sporozoites of the Nigerian P. berghei is similar at 21°C and 24°C. The smaller yield of sporozoites from mosquitoes incubated at the lower temperature was due to a delayed maturation; on the day sporozoites were harvested, there were many oocysts which had not reached maturity on the midguts of this batch.

The greater size of the oocysts at the lower temperature is similar to that reported by Dissanaik et al., (1965) for P. cynomolgi ceylonensis. It emphasises the need to regulate temperatures with great care when comparisons are made of the sizes of oocysts of murine parasites from different localities.

(11) Sporogony at 28°C.

The nominate subspecies of P. berghei is intolerant of high temperatures during sporogony (Yoceli, 1965; Wéry, 1968). A second experiment of similar design was therefore performed to investigate the effect of a temperature of 28°C on the sporogony of the Nigerian strain in A. stephensi.

Mosquitoes (177K) were permitted to feed on 2 mice 8 days after the inoculation of sporozoites. Unfed female mosquitoes were removed, and, as in the first experiment, the cage was kept at 24°C for 24 hours. The mosquitoes were then divided into two groups, one of which was kept at 24°C as a control, while the other was moved to an insectary at 28°C.

Dissections from day 5 onwards revealed similarly high rates of infections in both groups (see Table 2), but there were fewer oocysts (1-50) on the midguts of the mosquitoes at the higher temperature than on those at 24°C (100-250).

On day 6, the oocysts in mosquitoes kept at 28°C were maturing, and were much larger than those at 24°C. The

mean diameter of 10 oocysts at the lower temperature was $39\ \mu$ ($27-46\ \mu$), but that of 10 oocysts, all mature, in the other batch was $61\ \mu$ ($54-71\ \mu$). Sporozoites were seen in the salivary glands of one out of three mosquitoes at 28°C , whereas in the control mosquitoes on the same day there was no sign of the formation of sporozoites in the oocysts.

On day 8 there were sporozoites in the glands of 3 mosquitoes of the experimental batch, on the stomachs of which there were only few unruptured oocysts. Since it was then 2 days since the first invasion of the glands, it was decided to harvest the sporozoites from this group without waiting for infections to mature in the control mosquitoes. The yield was low, with a mean of only 194 sporozoites harvested per mosquito. A mouse inoculated intravenously with 13,600 sporozoites developed a patent parasitaemia on the 3rd day.

On day 9, mature oocysts of the control group attained the same mean diameter ($60\ \mu$) as that reached by oocysts of the experimental group 3 days earlier. Sporozoites invaded the glands of the control group on day 10, and were harvested 2 days later. The yield was more than 30 times greater than from the experimental group, with a mean of 6,650 sporozoites collected per mosquito. Three mice each inoculated intravenously with 14,700 sporozoites had patent parasitaemias 3 days later.

The small number of oocysts on the midguts of the

mosquitoes incubated at 28°C was probably due to interference with the penetration of ookinetes after the first 24 hours at 24°C. In the control group, ookinete penetration and oocyst formation is presumed to have continued beyond 24 hours. The higher temperature much increased the rate of growth of oocysts, but their mean size when mature was the same as at 24°C.

Even when the lower number of oocysts is taken into account, the higher temperature appeared to cause a reduction in the numbers of sporozoites invading the glands; they were, nevertheless, infective to mice.

It is concluded that the sporogony of the Nigerian P. berghei is adversely affected by incubation at 28°C, but the parasite is more tolerant than P. b. berghei of the high temperature.

Infectivity of Sporozoites of the NigerianP. berghei

During the present study, other work was in progress in the same laboratory to determine the possible influence of varying doses of sporozoites of P. vivax on the course of infection in man. To practice the preparation of ten-fold dilutions of suspensions of sporozoites, an experiment was carried out with the Nigerian P. berghei. It was hoped the results would also (i) provide information on the infectivity of sporozoites of the Nigerian parasite produced in A. stephensi at 24°C, and (ii) indicate the feasibility of preserving suspensions of sporozoites of known infectivity of a murine malaria parasite at low temperatures in the same way trypanosomes can be stored (Lumsden et al., 1963)

A large batch of A. stephensi which had fed on mice carrying gametocytes of strain N67, were incubated for 15 days at 23-25°C. The salivary glands of 200 of the mosquitoes were then dissected into cold tissue culture medium "199" by a team of three dissectors. The glands were crushed in a grinding tube with a "Teflon" pestle, and sporozoites were counted in a counting chamber. It was calculated that the suspension contained 8,000 sporozoites per 0.1 ml. A series of ten-fold dilutions was then prepared to provide 6 doses each of 8,000, 800, 80 and 8 sporozoites per 0.1 ml.

The dissection and the preparation of the

dilutions took 2 hours to complete. Throughout this time, tubes containing glands or free sporozoites were kept cool by immersion in iced water. The suspensions were thoroughly mixed and, beginning with the highest dilutions, 0.1 ml. doses of sporozoites were inoculated intravenously into 4 groups of 6 male mice of 15 gms. body weight; the 24 inoculations took 33 mins.

Blood films from the mice were examined for the first time 4 days later. The results are shown below:

TABLE 3

The results of the examination of blood films
from 4 groups of 6 mice given ten-fold doses of
sporozoites of Nigerian *P. berghei*; 4th day after
intravenous inoculation.

<u>Mice</u>	<u>Doses</u>	<u>Blood films</u>
184K/1-6	8	All - ve
185K/1-6	80	All + ve
186K/1-6	800	All + ve
187K/1-6	8000	All + ve

The six mice given the smallest number of sporozoites (8) were kept under observation for 15 days, but none became infected.

The most striking result was the unexpectedly

short prepatent period in all six mice given only 80 sporozoites. In a second experiment with sporozoites of P. b. yoelii (strain 17X), infections arose in 6/6 mice given 700 sporozoites, 3/6 mice given 70 sporozoites and 1/6 mice given 7 sporozoites; the longest prepatent period was 5 days. It is concluded that in work, such as drug trials, in which it is necessary to infect many mice, doses of only a few hundred sporozoites should ensure that every mouse becomes infected. In practical terms, this means that with a yield of 2,500 sporozoites per mosquito (the minimum recorded for the Nigerian P. berghei in A. stephensi incubated at 23-25°C), as few as 10 mosquitoes would provide 50 doses of 500 sporozoites.

Using the method for measuring the infectivity of trypanosomes published by Lumsden et al., (1963), Dr. J.R. Baker calculated from the results tabulated above that approximately 3 per cent of the sporozoites of the Nigerian strain were infective. It seems unlikely, however, that the method of estimating the infectivity of trypanosomes can be applied to sporozoites without modification. The method is based on the assumption that a single trypanosome is capable of giving rise to an infection. While this may be theoretically true for sporozoites also, the malaria parasite has a more hazardous journey before it is established in the host.

Trypanosomes inhabit the blood, but sporozoites of a mammalian Plasmodium must first gain entry into a parenchymal cell and complete a ²schizogonic cycle before the blood is invaded.

Wéry (1968) inoculated known numbers of sporozoites of P. b. yoelii intravenously into mice and estimated the total number of tissue schizonts in the liver at 48 hours. From his results, he calculated that not more than 10 per cent of the sporozoites gave rise to tissue schizonts. It is not known whether this is because 90 per cent of the sporozoites produced in A. stephensi are not viable, or whether the majority are simply lost by the wayside. Bray and Killick-Kendrick (unpublished) found that viable sporozoites of P. b. yoelii had completely left the circulation 5-10 minutes after being injected into the vein of a mouse, whereas carbon particles are known to circulate for 30-35 minutes. Presumably, therefore, infective sporozoites which are destined to develop further actively penetrate parenchymal cells whereas others are passively removed from the circulation by phagocytic cells more rapidly than inert particles of carbon. To ensure that an infection arises in a rodent, it is probable that more than one infective sporozoite must be inoculated because of the hazards they must face before gaining access to the only site, the parenchymal cell, in which development can take place.

In spite of the lack of knowledge of the precise fate of the sporozoite when it is inoculated intravenously (or, indeed, by any other route), some method of preserving suspensions of sporozoites of known infectivity at low temperatures may be feasible. The problem is, however, not as simple as that of preserving salivarian trypanosomes. The sporozoite is less tolerant than the trypanosome of current techniques of preservation, and sporozoites in suspensions prepared by crushing glands (Molinari, 1961; Bafort, 1968; Killick-Kendrick and Bruce-Chwatt, in press) appear to survive less well than those frozen in the intact gland (Jeffery and Rendtorff, 1955; Jeffery, 1957). The first step in devising a method for the low-temperature preservation of sporozoites of murine malaria parasites is to discover how better to protect the sporozoite during freezing storing and thawing when it is free from the salivary gland.

Primary Exoerythrocytic Schizogony of the
Nigerian *P. berghei* (Plates 17,18 & 19)

Tissue schizonts were obtained in the livers of white mice and rats given single intravenous inoculations of suspensions of sporozoites of the Nigerian *P. berghei* from the salivary glands of experimentally infected *A. stephensi*.

The maturation time of the tissue stages was found to be approximately 2 days. Seven mice, given intravenous inoculations of sporozoites, were sacrificed at hourly intervals 43-49 hrs after inoculation. Samples of blood 0.5 - 1.0 ml in volume were collected from the heart of each mouse, and were inoculated intravenously into a second series of mice. Only those given blood at 48 and 49 hrs became infected, and the minimum maturation time of the exoerythrocytic schizonts in the white mouse was, therefore, 47-48 hrs.

To provide material for a comparison of the tissue stages of the Nigerian *P. berghei* with those of strains from other localities, samples of liver were taken from white rats inoculated intravenously with sporozoites. Three rats (No. 165K) were given inoculations of sporozoites from *A. stephensi* (batch 161K) which had taken infective blood-meals from mice (No. 160K) 11 days earlier (see fig.5).

Each rat was inoculated with a suspension prepared from the glands of 30 mosquitoes, and although no count was made, it may be assumed from later work in which the yield of sporozoites was never found to be less than 2,500 per mosquito, that the number of sporozoites received by each rat was 75,000 or more.

Liver was taken from (i) rat 165K/3 at 25 hrs at biopsy (by laparotomy) and 50 hrs at autopsy, (ii) rat 165K/1 at 30 hrs (biopsy) and 36 hrs (autopsy) and (iii) rat 165K/2 at 45 hrs (biopsy). Primary tissue schizonts were found only at 36 and 50 hrs. In spite of a long search, no schizonts were seen in the pieces of liver taken from the same rats at biopsy (30 and 25 hrs respectively).

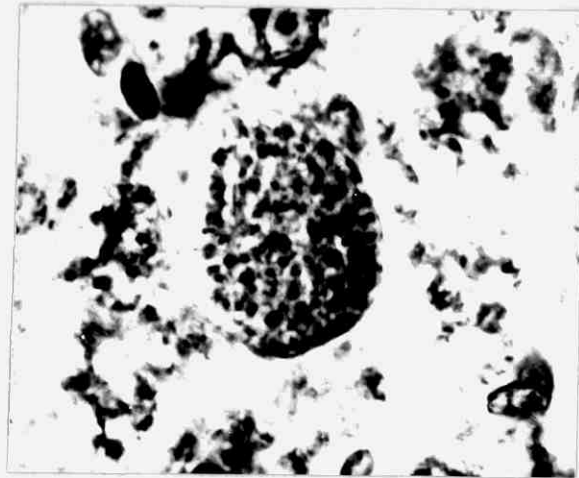
The tissue forms were very similar to those of P. b. yoelii described in detail in earlier work (Landau and Killick-Kendrick, 1966b), except that (i) the Nigerian parasite caused hypertrophy of the nuclei of some infected parenchymal cells, similar to that seen in infections of P. b. killicki (Landau et al., 1968), and (ii) mature schizonts at 50 hrs were a little larger than those of P. b. yoelii.

At 36 hrs the schizonts were oval in shape, and measured approximately 25 x 20 μ . The parasite completely filled the host-cell, the nucleus of which was pushed to one side. Although the nuclei of infected cells were not

enlarged at 36 hrs, they were already affected by the presence of the parasite. They stained palely, and the nuclear material lay in a few peripheral masses (fig. (1), plate 19); this appearance is the same as that seen in cells infected with older schizonts of P. b. yoelii (illustrated in fig. 9, plate 1 in the paper by Landau and Killick-Kendrick, 1966b).

As with young tissue forms of all murine malaria parasites, the cytoplasm of schizonts of the Nigerian P. berghoi stained a deep blue colour, and obscured the nuclei of the parasite; deeply staining cytoplasmic flocculi were usually present in 36 hr-old schizonts. Small indistinct vacuoles were common, and were most numerous at the periphery of the parasite, lying close to a fine limiting membrane (plate 17; fig. (1), Plate 19).

The last stages of growth of the tissue forms were observed in the livers of a rat taken at 50 hrs, and a mouse at 49 hrs. Mean diameters of mature schizonts in the rat were larger than those of P. b. yoelii in the same animal, and measured 42-50 μ (compared with 37 μ ; Landau and Killick-Kendrick, 1966b). In large immature forms, the cytoplasmic masses seen at 36 hrs persisted; they were irregularly shaped and measured 1-3.5 μ across. The nuclei of these forms remained obscured by the deeply staining cytoplasm. Small vacuoles were a common feature, and were often more numerous at one side of the parasite.

PLATE 17

Nigerian P. borghoi

36 hr-old primary exoerythrocytic
schizont in the liver of a white rat.

The final stages of maturation were the same as those described for P. b. yoelii. The schizont shrank a little in size, and in section appeared to lie in a large vacuole; pseudocytomeres arose giving a mosaic-like appearance (fig.(i), plate 18), and finally numerous merozoites were formed (figs (ii) & (iii) plate 18). These were small round bodies less than $1\ \mu$ in diameter, with a large nucleus surrounded by a rim of cytoplasm.

The nuclei of infected parenchymal cells became much enlarged by 50 hrs, and measured up to $10\ \mu$ across (fig. (i), plate 19). The nuclear material was clumped into a few deeply staining masses, but the rest of the nucleus stained very palely.

No differences were seen between schizonts in the liver of the rat, and those in the mouse.

PLATE 18Nigerian P. berghei

49 hr-old primary exoerythrocytic
schizonts in the liver of a laboratory
mouse (XL,500)

Fig. (i) A contracted schizont showing
pseudocytomeres.

Fig.(ii) A schizont with merozoites
forming.

Fig.(iii) A fully mature schizont with
free merozoites (M) in a
nearby sinusoid.

PLATE 18

fig.(i)

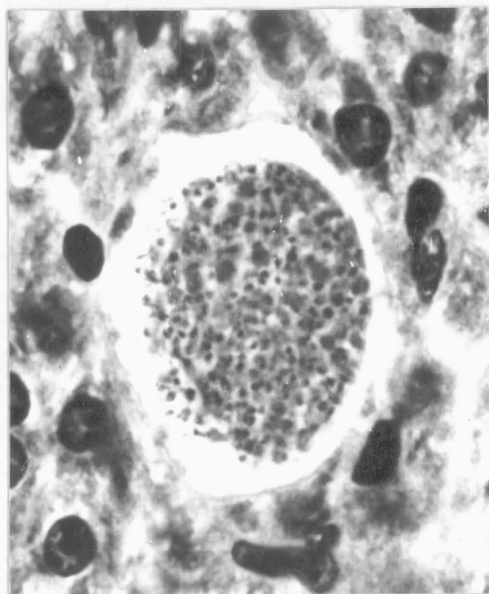


fig.(ii)

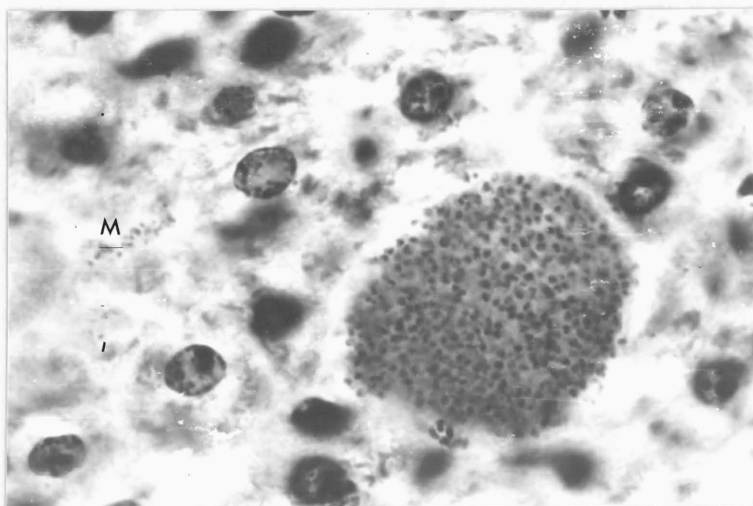


fig.(iii)

PLATE 19

Showing palely stained and enlarged nuclei
of parenchymal cells infected with primary
exoerythrocytic schizonts of Nigerian
P. berghei (X1,500)

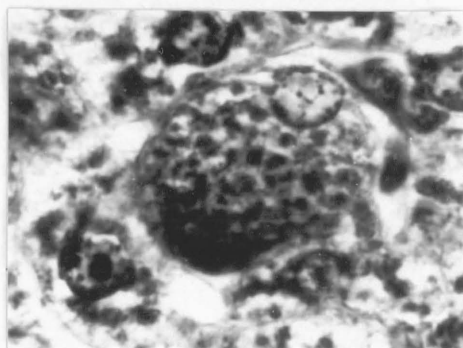


Fig. (i) 36 hrs.

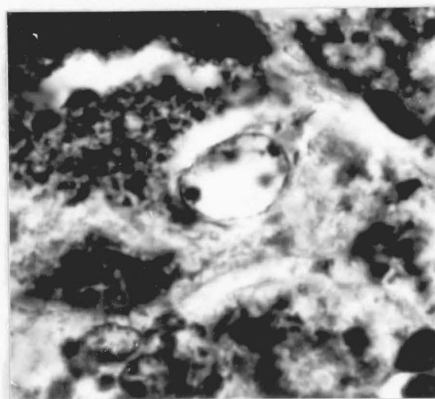


Fig.(ii) 50 hrs.

The Nigerian *P. berghei* in the blood of
white mice (Plate 20)

The morphology of the blood stages of strain N67 is the same as that of *P. b. berghei* and *P. b. yoelii*, with a few minor differences. As with all strains of *P. berghei* subspecies, polyparasitism was commonly seen, especially when parasitaemias were high. The usual predilection for immature erythrocytes was marked in many mice, but in about a quarter of the animals, the usual reaction of the white mouse (Yoeli, 1965) - the production of polychromatophils - was absent, and all parasites were then in normocytes. These mice suffered severely from the infection, and their parasitaemias were higher than in mice with many immature red-cells. Except for the mice which lacked polychromatophils, there was usually a spontaneous cure in the 2nd or 3rd week of infection; mice with heavy infections of normocytes seldom survived more than 2 weeks. This observation supports the work of Peters (1963b), who related the apparent virulence of a number of strains of *P. berghei* to the kind of red-cell mainly parasitised. He found the course of infection was most severe in mice with only few polychromatophils.

The smallest parasites in the blood of white mice were tiny compact bodies composed mainly of a nucleus

with a tiny crescentic wisp of cytoplasm. They measured 1.0 - 1.2 μ in diameter. As growth began, a central vacuole formed, and the parasite then adopted the typical ring form of the genus Plasmodium. Unlike the Nigerian P. vinckei, ring forms of strain N67 seldom had twin nuclear masses.

As growth continued, the vacuole was quickly lost, and young trophozoites were typically round compact bodies with no sign of amoeboidicity. Very fine grains of golden pigment first became visible in the late trophozoite as its diameter reached about half that of the infected cell. The presence of these larger stages affected the shape of the host-cell which was often stellate. No stippling was seen.

Division of the nucleus was delayed until the diameter of the parasite was a little more than half that of the host-cell. Nuclei then continued to divide until 8-16 merozoites were produced. Schizonts appeared to grow until they completely filled the infected erythrocyte, but just as they matured they shrank leaving visible a rim of the host-cell. At maturity the golden pigment clumped into one or two masses, often lying in the centre of the parasite.

Usually large schizonts measuring 8.5 - 9.0 μ in diameter, and always filling the host-cell completely were occasionally encountered. Such forms had up to 20 nuclei but none was ever seen to mature. These parasites were

possibly pseudoschizonts - aberrant forms resulting from polyparasitism.

Gametocytes were indistinguishable from those of P. b. yoelii (see Wery, 1968). The usual sexual dimorphism was seen. The nuclei of the female parasites were smaller, more compact and more strongly staining than those of the male gametocytes. The cytoplasm of the female stained a bright blue colour, whereas that of the male was pink, tinged with light brown by the finely scattered pigment. In both macro and microgametocytes, the pigment was a darker golden colour than in the asexual stages, and lay in discrete granules throughout the cytoplasm; female gametocytes seemed to contain more pigment than the males, but this appearance was perhaps due simply to the fineness of the pigment in the males. Some gametocytes, especially the males, contained a large central vacuole; this is probably an indication of senility, since vacuolated forms became more numerous as infections progressed.

The blood forms of strain N67 were typical of the berghei-group, and these stages in the life-cycle of the Nigerian parasite are indistinguishable from those of subspecies of P. berghei from Katanga, the C.A.R. and Brazzaville.

PLATE 20

Nigerian P. berghei (N67) in the
blood of white mice.

Fig. (i) Rings, trophozoites, a
microgametocyte (M) and
a macrogametocyte (F).

Fig. (ii) Four developing schizonts

Fig. (iii) A mature schizont with
ten merozoites.

Fig. (iv) Ten free merozoites and
a single mass of pigment

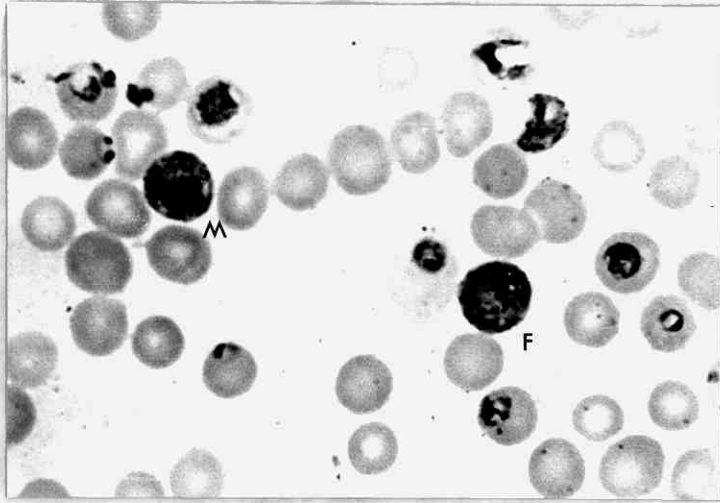
PLATE 20

Fig. (i)



Fig. (ii)

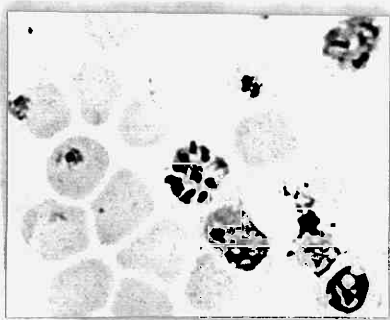


Fig. (iii)

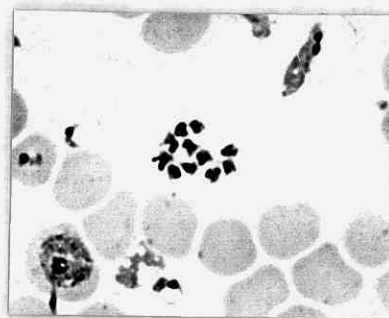


Fig. (iv)

Distribution of Blood-Sporozoa of Rodents

Rodents are so very ^{n m} numerous and diverse that it is difficult to draw many firm conclusions about the geographical and hostal distribution of their blood-sporozoa from the comparatively few reports (see check-list in Appendix). Some patterns nevertheless emerge, and a brief review points the way to new work. The genera of rodents in which blood-sporozoa are known are listed in Table 10 (in the Appendix) according to their systematic position.

Hepatozoon is the commonest blood sporozoan of sciuromorphs and myomorphs, with many records from rodents indigenous to Europe, Africa and Asia, and a few to America. Presumably this wide distribution is at least partly due to the ubiquity of suitable invertebrate hosts, fleas, mites and lice, and possibly to an ability of the parasite to undergo sporogony unaffected by extremes of ambient temperature. Blood forms of haemogregarines of all squirrels and most myomorphs are in leucocytes. The exceptions are the species parasitic in the dipodids, Jaculus and Alactagulus, and two of the murids Cricetomys and Dendromus; gametocytes of the haemogregarines of these rodents inhabit erythrocytes.

Piroplasms of rodents are less evenly distributed both hostally and geographically, than the haemogregarines. There are many records of Babesia in myomorphs, but only one

in a sciurormorph (see Table 10). Many squirrels are terrestrial, and the dearth of reports of piroplasms in these rodents is not satisfactorily explained by lack of possible contact with the only proven vectors, ticks, in an arboreal habitat.

No records were found of Babesia in rodents of Asia and S. America. Dunn et al. (1968) looked at the blood of Asian rodents but recorded no infections of piroplasms, and Lainson (personal communication) has examined hundreds of Brazilian cricetids without encountering Babesia. This unexplained restriction in the distribution of piroplasms of rodents is important when considering the source of infection of rare cases of piroplasmosis of splenectomised man such as those recorded from Yugoslavia (Skralbalo and Deanovic, 1957), the U.S.A. (Scholtens et al. 1969) and the Republic of Eire (Fitzpatrick et.al. 1968).

Hepatocystis is unknown in any host in the New World or Australasia, and it appears to be restricted to the Old World Tropics where it has been recorded from monkeys, the mouse-deer, the hippopotamus, bats and squirrels. The species infecting squirrels are from Asia, and although Hepatocystis is common in monkeys in Africa, it has never been recorded from an African rodent. (see Garnham, 1966).

As with Hepatocystis, Plasmodium is not recorded from New World rodents. True malaria parasites of mammals other than man are poorly represented in that continent, with

only two species, P. simium and P. brasilianum, known in monkeys. Murine rodents, among which are found hosts of Plasmodium in Africa, are not indigenous to the New World, and it is tempting to assume that true malaria parasites are absent in South American rodents of other families.

Species of Plasmodium of rodents are known from (i) semi-arboreal murines of the great Congo Forest, (ii) one African and two Asian squirrels and (iii) the African brush-tailed porcupine. With the exception of the last of these animals, infections of which are acquired in a restricted biotope in caves (Mouchet et al., 1957), terrestrial rodents seem not to occupy a niche where they are likely to become part of an anopheline - malaria - rodent complex. In the vast forests of South America, it is arboreal rodents which would appear most likely to harbour malaria parasites.

In Africa, the malaria parasites of murine rodents appear to be limited to the periphery of the Congo forest block which extends from Katanga in the east, to Nigeria in the west. (Garnham et al., 1966; Wéry, 1968). The principal vertebrate hosts are species of Thamnomys, a rodent which flourishes in forest galleries or secondary forest formed by the farming activities of man. A number of surveys have been carried out in the Guinea Forest west of the Dahomey Gap. Details have not been published, but Gunders and Bray

unsuccessfully examined rodents in Liberia, and Cross is currently looking at rodents in Sierra Leone. In this part of Africa, arboreal murids are scarce and difficult to catch. Thamnomys, and its relative Grammomys, occur, but are seldom encountered. Bellier (personal communication) suggests they have been largely replaced by another semi-arboreal rodent, the little-known Dephomys, following harsh competition among sylvatic rodents during dessication and fragmentation of the Guinea block some time well into the Late Pleistocene (Moreau 1963); the vicissitudes of this part of the African forest must also have affected the sylvatic anophelines. Possibly malaria parasites are no longer present in murids in this western part of the continent.

Malaria parasites of mammals other than

Supralemuroid primates are grouped in the subgenus Vinckeia Garnham, 1964. Fifteen species are known (Garnham, 1964, 1966; Lien and Cross, 1968). These are:

P. girardi and P. lemuris of lemurs

P. bubalis of the water buffalo

P. cephalophi and P. brucei of the duiker

P. traguli of the chevrotain

P. roussetti and P. voltaicum of bats

P. sandoshami of the colugo

P. anomaluri, P. booliati and P. Watteni of flying squirrels

P. atheruri of the brush-tailed porcupine

P. berghei and P. vinckei of murine rodents

The characters of the subgenus are:

- (i) blood stages cause no stippling of red cells,
- (ii) erythrocytic schizonts do not fill the host-cell, and produce not more than eight merozoites,
- (iii) gametocytes are round,
- (iv) sporogony is in anopheline mosquitoes,
- (v) exoerythrocytic schizogony is of short duration, taking 3 days or less,
- and (vi) secondary tissue stages are rare or absent.

Very little is known of the life-cycles of most

species of Vinckeia, and possibly the definition will have to be revised in due course, or perhaps the subgenus will be divided into two or more subgenera. Meanwhile, it provides a convenient grouping for the fifteen species of malaria parasite of mammals other than primates.

Murine malaria parasites fit well in the subgenus as presently defined, except for two characters. Firstly, erythrocytic schizonts of P. berghei and P. vinckei commonly produce more than eight merozoites and secondly, presumed secondary tissue stages have been found in the livers of thicket-rats with mixed natural infections of P. b. yoelii and P. v. chabaudi (Landau and Chabaud, 1965, 1968; Landau and Killick-Kendrick, 1966b; Landau et al., 1967, 1968^b). The numbers of merozoites produced by erythrocytic schizonts tend to vary according to host, and descriptions of the blood stages have been made from unnatural hosts (hamsters, white rats and mice). However, the study of the Nigerian P. vinckei in the blood of its natural host in the present work shows that the normal number of merozoites of this subspecies is 8-12, and in this respect P. vinckei does not fit with the definition of Vinckeia. But Garnham (1964) points out that within the subgenus there are a few minor variations from the definition.

With regard to Landau's tissue schizonts, it is significant that they have never been demonstrated in the

TABLE 4

The distribution of murine malaria parasites

Parasite	Locality	Vertebrate host	Invertebrate host
<u>P. b.berghei</u>	Katanga	<u>T.surdaster</u> (occasionally <u>P. jacksoni</u> and <u>L.bella</u>)	<u>A. dureni</u> <u>millecampsi</u>
<u>P. v.vinckei</u>	Katanga	? <u>T.surdaster</u>	<u>A. dureni</u> <u>millecampsi</u>
<u>P.berghei</u> ssp	Nigeria	<u>T.rutilans</u>	?
<u>P.vinckei</u> ssp	Nigeria	<u>T.rutilans</u> (rarely <u>P.tullbergi</u>)	?
<u>P.b.yoelii</u>	C.A.R.	<u>T.rutilans</u>	?
<u>P.v.chabaudi</u>	C.A.R.	<u>T.rutilans</u>	?
<u>P.b.killicki</u>	Brazzaville	<u>T.rutilans</u>	? <u>A.cinctus</u>
<u>P.vinckei</u> ssp	Brazzaville	<u>T.rutilans</u>	? <u>A.cinctus</u>

livers of laboratory bred Thamnomys with experimental infections, and until this is done it is not certain that they are stages in the life-cycle of Plasmodium.

In the four localities from which murine malaria parasites have been reported (Katanga, Nigeria, the C.A.R. and Brazzaville), P. berghei and P. vinckei are found together (see Table 4). The species are recognised by differences in the infectivity of newly isolated strains to laboratory rats, and the morphology of the stages in the blood of laboratory animals (Garnham, 1966, 1967, 1968; Garnham et al., 1967). In general, young rats are susceptible to P. berghei, but not to P. vinckei. Rings with double nuclear masses are common in P. vinckei, but rare in P. berghei. Unlike P. vinckei, P. berghei is normally characterised by a marked predilection for the immature erythrocytes (reticulocytes and "polychromatophils") of laboratory rodents (Ladda and Lalli, 1966). A striking difference is in the size of fully-grown gametocytes; those of P. berghei are 2 μ larger in diameter than those of P. vinckei.

A number of subspecies of P. berghei and P. vinckei have been recognised and named (Table 4) (Landau and Killick-Kendrick, 1966a; Bafort, 1968; Landau et al., 1968 and in press). The differential characters of the subspecies given by Garnham (1967, 1968) and Garnham et.al. (1967) are:

(i) the geographical and hostal distribution,

(ii) minor differences in the blood stages

(iii) the size of the oocyst,

(iv) the length of the sporozoite,

and (v) the size of the primary exoerythrocytic schizont, its rate of growth and the number of merozoites produced.

Some of these differential criteria, such as the numbers of merozoites produced by schizonts in the blood (a character which may vary according to host, or differ during the course of infection), or the size of the tissue forms at maturity (which may not be the same in different hosts; Yoeli et al., 1966; Bafort et al., 1968), are perhaps too variable in the hands of different workers. This led Landau et al. (in press) to select what they thought to be the most constant characters by which the subspecies could be recognised. These are:

(i) the mean diameter of the oocyst,

(ii) the mean length of the sporozoite,

and (iii) the growth rate of the primary exoerythrocytic schizont in the liver of a standard animal, the laboratory rat, obtained by plotting mean diameters against age. Since some degree of isolation is thought to be necessary for the subspecies to have evolved (see below), geographical and hostal distribution is important in the recognition of subspecies of the

murine malaria parasites.

Some workers dislike the use of subspecific names for malaria parasites and consider the differences as simply those of strains. Before considering the taxonomic position of the Nigerian parasites, it is therefore, necessary to examine the case for recognising subspecies of P. berghei and P. vinckei.

The modern view of a subspecies is that it is essentially a geographically determined taxon, with morphological differences as an expected, but not invariable sequel (Mayr et.al., 1953; Mayr, 1963; Gilles and de Meillon, 1968). Mayr (1963) emphasises that "it is the geographical element that is the essence of the definition, not the degree of taxonomic difference." No rank in the hierarchy of animals can be defined in absolute terms, however, and whether a population is viewed as a species or subspecies, or a biological race or deme (Hoare, 1952) often depends upon a slowly growing consensus of opinion. Subspecies are perhaps the most vague of the taxa, neatly described by Simpson (1945) as more or less arbitrary subdivisions of a continuum. Their use is particularly likely to cause controversy, but a common-sense approach is helpful and refreshing. If a malaria parasite from a given locality has hosts which do not migrate, and if it displays several constant morphological or biological characteristics enabling one to recognise its source, it seems sensible to give it a name. If strains of the parasite vary,

and morphological characters overlap with those of related parasites from other localities, it would not be possible to recognise the source and the parasite would not merit formal taxonomic recognition.

In the discussion below on the taxonomic position of P. berghei and P. vinckei from Nigeria, support is given to the view that differences between strains from some localities are of subspecific order.

The Identities of the Nigerian Parasites.

The climate in the Nigerian locality is similar to that of the localities in the C.A.R. and Brazzaville. There are some differences in the times of the seasons, but basically all three places are in warm, damp secondary forest at low altitude. In contrast, the climate of the Katanga highlands is cooler, and the biotope is one of forest galleries.

In the hostal distribution, there are similarities which again set the parasites of Nigeria, the C.A.R. and Brazzaville, apart from those of Katanga. The subspecies of the 3 localities have a common vertebrate host, T. rutilans, whereas the principal vertebrate host of at least one of the parasites of Katanga is T. surdaster (Table 4). Superficially these two thicket-rats resemble one another, but Rosevear (1969) found differences in the microscopic appearance of the hair which, with differences in the cranium and dentition, led him to support the removal of the species surdaster to Grammomys, a genus close to Thamnomys. Unfortunately, little is known of the habits of thicket-rats but Vincke's (1954) view that T. surdaster was wrongly described as arboreal is contradicted by Bafort's observation (personal communication) of nests of T. surdaster, similar to those of T. rutilans described in the present work, many metres above ground. Thus it seems that T. rutilans and

T. surdaster occupy similar ecological niches, but are perhaps less closely related than their superficial appearance would suggest.

The invertebrate hosts of the parasites of Nigeria, the C.A.R. and Brazzaville are not known, but they are certainly different from that of the Katanga parasites which are transmitted by A. d. millecampsi, a mosquito of limited distribution which is absent from the other localities.

Another similarity of the parasites of Nigeria, the C.A.R. and Brazzaville is in their prevalence in the rodent hosts. In all three places, the subspecies of P. vinckei are much more common than are those of P. berghei, and patent infections of T. rutilans are often met (Landau and Chabaud, 1965; Adam et al., 1966; Killick-Kendrick et al., 1968b). In contrast, P. b. berghei is found in a very low percentage of T. surdaster (Vincke and van den Bulcke, 1949; Vincke, 1954), and there are no reports of patent infections. P. v. vinckei is so rare in Katangan rodents that a natural infection in the vertebrate host has never been found, and our knowledge of this subspecies is limited to 2 isolations from A. d. millecampsi (Rodhain, 1952; Bafort, 1967).

Similarities in the localities, the hosts and the prevalence of the murine malaria parasites from Nigeria, the C.A.R. and Brazzaville mark them off from the Katangan

subspecies, P. b. berghei and P. v. vinckei. This suggests some degree of speciation is likely to have occurred; indeed, as Landau et al. (in press) point out, it would be surprising if this were not so. Garnham (1968), however, has stressed the need for more knowledge of the invertebrate hosts and zoogeography of the parasites before speculating further on the nature of the speciation. Without intensive surveys in places lying between the known localities, it is impossible to be certain there are never links in the distribution. But it is difficult to believe the Katangan subspecies have a genetic pool in common with the parasites from other places.

The Identity of the Nigerian P. berghei

From a comparison of all stages of the life-cycles of the Nigerian P. berghei and related parasites from Katanga, Brazzaville and the C.A.R., it is concluded that the new parasite does not warrant separate subspecific status. At first it was thought to be close to P. b. killicki (Killick-Kendrick et al., 1968b), but a re-examination of this Brazzaville parasite has led to the conclusion that it too should not be regarded as a subspecies. It is suggested that the berghei-like parasites of Brazzaville and Nigeria are best viewed as strains of P. b. yoelii.

The blood forms of the Nigerian parasite are

indistinguishable from those of P. berghei from Katanga, the C.A.R. and Brazzaville. The suggestion that the parasites from places other than Katanga should be grouped into one subspecies is based on distribution, the morphology of the sporogonic stages and growth rate of the primary tissue stages--the criteria chosen by Landau et al. (in press) listed in the discussion above on the systematics of the murine malaria parasites.

Landau et al. (in press) plotted the growth rates of tissue schizonts of P. berghei from Katanga, the C.A.R. and Brazzaville and found that schizonts of strains from the last two places grew at the same rate as each other, and differed from those of P. b. berghei. The mean diameters of the tissue schizonts of the Nigerian strain at two points of growth are so similar to those of the strains from the C.A.R. and Brazzaville that this character appears of value only to separate P. b. berghei from the others. A feature of the Nigerian parasite, the enlargement of the nuclei of infected parenchymal cells (plate 19), is shared by the Brazzaville parasite (Landau et al., 1968).

Mature oocysts of the Nigerian strain measure approximately 60 μ in diameter; those of the 17X strain of P. b. yoelii measure 75 μ (Landau and Killick-Kendrick, 1966b); of the 194ZZ strain of "P. b. killicki", 60 μ (Landau et al. 1968); and of the ANKA and R.L.L. strains of P. b. berghei,

TABLE 5

Sizes of mature oocysts and sporozoites
of strains of P. berghei from
four localities

subspecies	strain	Mean diameter of mature oocysts*	Mean length of 50 sporozoites(1)**
<u>berghei</u>	R.L.L.	34 μ (1)	10.9 $\mu \pm 0.22$
<u>yoelii</u>	17X	75 μ (2)	14.7 $\mu \pm 0.12$
" <u>killicki</u> "	194ZZ	60 μ (3)	14.9 $\mu \pm 0.25$
Nigerian	N67	60 μ (1)	16.2 $\mu \pm 0.22$

* On fresh midguts of A. stephensi gently
flattened under a coverslip.

** In stained smears of infected glands of
A. stephensi

(1) Original

(2) Landau and Killick-Kendrick (1966b)

(3) Landau et al. (1968)

approximately 40 μ (personal observation). In this character, therefore, the strain from Nigeria resembles the one from Brazzaville (see Table 5).

A statistical analysis was made of the length of sporozoites of strains of P. berghei from all 4 localities. Published data were inadequate for this purpose, and new measurements were therefore made. Stained smears of sporozoites of P. b. yoelii from the C.A.R. (strain 17X) were prepared from experimentally infected A. stephensi. Mme I. Landau kindly loaned two preparations of sporozoites of P. b. killicki (strains 193L and 194ZZ). Two freshly isolated strains of P. b. berghei (ANKA and R.L.L.) were kindly supplied by Dr. J. Bafort; sporogony was obtained in A. stephensi incubated at 19-21°C, and dried stained smears of sporozoites were prepared. The technique of measurement is described in the section on materials and methods in the present work.

Preliminary results of the statistical comparison of sporozoites of P. berghei are given in figure 6 in the form of Dice-Lerass diagrams; the data on which the diagrams are based are given in Table 6. The method of Dice and Lerass (1936) for comparing sets of measurements was modified by Davis (1952) to compare lewisii-like trypanosomes of Californian mammals. It has since been used in comparisons of trypanosomes of livestock (Hoare, 195⁹₂, 1956; Keymer, 1969),

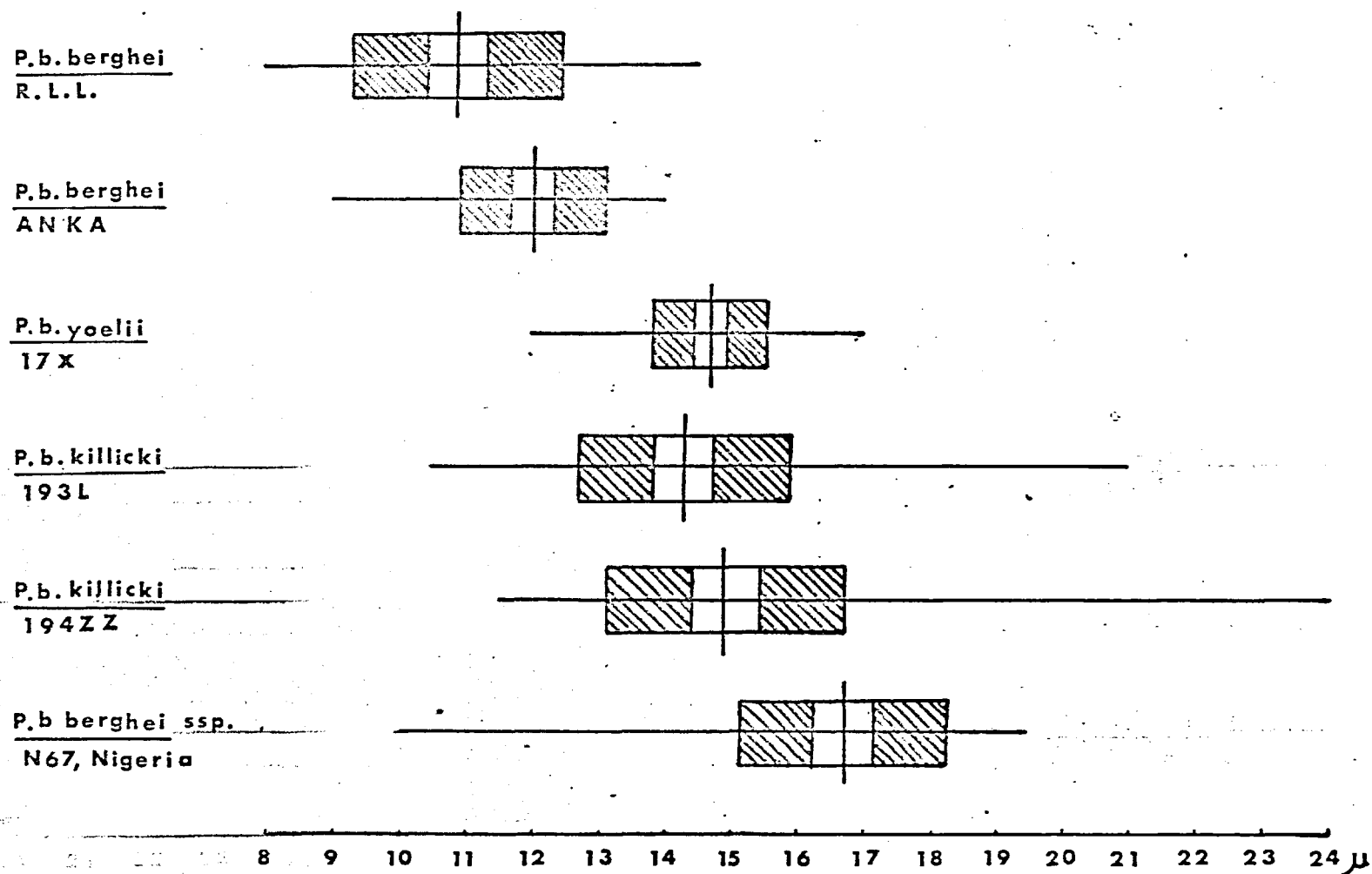


FIG. 6. DICE-LERASS DIAGRAMS OF LENGTHS OF SPOROZOITES OF P. berghei spp.

TABLE 6

Mensural data of 50 sporozoites of strains of
P. berghei from four localities

subspecies	strain	locality	mean μ	range μ	coeff of variation	Std dev.	Std error X2
<u>berghei</u>	R.L.L.	Katanga	10.90	8.0-14.5	14.5%	1.578	0.446
<u>berghei</u>	ANKA	Katanga	12.04	9.0-14.0	9.1%	1.100	0.312
<u>yoelii</u>	17X	C.A.R.	14.72	12.0-17.0	5.9%	0.870	0.246
<u>killicki</u>	194ZZ	B'ville	14.94	11.5-24.0	12.0%	1.797	0.508
?ssp.	N67	Nigeria	16.72	10.0-19.5	9.3%	1.556	0.440

shrews (Davis, 1969), amphibia (Diamond, 1965) and fishes (Needham, unpublished).

The Dice-Lerass diagrams are a simple graphic way of comparing mensural data. The horizontal line, which is the range of measurements, represents the extreme limits of variation of the sample. The arithmetical mean is represented by a cross-bar on the horizontal line. The white rectangle is twice the standard error of the mean plotted on each side of the cross-bar. The shaded rectangles represent the standard deviation of the series, again plotted from the cross-bar.

If the shaded rectangles of any two diagrams do not overlap, at least 84 per cent of the specimens in each sample are separable, a difference in trypanosomes which Hoare (1959) considered of subspecific order. According to Trevan (Hoare, 1956), if the white rectangles of any two diagrams based on samples of more than 30 and with the same standard deviation do not overlap, there is a reasonable probability ($p = 0.02$) that the differences in the means are statistically significant, the significance increasing with the distance between the rectangles. Even when samples are of unequal sizes or have dissimilar standard deviations, if the white rectangles do not overlap the probability that the samples are from the same population is not more than one in twenty ($p = 0.05$).

The populations examined fall into 2 groups, (i) P. b. berghei from Katanga and (ii) P. berghei subspecies from Nigeria, the C.A.R. and Brazzaville. There is, however, a slight overlap in the standard deviations of the series (the shaded rectangles) of the ANKA strain of P. b. berghei and strain 193L of P. b. killicki. Within the first group, sporozoites of the 2 strains of P. b. berghei have significantly different means. Within the second group, sporozoites of the Nigerian strain have a mean length significantly different from those of the strains from the C.A.R. and Brazzaville, but the sporozoites of P. b. yoelii and P. b. killicki appear to be inseparable.

There is an unexplained discrepancy in the measurements obtained of sporozoites of P. b. killicki, and those published by Landau et al. (1968). These workers found the mean length of 20 sporozoites of strain 193L was 18 μ , with a range of 15.5-20.15 μ . In the present study the mean length of 50 sporozoites of the same strain was only 14.32 μ (10.5-21.0 μ), and of the same number of sporozoites of strain 194ZZ, 14.94 μ (11.5-24.0 μ). Possibly the methods of sampling differed. In the present work, sporozoites were drawn in the order in which they were seen, and the only selection was the exclusion of damaged parasites, or parasites partially obscured by débris. In the recognition of the Brazzaville parasite as a new subspecies, the length of the

sporozoite was considered to be an important differential character (Landau et al., 1968), and before P. b. killicki is formally made a synonym of P. b. yoelii the contradictory results obtained in the present work must be confirmed by examining new material.

From the present evidence, however, it appears as though strains of P. berghei from places other than Katanga represent one polymorphic population. To recognise strains from each place as new subspecies obscures the differences between the Katangan subspecies and the others, and since classification is most useful when it shows similarities as well as differences, there is much to be said for grouping the parasites other than P. b. berghei into one taxonomic unit. In time, the subspecies may be raised to specific rank, and within the species yoelii geographical races may be recognised as subspecies. Such a step would still leave revealed the differences between P. b. berghei and the P. berghei-like parasites from other parts of Africa.

It may be argued that the parasites from Nigeria, the C.A.R. and Brazzaville are recognisable by differences in the morphology of the sporogonic stages (see Table 5). The sporozoites of the Nigerian parasite are longer (16.2μ) than those of the strains from the C.A.R. (14.7μ) and Brazzaville (14.9μ). And the mature oocysts of strains from the C.A.R. are larger (75μ) than those from

Nigeria (60 μ) and Brazzaville (60 μ). The Brazzaville parasite shares one character (the length of sporozoite) with strains from the C.A.R., and another (the size of oocyst) with the Nigerian parasite. At the moment it seems unwise to divide geographical races of P. ^{b.}yoelii into subspecies on such shared differences based on studies of only one strain from Nigeria, and 2 from Brazzaville.

The revised differential criteria of P. b. berghei and P. b. yoelii are summarised in Table 7. The Katangan subspecies is seen to differ from P. b. yoelii from Nigeria, the C.A.R. and Brazzaville in its biotope, hosts size of oocyst, length of sporozoite, and the rate of growth of its tissue stage.

The suggested division of P. berghei into only two subspecies is supported by the work of Carter (in press) who found that 4 enzymes of P. b. berghei (adenylate kinase, 6-phosphogluconate dehydrogenase, malate dehydrogenase and glucose phosphate isomerase) have electrophoretic patterns markedly different from those of P. b. yoelii from Nigeria, the C.A.R. and Brazzaville, all of which are closely similar to one another. It remains to be seen in what way this new finding will influence our view of the systematics of the group, but it is clearly wrong to ignore it, especially since similar differences of closely related ciliates are associated with an inability to interbreed.

TABLE 7

Differences between P. b. berghei and P. b. yoelii*

	<u>P. b. berghei</u>	<u>P. b. yoelii</u>
Geographical distribution:	Katanga only	Central Af.Rep., Brazzaville, W. Nigeria
Principal vertebrate hosts:	<u>Thamnomys surdaster</u>	<u>Thamnomys rutilans</u>
Invertebrate hosts:	<u>A.dureni millescampsii</u>	? <u>A. cinctus</u>
Optimum temp. of sporogony	<21°C	>21°C
Mean diameter of mature oocysts:	<45 μ	>50 μ
Mean length of sporozoites:	<13 μ	>13 μ
Primary e.e. schizonts:	Growth rate slower than <u>P.b. yoelii</u>	Growth rate faster than <u>P.b. berghei</u>

* Carter (in press) has demonstrated clear differences in the electrophoretic patterns of enzymes of P.b. berghei (from Katanga), and P.b. yoelii (from the C.A.R., Brazzaville and Nigeria).

In Carter's work, one isolate out of five of P. b. yoelii from the C.A.R. was recognised as a variant, and this was viewed as a strong indication of genetic polymorphism in the population. This is the key to the problem of giving formal taxonomic status to strains of P. berghei; it is difficult to grasp constant morphological or biological characters. Since the sporozoite, like the gametocyte, is at a point in the life-cycle where further development depends upon entry into a new host, it lends itself particularly well to precise measurement, and in current work the diagnostic value of statistical analyses not only of sporozoites of murine malaria parasites but also those of the P. cynomolgi complex and the malaria parasites of man is being further investigated.

Several interesting points have already emerged. Firstly, ranges of lengths based on only two measurements - the longest and the shortest - are usually valueless in making comparisons. Secondly, more subtle comparisons are possible if standard deviations of the means and the series are calculated, than if means (often of badly selected samples) are the sole measurements available. Thirdly, Dice-Lerass diagrams are a good graphic way of comparing sets of measurements of sporozoites.

The Identity of the Nigerian P. vinckei

It is not yet possible to assign the Nigerian P. vinckei to a subspecies. The blood stages are similar to

those of P. v. vinckei. One difference is the distortion of infected erythrocytes seen in strains of the Nigerian parasite from both Thammomys and Praomys, and on this minor character the new parasite is distinguishable from P. vinckei subspecies from Katanga, the C.A.R. and Brazzaville.

Apparent differences are also seen in the sporogonic stages, but the unhealthy sporogony of the Nigerian parasite in all mosquitoes used in the present work casts doubt on any conclusions one is tempted to draw. In ascending order the mean diameters of mature oocysts of subspecies of P. vinckei from the four localities are: 40 μ for P. v. vinckei (personal observation); 47 μ for the new subspecies from Brazzaville (Landau et al., in press); 50-60 μ for the Nigerian parasite; and 75 μ for P. v. chebaudi (Landau and Killick-Kendrick, 1966b) (Table 8). The complete range seen in the Nigerian P. vinckei, however, included a few oocysts as small as 40 μ in diameter and some as big as 70 μ , and a true comparison is not possible without a statistical treatment of the data. Unfortunately, before this can be done, a new method of accurate measurement must be devised, since the present way of measuring living oocysts on freshly dissected guts is not good enough to reveal differences which, though significant, may be slight. The main objection to the present method is the variation in sizes of oocysts due to differences in the amount of saline under the coverslip; an oocyst with a diameter of 60 μ can be squashed

TABLE 8

Sizes of mature oocysts and sporozoites
of subspecies of *P. vinckei*.

Subspecies	Strain	Mean diameter of mature oocyst *	Mean length of 50 sporozoites (1)Ø
<u>vinckei</u>	67	40 μ (1)	12.3 $\mu \pm 0.14$
<u>chabaudi</u>	54X	75 μ (2)	14.3 $\mu \pm 0.17$
Brazzaville ssp.		47 μ (3)	^x 19.5 $\mu \pm 0.48$
Nigerian ssp.	N54	54 μ (1)	14.7 $\mu \pm 0.47$

* On fresh midguts of *A. stephensi* gently flattened under a coverslip.

Ø In stained smears of infected glands of *A. stephensi*.

x 30 sporozoites.

(1) Original

(2) Landau and Killick-Kendrick (1966)

(3) Landau et al. (in press)

to 70 μ . From the data at present available, the size of mature oocysts of the Nigerian parasite appears to be generally similar to that of the subspecies from Brazzaville.

Another similarity to the parasite from Brazzaville was the low infectivity of sporozoites produced in A. stephensi (Landau et al., in press), although infective forms of Landau's parasite could be produced with difficulty, whereas infective sporozoites of the Nigerian parasite were never obtained.

Sporozoites of the Nigerian P. vinckei were measured and comparisons were made with the sporozoites of P. vinckei from other localities; the data were analysed in the same way as in the study of strains and subspecies of P. berghei. All preparations were from the salivary glands of experimentally infected A. stephensi. The material studied was:

(i) smears prepared from mosquitoes infected with strain 67 of P. v. vinckei; the strain was generously provided by Dr. J. Safort;

(ii) a smear of sporozoites of the new subspecies from Brazzaville, kindly presented by Mme I. Landau;

(iii) a smear of sporozoites of strain 54X of P. v. chabaudi made in earlier work (Landau and Killick-Kendrick, 1966a,b); and

(iv) smears of strain N54 of the Nigerian parasite.

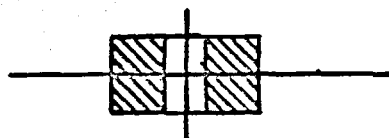
The results are shown as Dice-Lerass diagrams in Fig. 7; the data upon which the figures are based are given in Table 9.

The mean lengths in ascending order were 12.3 μ for P. v. vinckei, 14.3 μ for P. v. chabaudi, 14.7 μ for the Nigerian parasite and 19.5 μ for the subspecies from Brazzaville (Table 9).

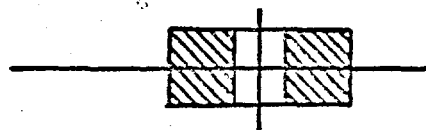
From the Dice-Lerass diagrams (Fig. 7), it is seen that there is a complete overlap between the white rectangles (twice the standard errors of means) of P. v. chabaudi and the Nigerian P. vinckei, and the means may therefore be considered as the same. The mean length of P. v. vinckei is significantly different from the other three populations, but the standard deviation of the series overlaps with those of P. v. chabaudi and the Nigerian P. vinckei. Similarly, the Brazzaville subspecies has a mean length significantly different from the other three, but the standard deviation of the series overlaps with that of the Nigerian strain. From these results, it is concluded that the sporozoites of P. v. vinckei from Katanga and P. vinckei ssp. from Brazzaville are separable from those of strains from the C.A.R. and Nigeria by highly significant differences in their mean lengths.

The data obtained from measurements of strains of P. vinckei ssp. were less uniform than those obtained in

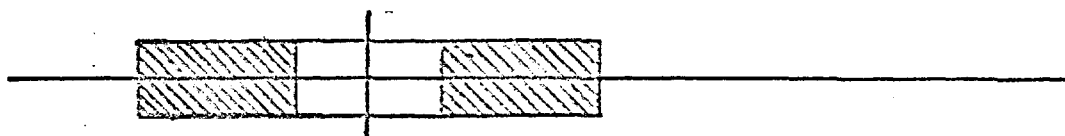
P.v.vinckei
67



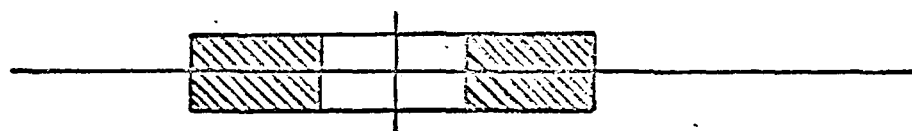
P.v.chabaudi
54X



P.vinckei spp.
N54, Nigeria



P.vinckei spp.
Brazzaville



10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27

FIG.7 DICE-LERASS DIAGRAMS OF THE LENGTHS OF SPOROZOITES OF P.vinckei spp.

TABLE 9

Mensural data of sporozoites of strains of
P. vinckei from four localities.

subspecies	strain	locality	mean μ	range μ	coeff of vari ation	stnd dev.	stud error x2
<u>vinckei</u> (1)	67	Katanga	12.30	10.0-14.5	8.0%	0.988	0.279
<u>chabaudi</u> (1)	54X	C.A.R.	14.26	11.0-16.5	8.3%	1.188	0.336
ssp. (2)		B'ville	19.52	14.5-26.5	13.5%	2.644	0.965
ssp. (1)	N54	Nigeria	14.68	10.0-24.0	20.7%	3.038	0.949

(1) 50 sporozoites

(2) 30 sporozoites

the study of P. berghei. Coefficients of variation of the sporozoites of strains from Brazzaville and Nigeria were very large (Table 9), and new material must be obtained so that more precise comparisons may be made. The range of lengths of sporozoites of the Nigerian parasite was 10-24 μ , the widest of any population measured in the present work giving the highest coefficient of variation (20.7 per cent; see Tables 6 and 9). The measurements are perhaps revealing a higher than usual proportion of malformed parasites, partially explaining their lack of infectivity. As with the oocysts, new measurements of the sporozoites of the Nigerian P. vinckei should be made when a healthier sporogony is obtained, either with other strains of the parasite, or with other species of mosquitoes.

Like the Nigerian P. berghei, the Nigerian P. vinckei appears to share the morphological characters of its sporogonic stages with more than one parasite from other places. The size of its mature oocyst is closest to that of the subspecies from Brazzaville, but its sporozoites cannot be distinguished from those of P. v. chabaudi from the C.A.R. (Table 8).

In spite of the striking differences in the localities and hosts of P. vinckei from Katanga on the one hand and P. vinckei from Nigeria, the C.A.R. and Brazzaville on the other, there is no clear indication that there are

only two clearly recognisable subspecies as in the case of P. berghei. P. vinckei is certainly polymorphic, but more work must be done on strains from all localities before it~~x~~ can be decided how many geographically separate populations there are warranting subspecific status. For a better understanding of the relationships within the group, it is necessary to know which mosquitoes are the natural vectors, and whether or not there is a continuous distribution between the known localities. In addition, it would be interesting to see the electrophoretic patterns of enzymes of the vinckei group similar to those of the berghei group shortly to be described by Carter (in press). Such a study may help in judging the value of this kind of observation in the systematics of malaria parasites.

REFERENCES

- ADAM, J.P., LANDAU, I., & CHABAUD, A.G. (1966).
 Découverte dans la région de Brazzaville de
 rongeurs infecté par des Plasmodium.
C.r. hebdomadaire. Séances Acad. Sci., Paris, 263, 140.
- BAFORT, J. (1967). La transmission cyclique de
Plasmodium vinckei. Annals Soc. belge Méd. trop.,
 47, 271.
- , (1968a). Primary exoerythrocytic forms of
Plasmodium vinckei. Nature, Lond., 217, 1264
- , (1968b). The effects of low temperature
 preservation on the viability of the sporozoites
 of Plasmodium berghei. Ann. trop. Med. Parasit.,
 62, 301.
- , (1969). An unidentified trypanosome, and a
 new Babesia sp. from wild rats from the Congo.
Trans. R. Soc. trop. Med. Hyg., 63, 12.
- , TIMPERMAN, G., & DELBAR, T. (1968).
 Observations on tissue schizogony and sporogony
 of rodent malaria. Annals Soc. belge Méd. trop.,
 48, 535.
- BAKER, J.R. CHITTY, D., & PHIPPS, E. (1963). Blood
 parasites of wild voles, Microtus agrestis, in
 England. Parasitology, 53, 297.
- BALFOUR, A. (1905). A haemogregarine of mammals,
H. jaculi (H. bafouri Laveran). J. trop. Med.
Hyg., 8, 240.
- , (1906). A haemogregarine of mammals and
 some notes on trypanosomes in the Anglo-Egyptian
 Sudan. Ibid., 9, 81.
- BECKER, E.R., & ROUNDABUSH, R.L. (1933). Trypanosoma
iowensis n. sp. and Babesia citelli n. sp. from
Citellus tridecemlineatus and Trypanosoma hixsoni
 n. sp. from Citellus franklini. Iowa State Coll.
J. Science, 8, 527.
- van den BERGHE, L. (1954). The history of the discovery
 of Plasmodium berghei. Indian J. Malar., 3, 241.

- , PEEL, E., CHARDÔME, M., & LAMBRECHT, F.L. (1968). Le cycle asexué de Plasmodium atheruri n. sp. du pore-épic Atherurus africanus centralis au Congo belge. Annls Soc. belge Méd. trop., 38, 971.
- , VINCKE, I.H., CHARDÔME, M., & van den BULCKE, M. (1950). Babesia rodhaini n. sp. d'un rongeur du Congo belge transmissible à la souris blanche. Ibid., 30, 83.
- BRAY, R.S. (1964). A check-list of the parasitic protozoa of West Africa with some notes on their classification. Bull. Inst. fr. Afr. noire, Ser. A., 26, 238.
- , & GARNHAM, P.C.C. (1962). The Giemsa-colophonium method for staining protozoa in tissue sections. Indian J. Malar., 16, 153.
- BRUCE, D., HAMERTON, A.E., & BATEMAN, H.R. (1909). A trypanosome from Zanzibar. Proc. R. Soc. B., 81, 14.
- , -----, -----, MACKIE, F.P. & LADY BRUCE. (1911). The occurrence of piroplasmosis in a white mouse. Rep. sleep Sickn. Comm. R. Soc., 11, 189.
- , HARVEY, D. HAMMERTON, A.E., DAVEY, J.B., & LADY BRUCE. (1915). Trypanosomes and other parasites of animals in Nyasaland. Piroplasm of edible rat. Ibid., 16, 206.
- BRUCE-CHWATT, L.J. ARCHIBALD, N.A., ELLIOT, R., FITZJOHN, R.A. & BALOGAN, I.A. (1955). An experimental control scheme in Ilaro, a semi-rural holoendemic area of southern Nigeria. Report on five years results 1949-1953. Information Bulletin No. 3, Malaria Service Dept. of Medical Services, Federation of Nigeria.
- , & GIBSON F.D. (1955). I: A Plasmodium from a Nigerian rodent. II: A Babesia from wild Nigerian rodents. Trans. R. Soc. trop. Med. Hyg., 49, 9.
- BRUMPT, E. (1946). Contribution à l'étude d' Hepatozoon muris. Utilisation du xénodiagnostic pour l'identification des espèces d'hémogregarines. Annls. Parasit. hum. comp., 21, 1.

- BÜTTNER, D.W. (1968). Vergleichende Untersuchung der Feinstruktur von Babesia gibsoni und Babesia canis. Z. Tropenmed. Parasit., 19, 330.
- CARINI, A., & MACIEL, J. (1915). Sur une hémogregarine et un trypanosome d'un Muridé (Akodon fuliginosus) Bull. Soc. Path. exot., 8, 165.
- CARTER, R. (in press). Enzyme variation in Plasmodium berghei. Trans. R. Soc. trop. Med. Hyg.
- ČERNA, Z. (1957). Further finds of Hepatozoon microti Coles, 1914 in Czechoslovakia (In Czech). Acta Soc. Zool. Bohem., 21, 95.
- CHRISTOPHERS, S.R. (1905). Haemogregarina gerbilli. Sci. Mem. Off. Med. San. Dep. Gov. India, N.S., vol. 18. (quoted from Wenyon, 1926).
- CLARK, M.G. (1958). Hepatozoon griseisciuri n. sp.; a new species of Hepatozoon from the grey squirrel (Sciurus carolinensis Gmelin, 1788); with studies on the life-cycle. J. Parasit., 44, 52.
- COATNEY, G.R., ELBEL, R.E., & KOCHARATANA, P. (1960). Some blood parasites found in birds and mammals from Loei Province, Thailand. Ibid., 46, 701.
- COLES, A.C. (1914). Blood parasites found in mammals, birds, and fishes in England. Parasitology, 7, 17.
- DASGUPTA, B. (1965). Blood parasites in the Himalayan flying squirrel. Trans. R. Soc. trop. Med. Hyg., 59, 716.
- , (1967). A new malarial parasite of the flying squirrel. Parasitology, 57, 467.
- , & CHATTERJEE, A. (1969). Further observations on the malaria parasite of the Himalayan flying squirrel. Ibid., 59, 733.
- DAVIS, B.S. (1952). Studies on the trypanosomes of some California mammals. Univ. Calif. Publ. Zool., 57, 145.
- , (1969). Trypanosoma (Megatrypanum) hoare sp. nov. (Protozoa: Trypanosomatidae) from shrews of the genus Sorex in California. Parasitology, 59, 469.

- DAVIS, D.H.S. (1963). Wild rodents as laboratory animals and their contribution to medical research in South Africa. S. Afr. J. med. Sci., 20, 53.
- (1965). Classification problems of African Muridae. Zoologica afr., 1, 121.
- DIAMOND, L.S. (1965). A study of the morphology, biology and taxonomy of the trypanosomes of Anura. Wildl. Dis., 44, 1
- DICE, L.R. & LERIAS, H.J. (1936). A graphic method for comparing several sets of measurements. Contr. Lab. Vert. Gen. Univ. Mich. 3, 1.
- DISSANAIKE, A.S. (1963). On some blood parasites of wild animals in Ceylon. Ceylon vet. J., 11, 73.
- , NELSON, P. & GARNHAM, P.C.C. (1965). Two new malaria parasites, Plasmodium cynomolgi ceylonensis subsp. nov. and Plasmodium fragile sp. nov., from monkeys in Ceylon. Ceylon J. med. Sci., 14, 1.
- DONOVAN, C. (1920). Malaria of monkeys. At the foot of the Nilgiris. During the months of May and June 1919. Indian J. med. Res., 7, 717.
- DUNN, F.L., LIM, B.L. & YAP, L.F. (1968). Endoparasite patterns in mammals of the Malayan rain forest. Ecology, 49, 1179.
- ELLERMAN, J.R. (1940, 1941). The families and genera of living rodents. I: Rodents other than Muridae. II: Family Muridae. British Museum, London.
- FANTHAM, H.B. (1906). Piroplasma muris Fant. from the blood of the white rat, with remarks on the genus Piroplasma. Q. Jl microsc. Sci., 50, 493.
- FIELD, J.W. & EDESON, J.F.B. (1950). A malaria parasite of the Malayan squirrel. Bull Inst. med. Res. Fed. Malaya, No. 2, 1949.
- FITZPATRICK, J.E.P., KENNEDY, C.C., McGEOWN, M.G., OREOPOULDOUS, D.G., ROBERTSON, J.H., & SOYANNWO M.A.O. (1968). Human case of piroplasmosis (babesiosis). Nature, Lond., 217, 361.

- FRANÇA, C. (1912). Sur la classification des piroplasmés et description de deux formes de ces parasites Archos Inst. Bact. Cam. Pest., 3, 11.
- FRANCHINI, G. (1924). Piroplasmose chez un petit loir (Myoxus avellanarius). Bull. Soc. path. exot., 17, 881.
- (1932). Su di una leucocito-gregarina dello scoiattolo (Sciurus vulgaris). Archo ital. Sci. Med. Colon., 13, 385. (quoted from Clark, 1958).
- FRIEDHOFF, K., & SCHOLTYSECK, E. (1968). Feinstrukturen von Babesia ovis (Piroplasmidea) in Rhipicephalus bursa (Ixodoidea): transformation sphäroider formen zu Vermiculaformen. Z. ParasitKde, 30, 347.
- FURMAN, D.P. (1966). Hepatozoon balfouri (Laveran, 1905). Sporogonic cycle, pathogenesis and transmission by mites to jerboa. J. Parasit., 52, 373.
- GARNHAM, P.C.C. (1948). The developmental cycle of Hepatocystes (Plasmodium) kochi in the monkey host. Trans. R. Soc. trop. Med. Hyg., 41, 601.
- (1964). The subgenera of Plasmodium in mammals. Annls Soc. belge Méd. trop., 44, 267.
- (1966). Malaria parasites. Blackwell, Oxford.
- (1967). Malaria in mammals excluding man. Advances in Parasitology, 5, 139.
- (1968). Recent researches on rodent malaria parasites. Israel J. med. Sci., 4, 1057.
- LANDAU, I., KILLICK-KENDRICK, R., & ADAM, J.P. (1967). Répartition et caractères différentiels des Plasmodiums de muridés. Bull. Soc. Path. exot., 60, 118.
- GILLES, M.T. & de MEILLON, B. (1968). The Anophelinae of Africa south of the Sahara (Ethi- an zoogeographical region). 2nd Ed., S. African Institute for Medical Research.
- GREEN, R. (1933). Ann. Rep. Inst. med. Res., Kuala Lumpur.

HAMERTON, A.E. (1930). Report on the deaths occurring in the Society's gardens during the year 1929. Proc. zool. Soc. London, p. 357.

----- (1931). Report on the deaths occurring in the Society's gardens during the year 1930. Ibid., p. 527.

----- (1941). Report on the deaths occurring in the Society's gardens during the years 1939-1940. Ibid., p. 151.

HAMON, J. ADAM, J.P. and GRJEBINE, A. (1956). Observations sur la répartition et le comportement des Anophèles de l'Afrique Equatoriale Française, du Cameroun et de l'Afrique Occidentale. Bull. Wild Hlth Org., 15, 549.

HERMAN, C.M. & PRICE, D.L. (1954) Hepatozoon in grey squirrels, Sciurus carolinensis. J. Protozool., 1, 11.

HOARE, C.A. (1952). The taxonomic status of biological races in parasitic protozoa. Proc. Linn. Soc. Lond., 163, 44.

----- (1956). Morphological and taxonomic studies on mammalian trypanosomes. VIII: Revision of Trypanosoma evansi. Parasitology, 46, 130.

----- (1959). Morphological and taxonomic studies on mammalian trypanosomes. IX: Revision of Trypanosoma dimorphon. Ibid., 49, 210.

HOOGSTRAAL, H. (1959). Observations on some blood protozoa of Egyptian vertebrates with special reference to Hepatozoon balfouri (Laveran, 1905). PhD. Thesis London University.

----- (1961). The life-cycle and incidence of Hepatozoon balfouri (Laveran, 1905) in Egyptian jerboas (Jaculus spp.) and mites (Haemolaelaps aegyptius Keegan, 1956). J. Protozool., 8, 231.

JACOBS, B. (1953). Some blood parasitic protozoa of small wild mammals in England. Trans. R. Soc. trop. Med. Hyg., 47, 10.

- JEFFERY, G.M. (1957). Extended low-temperature preservation of human malaria parasites. J. Parasit., 43, 488.
- & RENDTORFF, R.C. (1955). Preservation of viable human malaria sporozoites by low-temperature freezing. Expl. Parasit., 4, 445.
- KAKABADZE, M.G. & SASSUCHIN, D.N. (1969). Hepatozoon epsteini sp.n. - a parasite of rats. Progress in Protozoology, Nauka, Leningrad, 3, pp 337-338.
- KEYMER, I.F. (1969). Investigations on the duiker (Sylvicapra grimmia) and its blood protozoa in Central Africa. Phil. Trans. R. Soc. B, 255, 33.
- KILLICK-KENDRICK, R. & BRUCE-CHWATT, L.J. (in press) Preservation of sporozoites of Plasmodium berghei yoelii in liquid nitrogen. J. Protozool.
- , SHUTE, G.T., LAMBO, A. & GUY, N.W. (1968a). Rodent plasmodia of Nigeria. Trans. R. Soc. trop. Med. Hyg., 62, 2.
- , -----, & LAMBO, A.O. (1968b). Malaria parasites of Thomomys rutilans (Rodentia, Muridae) in Nigeria. Bull. Wld Hlth Org., 38, 822.
- KIRNER, S.H. BARBEHENN, K.R. & TRAVIS, B.V. (1958). A summer survey of the parasites of two Microtus pennsylvanicus pennsylvanicus (Ord) populations. J. Parasit., 44, 103.
- KLEINE, F.K. (1910). Trypanosomenbefunde am Tanganyika und andere Beobachtungen. Dt. med. Wschr., 36, 1400.
- KRAMPITZ, H.E. (1957). Ricerche sugli emoparassiti dei micromammiferi selvatici della Sicilia. Riv Parassit., 38, 219.
- (1964). Über das Vorkommen und Verhalten von Haemococcidien der Gattung Hepatozoon Miller, 1908 (Protozoa, Adeleidea) in mittel- und südeuropäischen Säugern. Acta trop., 21, 114.
- , SACHS, R., SCHALLER, G.B., and SCHINDLER, R. (1968). Zur Verbreitung von Parasiten der Gattung Hepatozoon Miller, 1908 (Protozoa, Adeleidea) in ostafrikanischen Wild säugetieren. Z. ParasitKde, 31, 203.

- KUSAMA, S., KASAI, K., & KOBAYASHI, R. (1919). The leucocytozoores of the wild rat with special reference to its life-history. Kitasato Archs exp. Med., 3, 103.
- LADDA, R. & LALLI, F. (1966). The course of Plasmodium berghei infection in the polycythemic mouse. J. Parasit., 52, 383.
- LANDAU, I. (1965). Description de Plasmodium chabaudi n. sp., parasite de rongeurs africains. C.r. hebd. Séanc. Acad. Sci., Paris, 260, 3758.
- , ADAM, J.P. & BOULARD, Y. (1969). A malaria parasite of the brush-tailed porcupine in Calomys and white mice. Trans. R. Soc. trop. Med. Hyg., 63, 8.
- , BOULARD, Y., & HOUIN, R. (1969). Anthemiosoma garnhami n.g. n.sp., premier Dactylosomidae connu chez un mammifère. C.r. hebd. Séanc. Acad. Sci., Paris, 268, 373.
- , & CHABAUD, A.G. (1965). Infection naturelle par deux Plasmodium du rongeur Thamnomys rutilans en République Centrafricaine. Ibid., 261, 230.
- , ----- (1968). Schizogonie hépatique secondaire dans le paludisme spontané des rongeurs. Ibid., 266, 1730.
- , -----, ADAM, J.P., MICHEL, J.C. & BOULARD, Y. (1968). Morphologie et évolution des schizontes hépatiques secondaires dans le paludisme spontané des rongeurs de la Maboké. Cahiers de la Maboké, Paris, 6, 63.
- , & KILLICK-KENDRICK, R. (1966a). Note préliminaire sur le cycle évolutif des deux Plasmodium du rongeur Thamnomys rutilans de la République Centrafricaine. C. R. hebd. Séanc. Acad. Sci., Paris, 262, 1113.
- , -----, (1966b). Rodent plasmodia of the République Centrafricaine: the sporogony and tissue stages of Plasmodium chabaudi and P. berghei yoelii. Trans. R. Soc. trop. Med. Hyg., 60, 633.

- LANDAU, I., MICHEL, J.C. & ADAM, J.P. (1967). The growth of exoerythrocytic schizonts of a malaria parasite in the liver of a naturally infected rodent Ibid., 61, 7.
- , -----, -----, (1968). Cycle biologique au laboratoire de Plasmodium berghei killicki n. sub.sp. Annls Parasit. hum. comp., 43, 545.
- , & colleagues (in press). The life cycle of Plasmodium vinckei lentum n. sub.sp. in the laboratory; comments on the nomenclature of the murine malaria parasites. Ann. trop. Med. Parasit.
- LANGUILLON, J. (1957). Note sur deux parasites sanguicoles de mammifères du Cameroun. Bull. Soc. Path. exot., 50, 362.
- LATYSHEV, N.I. (1949). Some parasitological discoveries among animals of the Murgab River (Turkmenia) (in Russian). Vopr. Kraev. Obsh. i Ekper. Parazitol, 4, 83. (quoted from Clark, 1958).
- LAVERAN, A. (1905). Sur une hémogregarine des gerboises. C.r. hebdom. Séanc. Acad. Sci., Paris. 141, 294.
- (1905). Haemocytozoa. Essai de classification. Bull. Inst. Pasteur, 3, 809.
- LAVIER, G. (1921). Hémogrégaires, Grahamella, spirochète et trypanosome du campagnol indigène Microtus arvalis Pallas. Bull. Soc. Path. exot., 14, 569.
- LEGER, M. (1922). Parasites sanguicoles des animaux de l'A.O.F. Leur dénombrement et classification. Bull. Com. Etudes Hist. Sci. A.O.F., 5, 348.
- & BEDIER, E. (1922). Plasmodium du lérot, Myoxus murinus. C. r. Séanc. Soc. Biol., 87, 1336.
- , ----- (1923). Piroplasme d'un muride, Golunda campanae, du Sénégal. Ibid., 88, 424.
- LEVADITI, C. & SCHOEN, R. (1932). Sur un parasite sporulé du singe. Ibid., 109, 343.
- LIEN, J.C., & CROSS, J.H. (1968). Plasmodium (Vinckeia) watteni sp. n. from the Formosan flying squirrel, Petaurista petaurista grandis. J. Parasit., 54, 1171

- LUMSDEN, W.H.R., CUNNINGHAM, M.P., WEBBER, W.A.F., van HOEVE, K., & WALKER, P.J. (1963). A method for the measurement of the infectivity of trypanosome suspensions. Expl Parasit., 14, 269.
- MACFIE, J.W.S. (1914). Notes on some blood parasites collected in Nigeria. Ann. trop. Med. Parasit., 8, 439.
- (1915). Babesiasis and trypanosomiasis at Accra, Gold Coast, West Africa. Ibid., 9, 457.
- MANWELL, D. & KUNTZ, R.E. (1966). Hepatozoon in Formosan mammals with a description of a new species. J. Protozool., 13, 670.
- MARCHIAFAVA, E. & CELLI, A. (1885). Weitere Untersuchungen über die Malariainfektion. Fortschr. Med., 3, 787.
- MARTOGLIO, F. (1913). Contributo alla conoscenza delle leucocytogregarine. Annali Ig. sper., 23, 161.
- MAYR, E. (1963). Animal species and evolution. Oxford Univ. Press.
- LINSLEY, E.G. & USINGER, R.L. (1953). Methods and principles of systematic zoology. McGraw-Hill, N.Y.
- MILLER, W.W. (1908). Hepatozoon perniciosum (n.g., n.sp.): a haemogregarine pathogenic for white rats: with a description of the sexual cycle in the intermediate host, a mite (Laelaps echidnius). Bull. No. 46, Hyg. Lab. Washington.
- MIYAIRI, K. (1934). Zur Entwicklungsgeschichte einer Haemogregarine (richtiger Karyolysus) aus der Feldmaus Arvicola hatanezumii Sasaki. Proc. Imp. Acad. Tokyo, 8, 328.
- MOLINARI, V., (1961). The action of low temperatures on Plasmodia. J. trop. Med. Hyg., 64, 225.
- MOLYNEUX, D.H. (1969). The morphology and life-history of Trypanosoma (Herpetosoma) microti of the field-vole, Microtus agrestis. Ann. trop. Med. Parasit., 63, 229.

- MOREAU, R.E. (1963). Vicissitudes of the African Biomes in the late Pleistocene. Proc. Zool. Soc. London. 141, 395.
- MORGAN, J.F., MORTON, H.J. & PARKER, R.C. (1950). Nutrition of animal cells in tissue culture. 1. Initial studies on a synthetic medium. Proc. Soc. exp. Biol. Med., 16, 113.
- MOUCHET, J., GARIOU, J. & RIVOLA, E. (1957). Observations sur la biologie d'Anopheles smithi var rageauli Mattingly et Adam, 1954. Vecteur d'un Plasmodium de mammifères aux environs de Yaoundé (Sud Cameroun). Bull. Soc. Path. exot., 50, 157.
- MULLIGAN, H.W., & SOMERVILLE, T. (1947). Malaria in the Malabar squirrel. Indian J. Malar., 1, 329.
- NAJERA ANGULO, L. (1937). Sur le parasitisme de Trypanosoma blanchardi et Hepatozoon lusitanicus n.sp. en Espagne. 12th Congress on International Zoology, Lisbon, 1935, 31, 1921.
- ^C
NAUK, E.G. (1927). Ueber Befund in Blut splenektomierter Nager. Arch. Schiffs-Tropenhyg., 31, 322.
- NICOLLE, C. (1907). Sur un piroplasmose nouvelle d'un rongeur. C. r. Séanc. Soc. Biol., 63, 213.
- NIETZ, W.O. (1956). Classification, transmission, and biology of piroplasms of domestic animals. Ann. N.Y. Acad. Sci., 64, 56.
- (1957). Genus I. Anaplasma Theiler, 1910. In Bergey's manual of determinative bacteriology, ed. by R.S. Breed, E.G.D. Murray, and N.R. Smith, 7th ed., Williams and Wilkins, Baltimore, 981-984.
- NÖLLER, W. (1922). Die Blutprotozoen des Hamsters (Cricetus frumentarius Pall.) und ihre Übertragung. (Vorl. Mitt.). Arch. Protistenk., 25, 377.
- NUTTALL, G.H.F. & GRAHAM-SMITH, G.S. (1908). The mode of multiplication of Piroplasma bovis and P. pitheci in the circulating blood compared with that of P. canis, with notes on other species of Piroplasma. Parasitology, 1, 134.

- OXBROW, A.I. (1969). Some growth characteristics of Plasmodium berghei strains. Trans. R. Soc. trop. Med., 63, 6.
- PATTON, W.S. (1906). On a parasite found in the white corpuscles of the blood of palm squirrels. Sci. Mem. Off. Med. and San. Dept. Govt. India, 24, 13 pp. (quoted from Clark, 1958).
- van PEENEN, P.F.D., HOOGSTRAAL, H., DUNCAN, J.F. & RYAN P.F. (1968). Hematozoa from mammals of South Vietnam. J. Protozool., 15, 608.
- , & DUNCAN, J.F. (1968). Piroplasms (Protozoa: Sarcodina) of wild mammals in California. Bull. Wildlife Diseases Assoc., 4, 3.
- PEREZ-ALVA, S., & GIUNTINI, J. (1957). Etude morphologique de Bartonella bacilliformis au microscope électronique. Bull. Soc. Path. exot., 50, 188.
- PETERS, D. & WIGAND, R. (1955). Bartonellaceae. Bact. Rev. 19, 150.
- PETERS, W. (1963a). The chemotherapy of rodent malaria, 1. Host-parasite relationships, part 1: The virulence of infection in relation to drug resistance and time elapsed since isolation of the "wild" strain. Ann. trop. Med. Parasit., 62, 238.
- (1963b). The chemotherapy of rodent malaria, 11. Host-parasite relationships, part 2: The relationship between chloroquine sensitivity and the age of the host cell. Ibid., 62, 246.
- PHILIP, C.B. (1957). Family IV. Anaplasmataceae Philip fam. nov. In Bergey's manual of determinative bacteriology, ed. by R.S. Breed, E.G.D. Murray, and N.R. Smith, 7th ed., Williams and Wilkins, Baltimore, 980.
- PORTER, A. (1908). Leucocytozoon musculi sp. n., a parasitic protozoon from the blood of white mice. Proc. zool. Soc. Lond., 2, 703.
- PRINGLE, G. (1960). Two new malaria parasites from East African vertebrates. Trans. R. Soc. trop. Med. Hyg., 54, 411.

- RAY, H.N. (1949). Exoerythrocytic schizogony in Plasmodium sp. in the Himalayan flying squirrel Petaurista inornatus (Geoffrey). A preliminary note. Proc. natn. Inst. Sci. India, 15, 241.
- RIOUX, J.A. & GOLVAN, Y.T. (1969). Epidémiologie des leishmanioses dans le sud de la France. Monograph 37 of l'Institut de la Santé et de la Recherche Médicale, Paris.
- RODHAIN, J. (1915). Quelques hématozoaires de petit mammifères de l'Uele (Ouélé) Congo belge. Bull. Soc. Path. exot., 8, 726.
- (1952). Un deuxième Plasmodium parasite de rongeur sauvages au Katanga. Annls Soc. belge Méd. trop., 32, 275.
- , PONS, C., VANDENBRANDEN, F. & BEQUAERT, J. (1913). Notes sur quelque hématozoaires du Congo belge. Arch. ProtistKde, 29, 259.
- ROMANA, C (1945). Infección de un Graomys medius Thomas pr diversos haemoparasitos. An. Inst. Med. reg., Tucumán, 1, 177.
- ROSEVEAR, D.R. (1950). Rodents of Nigeria. IV: True rats and mice. Niger. Fld., 15, 148.
- (1953). Checklist and atlas of Nigerian mammals, with a foreword on vegetation, Crown Agents for the Colonies, London.
- (1969). Rodents of West Africa, Publ. No. 677, British Museum, London.
- ROUSSELOT, R. (1953). Notes de parasitologie tropicale. Parasites du sang des animaux. Vigot Frères, Paris. (quoted from Bray, 1964).
- SANDOSHAN, A.A., YAP, L.F., & OMAR, I., (1965). A malaria parasite, Plasmodium (Vincelae) booliati sp. nov. from a Malayan giant flying squirrel. Med. J. Malaya, 20, 3.
- SANGIORGI, G. (1912). Leucocytogregarina musculi. Zentbl. Bakt. ParasitKde, 66, 287.

- SASSUCHIN, D. (1931). Grahamia-und Hepatozoon - Blutparasiten der Nager in Süd-Osten R.S.F.S.R. Arch. Protistenk., 74, 523.
- SCHWETZ, J. & COLLART, A. (1930). Notes on protozoa found in the blood of rats and mice in the district of Lake Albert. Trans. R. Soc. trop. Med. Hyg., 23, 529.
- SCOTT, H.H. (1927). Report on the deaths occurring in the Society's gardens during the year 1926. Proc. Zool. Soc. London, p. 173.
- SERGEANT, E. (1921). Haemogregarine (Hepatozoon) de l'écureuil de Gétulie. Archs Inst. Pasteur Afr. nord, 1, 56.
- SHORTT, H.E. & BLACKIE, E.J. (1965). An account of the genus Babesia as found in certain small mammals in Britain. J. trop. Med. Hyg., 68, 37.
- SHUTE, P.G. & MARYON, M. (1966). Laboratory technique for the study of malaria. 2nd ed., Churchill, London.
- SKRABALO, Z. & DEANOVIC, Z. (1957). Piroplasmosis in man. Documenta Med. geogr. trop., 9, 11.
- SCHOLTENS, R.G., BRAFF, E.H., HEALY, G.R., & GLEASON, N. (1969). A case of babesiosis in man in the United States. Am. J. trop. Med. Hyg., 17, 810.
- SIMPSON, G.G. (1945). The principles of classification and a classification of mammals. Bull. Am. Mus. nat. Hist., 85, 1.
- SOUTHERN, H.N. (1964). The handbook of British Mammals. Blackwell, Oxford.
- SPLENDRE, A. (1920). Sui parassiti delle arvicole. (2nd part). Annali Ig. sper., 30, 560.
- STAROVICI, C. (1893). Bemerkungen über den durch Babes entdeckten Blutparasiten und die durch denselben hervorgebrachten Krankheiten, die seuchenhafte Hämoglobinurie des Rindes (Babes), das Texasfieber (Th. Smith) und der Carceag der Schafe (Babes). Zentbl. Bakt. 1. Abt., 14, 1.

- TARTAKOWSKY, M. (1913). Katalogue der exponaten der Landwirtschaftlichen Ausstellung. Petersburg. (quoted from Hoogstraal, 1959).
- TOKURA, N., & WU, CHEN-LAN, (1961). A malaria parasite, Hepatocystes, of the flying squirrel in Taiwan. Endem. Dis. Bull. Nagasaki Univ., 3, 174
- TOMLINSON, T.H. Jr., SMITH, C.S., & FOGG, V.H. (1948). A piroplasm, Babesia wrighti, n. sp., from the rock squirrel (Citellus variegatus buckleyi). J. natn. Malar Soc., 7, 254.
- TYZZER, E.E. (1938). Cytoecetes microti n.g., n.sp., a parasite developing in granulocytes and infective for small rodents. Parasitology, 30, 242.
- (1939). Haemobartonella n.g. (Bartonella olim pro parte). H. microti n.sp. of the field vole Microtus pennsylvanicus. Am. J. Hyg., 30, 141.
- (1942). A comparative study of Grahamellae, Haemobartonellae and Eperythrozoo in small animals. Proc. Am. phil. Soc., 85, 359.
- VANDERBERG, J. & YOELI, M. (1965). Some physiological and metabolic problems related to the Plasmodium berghei cycle in Anopheles quadrimaculatus. Annls Soc. belge Méd. trop., 45, 419.
- VASSAL, J.J. (1905). Sur un hématozoaire endoglobulaire nouveau d'un mammifère. Annls Inst. Pasteur, Paris, 19, 224.
- (1907). Nouvelle contribution à l'étude de l'hématozoaire de l'écureuil (Haemomoeba vassali Lav) Ibid., 21, 851.
- VINCKE, I.H. (1954). Natural history of Plasmodium berghei. Indian J. Malar., 8, 245.
- (1967). The potential vectors of P. ohabaudi and P. b. yoelii. (correspondence) Trans. R. Soc. trop. Med. Hyg., 61, 154.
- , BAFORT, J. & SCHEEPERS-BIVA, M. (1966). Observations récentes sur la transmission cyclique du Plasmodium berghei. Annls Soc. belge Méd. trop., 46, 327.

- VINCKE, I.H. & van den SULCKE, M.A. (1949). Observations sur l'entretien de la souche K173 du Plasmodium berghei Vincke and Lips. Ibid., 29, 525.
- , & LIPS, M. (1948). Un nouveau Plasmodium d'un rongeur sauvage du Congo, Plasmodium berghei n. sp. Ibid., 28, 97.
- , ----- (1950). Note sur la transmission cyclique du Plasmodium berghei. Ibid., 30, 1605.
- WEINMAN, D. (1957). Genus II: Grahamella Brumpt, 1911, Genus III: Haemobartonella Tyzzer and Weinman, 1939. Genus IV: Eperythrozoon Schilling, 1928. In Bergey's manual of determinative bacteriology, ed. by R.S. Breed, E.G.D. Murray, and N.R. Smith, 7th ed., Williams and Wilkins, Baltimore, 971-980.
- WELLMAN, C. & WHERRY, W.B. (1910). Some new internal parasites of the California ground squirrel (Otospermophilus beecheyi). Parasitology, 3, 417.
- WENYON, C.M. (1909). Report of the travelling pathologist and protozoologist. 3rd Rept. Wellcome Trop. Res. Labs, Khartoum, 1908, p. 121.
- (1926). Protozoology, vols I and II, Ballière, Tindall and Cox, London.
- & LOW, G.C. (1914). The occurrence of certain structures in the erythrocytes of guinea-pigs and their relationship to the so-called parasite of yellow fever. J. trop. Med. Hyg., 17, 369.
- WÉRY, M. (1962). Studies on the sporogony of rodent malaria parasites. Annls Soc. belge Méd. trop., 48, 1
- , KILLICK-KENDRICK, R., & BRAY, R.S. (1963). Sporozoite-induced infections of Plasmodium berghei yoelii in rabbits and a guinea-pig. Trans. R. Soc. trop. Med. Hyg., 62, 1.
- WHARTON, R.H., EYLES, D.E., WARREN, M., MOORHOUSE, D.E. & SANDOSHAM, A. A. (1963). Investigations leading to the identification of the Anopheles umbrosus group as probable vectors of mouse deer malaria. Bull. Wild Hlth Org., 29, 357.

- WOLBACH, S.B. (1911). The use of colophonium in differentiating the eosin-methylene-blue and other stains. J. Am. med. Ass., 56, 345.
- WU, C.L. (1953). Contributions to the study on the mammalian malaria in the Formosa (Taiwan). Acta med. Fukuoka, 23, 1979.
- YAKIMOFF, W.L. & SAPHRONOWITSCH, R.A. (1917). Theileria chez le campagnol. Bull. Soc. Path. exot., 10, 99.
- & SCHOKHOR, N.J. (1917) Leucocytozoores musculi A. Porter à Petrograd. Ibid., 10, 100.
- YOELI, M. (1965). Studies on Plasmodium berghei in Nature and under experimental conditions. Trans. R. Soc. trop. Med. Hyg., 59, 255.
- , ALGER, N. & MOST, H. (1963). Tree rat, Thamnomys surdaster surdaster, in laboratory research. Science, N.Y., 142, 1585
- , MOST, H. & BONE, G. (1964). Plasmodium berghei: cyclical transmissions by experimentally infected Anopheles quadrimaculatus. Ibid., 144, 1580.
- , -----, ----- (1965). The natural history of Plasmodium berghei in the field and under experimental conditions. Annls Soc. belge Med. trop., 45, 267.
- , UPMANIS, R.S., VANDERBERG, J., & MOST, H. (1966). Life cycle and patterns of development of P. berghei in normal and experimental hosts. Milit. Med. Suppl. 131, 900.
- , VANDERBERG, J. NAWROT, R., & MOST H. (1965b). Studies on sporozoite-induced infections of rodent malaria. II: Anopheles stephensi as an experimental vector of Plasmodium berghei. Am. J. trop. Med. Hyg., 14, 927.

APPENDIXSporozoa in the blood of RodentsINTRODUCTION

The protozoa parasitising the erythrocytes or leucocytes of rodents lie in four of the families of the Sporozoa, namely Hepatozoidae and Plasmodiidae (Telosporea; Eucoccida), and Babesiidae and Dactylosomidae (Piroplasmae; Piroplasmida).

Hepatozoidae includes the genus Hepatozoon Miller, 1908, a widespread parasite of the leucocytes, or in a few instances the erythrocytes, of rodents. The only stage in the blood is the characteristic banana-shaped gametocyte. These haemogregarines have been given a number of generic names, but in the check-list below they are all referred to the single genus Hepatozoon. In reptiles and amphibia, which are hosts of several different haemogregarines, it is usually difficult to be certain of the genus unless the life-cycle is known, but, except for differences in size and host-cell, the gametocytes of the haemogregarines of rodents look very much alike, and the few life-cycles which have been seen (H. muris, H. pitymusi, H. balfouri, H. griseldiuri and H. erhardovae) are typical of the genus Hepatozoon. At the moment, therefore, there is no reason to suppose the haemogregarines of rodents are represented by more than this one genus.

Plasmodiidae contains two genera of malaria parasites of rodents, namely Plasmodium Marchiafava and Celli, 1885, and Hepatocystis Levaditi and Schoen, 1932 emend. Garnham, 1948. Both are erythrocytic parasites which produce malaria pigment.

The true malaria parasites (Plasmodium) of rodents form part of the subgenus Vinckeia Garnham, 1964, and the two species found in murine rodents of Africa are subdivided into a number of subspecies based largely on differences in the sporogonic and exoerythrocytic stages (Landau and Killick-Kendrick, 1966a; Garnham et al., 1967; Bafort, 1968; Landau et.al., 1968 and in press)

Among rodents, Hepatocystis is found ~~almost~~ exclusively in Asian squirrels. The commoner of the two known species, H. vassali (Laveran 1905), is divided into four subspecies based upon geographical distribution and minor morphological differences of the gametocytes in the blood of the vertebrate host (Garnham, 1966).

Babesiidae includes the piroplasms of rodents, which are here all placed in the only genus in the family recognised by Nietz (1956), Babesia Starovici, 1893. The identity of the piroplasms is as confused as that of the haemogregarines, and for the same reason; too few life-cycles have been seen. A revision of the family will no doubt become necessary when more complete life-cycles are known

(Nietz, 1956). The confusion extends to the higher taxa. In the present work, Piroplasmae is considered as a class of the Sporozoa because of new observations on the fine structure of piroplasms, which are now known to have several organelles typical of the subphylum (Büttner, 1968; Friedhoff and Scholtyseck, 1968)

In light infections in rodents, piroplasms are easily mistaken for malaria parasites. Both inhabit erythrocytes, and young rings of Plasmodium, in which no pigment is yet visible, are so similar to Babesia that misidentifications are understandably common. The true schizogony of Plasmodium in the blood is, however, absent in Babesia, and pigment is not produced by the piroplasm.

A new genus was recently created to accommodate an unusual parasite of the spiny mouse (Acomys). When first encountered, it was thought to be a piroplasm, but it was seen to undergo a quite different kind of multiplication in the erythrocytes of the host similar to the schizogony of Plasmodium; pigment was, however, completely absent. In parasitaemias heightened by splenectomy, stages thought to be gametocytes were found, and a resemblance to the dactylosomes of fishes and amphibia was immediately recognised. The new genus, Anthemosoma Landau, Boulard and Houin, 1969, was therefore placed in the family Dactylosomidae (Piroplasmae; Piroplasmida). When the life-cycles of the dactylosomes

become known, it seems unlikely that they will remain grouped so closely to the piroplasms.

Apart from the true protozoa parasitising the blood cells of rodents, there are a number of other organisms which share the same habitat, and which are commonly met in studies on the protozoa. Bartonella Strong et al., 1915, Anaplasma Theiler, 1910, Grahamella Brumpt, 1911, Haemobartonella Tyzzer and Weinman, 1939 and Eperythrozoon Schilling, 1928 are parasites of the erythrocytes of a great number of different mammals including many rodents. Their true affinities long remained in doubt, but Bartonella is now recognised as a bacterium (Peters and Wigand, 1955; Perez-Alva and Giuntini, 1957) and the others as rickettsiae (Nietz, 1957; Philip, 1957; Weinman, 1957); records of these organisms are not included in the check-list. They are easily recognised in the Giemsa-stained blood films, although less easily assigned to a genus (see Tyzzer, 1942); they are seen singly or in groups on erythrocytes, and appear as small dots or rod-shaped bodies.

A similar parasite of the leucocytes of voles was described by Coles (1914), who found it in the white-blood cells of an English Microtus agrestis. It was later found in the neutrophils and eosinophils of M. pennsylvanicus in the U.S.A. and named Cytoecetes microti Tyzzer, 1938. For the moment, Cytoecetes is most sensibly grouped with the rickettsiae

of the red-blood cells of rodents and other mammals. Morphologically it is similar to Grahamella, a very common parasite of the erythrocytes of voles, and C. microti is perhaps this organism in an unusual host-cell.

In the preparation of the check-list the exact identity of some of the rodents has given great difficulty. Whenever possible an attempt has been made to correct obvious errors, such as records from rodents totally unknown in the given locality. In a few reports, however, the name listed is little more than an informed guess. The names of some of the rodents have been changed over the period covered; as far as possible the generally accepted modern name has been substituted. Subspecies have largely been omitted because in so many groups the authorities are not in agreement. Specific names are those used by Ellerman (1940, 1941) and Rosevear (1953, 1969). Ellerman's (1941) subgenera of African murines which he viewed as parts of Rattus and Mus are here considered to be separate genera (Rosevear, 1969). In the higher taxa, Simpson's (1945) classification has been followed. The common English names given in the list of rodents have been culled from many sources.

Great significance cannot be placed on the geographical distribution suggested by some of the collected records; in many instances this appears to reveal the distribution of protozoologists rather than that of the parasites themselves.

WORLD CHECK-LIST OF THE SPOROZOA IN THE BLOOD
OF RODENTS.

The numbers after the names of the hosts below refer to the rodents in the host-index in which common-names are given, questions of the identity of some of the hosts are discussed, and records from each rodent are gathered together.

Class Telosporea

Order Eucoccida

Fam. Hepatozoidae

1. Hepatozoon akodon (Carini and Maciel, 1915);
Akodon fuliginosus (41), São Paulo, Brazil.
2. Hepatozoon alactaguli Brumpt, 1946; Alactagulus
umilio (87), U.S.S.R. (Sassuchin, 1931).
3. Hepatozoon arvalis (Martoglio, 1913); rodent, Ethiopia.

Martoglio identified the host as Arvicola (=Microtus) arvalis, but since this vole does not occur in Africa, the identification was presumably a mistake. Brumpt (1946) suggested Martoglio's rodent was Arvicanthis abyssinicus (=A. niloticus), and his parasite the same as H. arvicanthis Schwetz and Collart, 1930.

Lavier (1921) reported a haemogregarine which he considered as H. arvalis in an undoubted Microtus arvalis collected in France. Brumpt (1946) recognised Lavier's parasite as new, and named it H. lavieri.

4. Hepatozoon arvicanthi Schwetz and Collart, 1930;
Arvicanthi niloticus (64), Democratic Republic of
the Congo.
5. Hepatozoon arvicolae Brumpt, 1946; Microtus montebelloi
(52) Japan (Miyairi, 193⁴~~2~~).
6. Hepatozoon balfouri (Laveran, 1905); Jaculus gordonii (84)
Sudan (Balfour, 1905, 1906); J. orientalis (85),
Tunisia (Laveran, 1905), Egypt (Hoogstraal, 1961);
J. jaculus (86) Sudan (Balfour 1905, 1906), Egypt
(Hoogstraal, 1961; Furman, 1966).
7. Hepatozoon citellicolum (Weillman and Wherry, 1910);
Citellus beecheyi (32), U.S.A.
8. Hepatozoon criceti Nöller, 1912; Cricetus cricetus (44)
Austria.
9. Hepatozoon cricetomys Brumpt, 1946; Cricetomys gambianus
(79) Bambili, Democratic Republic of the Congo
(Rodhain, 1915).
10. Hepatozoon dendromysi Brumpt, 1946; Dendromus ?insignis
(80) Tanzania (Kleine, 1910).
11. Hepatozoon epsteini Kakabadze and Sassuchin, 1969;
"gray rats", Sukhumi, e. shore of Black Sea, U.S.S.R.

From the description of this parasite, it seems
certain it is different from the widespread species,
H. muris, a parasite of Rattus spp.

12. Hepatozoon erhardovae Krampitz, 1964; Clethrionomys glareolus (45) England (Jacobs, 1953; Baker et al., 1963), Sweden (Tadros, unpublished), Czechoslovakia (Černa, 1957), W. Germany (Krampitz, 1964);
Clethrionomys rutilus (46), W. Germany (Krampitz, 1964).
13. Hepatozoon funambuli (Patton, 1906); Funambulus pennanti (7), India.
14. Hepatozoon gerbilli (Christophers, 1905); Tatera indica (55), India.
15. Hepatozoon getulum Sargent, 1921; Atlantoxerus getulus (28), Tunisia.
16. Hepatozoon griseisciuri Clark, 19⁵/₈; Sciurus carolinensis (1), Maryland, U.S.A. (Herman and Price, 1954; Clark, 1958)
17. Hepatozoon groamysi Brumpt, 1946; Graomys medius (42) Argentine (Romaña, 1945) Brumpt misspelt the generic name of the rodent "Groamys", and perpetuated the error in the specific name of the parasite.
18. Hepatozoon lavieri Brumpt, 1946; Microtus arvalis (50), France (Lavie, 1921), Czechoslovakia (Černa, 1957), Austria, (Krampitz, 1964).
19. Hepatozoon lusitanicum Nájera-Angulo, 1937; Eliomys quernicus (82) Spain.
20. Hepatozoon mereschkowskii Tartakowsky, 1913; Citellus guttatus (33), C. musicus (34), U.S.S.R.

21. Hepatozoon microti (Coles, 1914); Microtus agrestis (49)
England (Coles, 1914; Jacobs, 1953), Germany
(Krampitz, 1964), Czechoslovakia (Černa, 1957).
22. Hepatozoon muris (Balfour, 1905); Rattus norvegicus (68)
R. rattus (69),

As these rats have moved around the world in the company of man, their parasites have moved with them; early records were listed by Kusama et al., (1919), and more recently Krampitz (1964) published a map showing 39 localities in Europe, Asia, Australasia and N. and S. America from which H. muris has been reported in Rattus. The common identity of the haemogregarines of R. norvegicus and R. rattus has not been confirmed by transmission experiments in the laboratory.

23. Hepatozoon musculi (Porter, 1908); Mus musculus (76),
England (Porter, 1908), Italy (Sangiorgi, 1912),
U.S.S.R. (Yakimoff and Schokhor, 1917).
24. Hepatozoon pitymysi Splendore, 1920; Pitymys savii (48)
Italy (Splendore, 1920), Sicily (Krampitz 1957).

Lavier's (1921) suggestion that the haemogregarines of M. arvalis and P. savii were the same is not supported by recent work of Krampitz (1964), who demonstrated a marked host restriction of a haemogregarine of voles. Krampitz's work raises the interesting possibility that haemogregarines of voles

are similar to the large group of lewisii-like trypanosomes which are morphologically indistinguishable but which exhibit a strong host restriction (see Molyneux, 1969).

25. Hepatozoon plicatum (Martoglio, 1913); rodent ?, Ethiopia

The host of H. plicatum was identified as a marmot (Arctomys (= Marmota) marmota) a rodent which is absent from Africa. Brumpt (1946) suggested the host was a hyrax mistaken for a rodent.

26. Hepatozoon sciuri (Coles, 1914); Sciurus vulgaris (5) England (Coles, 1914), China (Nauck, 1927), Italy (Francini, 1932), central and southern Europe (Krampitz 1964).

27. Hepatozoon sylvatici (Coles 1914); Apodemus sylvaticus (60) England (Coles, 1914), W. Germany (Krampitz, 1964) France (Rioux and Golvan, 1969); Apodemus flavicollis (59) central and southern Europe (Krampitz, 1964).

28. Hepatozoon sp. Bruce-Chwatt and Gibson (unpublished); Hybomys trivirgatus (67), Agege, W. Nigeria.

29. Hepatozoon sp. Bruce-Chwatt and Gibson (unpublished); Praomys tullbergi (73) Ilobi, W. Nigeria.

30. Hepatozoon sp. Dasgupta, 1965; Petaurista magnificus (36), Darjeeling, India.

31. Hepatozoon sp. Kleine, 1910; Myiomys dybowski (63) Tanzania.

32. Hepatozoon sp. Krampitz, 1964; Microtus oeconomus (53), central and southern Europe.
33. Hepatozoon sp. Lainson (unpublished); Holochilus brasiliensis (43), Brazil.
34. Hepatozoon sp. Lainson (unpublished); Oryzomys capito (40), Brazil.
35. Hepatozoon sp. Landau (unpublished); Hybomys univittatus (66), Central African Republic.
36. Hepatozoon sp. Landau (unpublished); Xerus rutilus (30) Omo Valley, Ethiopia.
37. Hepatozoon sp. Latyshev, 1949; Meriones erythraurus (56) Murgab Valley, Turkmenia, U.S.S.R.
38. Hepatozoon sp. Latyshev, 1949; Spermophilopsis leptodactylus (31), Murgab Valley, Turkmenia, U.S.S.R.
39. Hepatozoon sp. (Léger, 1922); Xerus erythropus (29) Republic of Senegal.
40. Hepatozoon sp. Rodhain, Pons, Vandenbranden and Bequaert, 1913; rodent (see note in the host-index under Fam. Dipodidae), Sankisia, Democratic Republic of the Congo.
Fam. Plasmodiidae
41. Plasmodium anomaluri Pringle, 1960; Anomalurus fraseri (39) Chemka, Tanzania.
42. Plasmodium atheruri van den Berghe, Peel, Chardôme and Lambrecht, 1958, Atherurus africanus, (88) Brazzaville, Republic of Congo (Languillon, 1957; Landau et al.

- 1939), Icangi Forest, Democratic Republic of the Congo (van der Berghe et al., 1958).
43. Plasmodium berghei borghei Vincke and Lips, 1948;
Thamnomys surdaster (62), Praomys jacksoni (72),
Leggada bella (77), Katanga, Democratic Republic of
the Congo (Vincke and Lips, 1948; Vincke, 1954;
Yoeli et al., 1965; Bafort et al., 1968).
44. Plasmodium berghei killicki Landau, Michel and Adam, 1968;
Thamnomys rutilans (61), Brazzaville, Republic of the
Congo (Adam et al., 1966)
45. Plasmodium berghei yoelii Landau and Killick-Kendrick 1966,
Thamnomys rutilans (61), la Maboké, Central African
Republic (Landau and Chabaud, 1965).
46. Plasmodium berghei ssp. indet. Killick-Kendrick, Shute and
Lambo, 1968; Thamnomys rutilans (61), Ilobi, W.
Nigeria.
47. Plasmodium booliati Sandosham, Yap and Omar, 1965;
Petaurista petaurista (37), Malaysia (Sandosham et al.,
1965), possibly Taiwan (Garnham, 1966, p. 511).
48. Plasmodium vinckei chabaudi Landau, 1965; Thamnomys
rutilans (61), la Maboké, Central Africa Republic
(Landau, 1965; Landau and Chabaud, 1965).
- This parasite was first named P. chabaudi but later
placed as a subspecies of P. vinckei (Bafort, 1968).
49. Plasmodium vinckei ssp. indet. Adam, Landau and Chabaud,
1966; Thamnomys rutilans (61), Brazzaville, Republic
of Congo.

50. Plasmodium vinckei vinckei Rodhain, 1952; Thamnomys
surdaster (?) (62), Democratic Republic of the Congo.

P. v. vinckei has never been found in a rodent, and is known only by two isolations from the mosquito Anopheles durenii millescampsii (Rodhain, 1952; Safort, 1967). Since A. d. millescampsii appears to feed exclusively on rodents (Gilles and de Meillon, 1968), there is no doubt that the natural vertebrate host is a sylvatic rodent in the forest galleries of Katanga, and the most likely animal is T. surdaster.

51. Plasmodium vinckei ssp. indet. Bruce-Chwatt and Gibson, 1955; Praomys tullbergi (73), (Bruce-Chwatt and Gibson 1955; Thamnomys rutilans (61), (Killick-Kendrick et.al., 1968), Ilobi, W. Nigeria.

A third of the T. rutilans in this locality were found to be infected with P. vinckei ssp. whereas only one out of 1,038 Praomys was infected. This suggests either that the single infected Praomys was a misidentified Thamnomys, or that the discoverer stumbled upon a Praomys with a very rare accidental infection of a parasite of Thamnomys.

52. Plasmodium watteni Lien and Cross, 1968; Petaurista
petaurista grandis (38), Taiwan.

53. Hepatocystis rayi Tokura and Wu, 1961; Petaurista
inornatus (35), Mukteswar, India (Ray, 1949);
P. p. grandis (38), Taiwan (Tokura and Wu, 1961;

Manwell and Kuntz, 1966; Lien and Cross, 1968];
P. magnificus (36), Darjeeling, India (Dasgupta, 1965,
 1967)

A new monospecific genus, Rayella, Dasgupta, 1967, was created to accommodate this parasite of giant flying squirrels. The new genus was said to differ from Hepatocystis by the morphology of the tissue stages, which were described as groups of small cystic schizonts. Hepatocystis is divided into species largely on the basis of the morphology of the asexual stages in the tissues (Garnham, 1966), and it is doubtful if the separation of Rayella solely on this character will be generally accepted. Another recently reported difference, however, is the unusually long time necessary for the exflagellation of H. rayi (8 mins) (Dasgupta and Chatterjee, 1969) which contrasts sharply with the characteristically short time (2 mins) of other species of Hepatocystis (Garnham, 1966, p. 21). Until the complete life-cycle of H. rayi is known, it is perhaps best to regard Rayella as a synonym of Hepatocystis.

54. Hepatocystis vassali malayensis Field and Edeson, 1950;
Sciurus vittatus (4) (Green, 1932); Callosciurus
notatus (22), C. tenuis (24) C. nigrovittatus (21),
C. caniceps (12) (Field and Edeson, 1950); Ratufa ssp

(11) Callosciurus spp. (26) (Wharton et al., 1965);
Malaysia.

55. Hepatocystis vassali ratufao (Donovan, 1920); Ratufa indica (9) India (Donovan, 1920); R. bicolor gigantea (8), Thailand (Coatney et al., 1960); R. macroura (10) Ceylon (Dissanaike, 1963).
56. Hepatocystis vassali vassali (Laveran, 1905); Callosciurus vittatus (25) (Vassal, 1905); Sciurus griseimanus (2) (Laveran, 1905; Vassal, 1905, 1907), Sciurus sp. indet. (6) (Vassal, 1907); C. flavimanus (18), (van ^PHeenen et al., 1968), Vietnam; C. maclellandii kongoensis (19), C. finlaysoni (17), Thailand (Coatney et al., 1960).
57. Hepatocystis vassali yokogawai Wu, 1953; Callosciurus erythraeus roberti (14), C. e. centralis (13), (Wu, 1953; Manwell and Kuntz, 1966), C. e. taiwanensis (15), C. maclellandii ssp. indet (20) (Wu, 1953), C. erythraeus ssp. indet. (16), (Manwell and Kuntz 1966), Taiwan.
58. Hepatocystis vassali ssp. indet; Callosciurus prevosti (23), S. stramineus grayanus (3), London Zoo (Scott, 1927; Hamerton, 1930, 1931, 1941).
59. Hepatocystis sp. indet. Dunn, Lim and Yap, 1968; Rhizomys sumatrensis (57), Lariscus insignis (27), Malaysia.

Lariscus is a squirrel, and the rare infections reported by Dunn et al., (1968) may be H. v. malayensis in a new host. Rhizomys is myomorph, and it is unlikely that the species of Hepatocystis found in this animal is the same as that of Lariscus.

Class Piroplasmoea

Order Piroplasmida

Fam. Babesiidae

60. Babesia arvicularis Wenyon, 1909; Lemniscomys barbarus (65) Sudan.
61. Babesia cobayae (Macfie, 1914); Cavia porcellus (89), Nigeria.

This parasite, first named Paraplasma cobayae, was found in laboratory guinea-pigs. The illustrations in Macfie's (1914) paper show a piroplasm-like body, with a red nucleus and compact blue cytoplasm, lying in a stippled erythrocyte. The lack of further records in such a commonly examined animal is puzzling. In the same paper, Macfie describes Paraplasma flavigenum from the same host. At the time, there was much interest in this so-called parasite because it had been claimed it was the causative organism of yellow fever. Wenyon and Low (1914) showed that these very tiny bodies were not true parasites, and caused no disease. While

it is certain that P. flavigenum is not a piroplasm, the description of P. cobayae is sufficiently convincing for it to be included here. The stippling of the infected cell is a most unusual feature for a piroplasm.

62. Babesia decurnai (Macfie, 1915); Rattus ⁿnorvegicus (68)
Ghana.
63. Babesia golundae (Léger and Bédier, 1923); Myiomys
dybowski (63), Senegal.
64. Babesia microti (França, 1912); Microtus incertus (51)
Portugal (França, 1912); Arvicola amphibius (47),
(Coles, 1914); Microtus agrestis (49), (Coles, 1914;
Baker et.al., 1963; Shortt and Blackie, 1965),
Apodemus sylvaticus (60) (Coles, 1914; Jacobs, 1953;
Shortt and Blackie, 1965), Clethrionomys glareolus
(45) (Jacobs, 1953; Shortt and Blackie, 1965),
England.

This parasite was also named Smithia microtia França, 1912, Nuttalia muris, Coles, 1914, and Nuttalia microti, Coles, 1914. In a laboratory study of the course of infection of strains from several English hosts, Shortt and Blackie (1965) found no differences in infectivity or morphology. There is thus good evidence that the piroplasm of small rodents in England is represented by the single species B. microti.

65. Babesia muris (Fantham, 1906); Rattus norvegicus (68) 210
England.

This parasite was seen in three laboratory white rats. It seems possible that the infection was acquired from arthropods in the bedding of the cage, and the parasite may have been Babesia microti, the common piroplasm of British small mammals. If this were so, the name muris would have priority over microti.

66. Babesia myoxi (Franchini, 1924); Muscardinus avellanarius
(81), Italy.

67. Babesia quadrigemina (Nicolle, 1907); Ctenodactylus gundi
(91), Tunisia.

The genus Nicolliia Nuttal, 1908 was created to accommodate this species. Nuttal and Graham-Smith (1908) compared its division in the blood with that of other piroplasms, and concluded that it was correctly placed in a new genus. Wenyon (1926) did not agree and, more recently, Nietz (1956) listed Nicolliia as a synonym of Babesia.

68. Babesia ratti Schewtz and Collart, 1930; Arvicanthis niloticus (64), Mastomys coucha (71), Democratic Republic of the Congo.

The discoverers of this parasite thought it was possibly the same as B. decumani Macfie, 1915 and B. avicularis Wenyon, 1909.

69. Babesia rigolletti (Léger and Bédier, 1922); Graphiurus murinus (83), Senegal.

B. rigolletti was first named as a Plasmodium.
Wenyon (1926) noted the lack of pigment, and Bray
(1964) referred it to the genus Babesia.

70. Babesia rodhaini van den Berghe, Vincke, Chardôme and van den Bücke, 1950. Thamnomys surdaster (62), Katanga, Democratic Republic of the Congo.

71. Babesia rossica (Yakimoff and Saphronowitch, 1917); voles, (54), Saratow and Transcaucasia, U.S.S.R.

This was first described as Theileria, but there was no real evidence it belonged to this genus (Wenyon, 1926).

72. Babesia taterilli (Rousselot, 1953); Taterillus gracilis (56), Republic of Mali.

73. Babesia sp. Adam and Landau (unpublished); Praomys jacksoni (72) Brazzaville, Republic of Congo.

74. Babesia sp. Adam and Landau (unpublished); Thryonomys swinderianus (90), Brazzaville, Republic of Congo.

75. Babesia sp. Bafort, 1969; Hylomyscus sp. indet. (75), Kinshasa, Katanga, Democratic Republic of Congo.

76. Babesia sp. Bray, 1964; Thryonomys swinderianus (90) Liberia.

77. Babesia sp. Bruce, Harvey, Hamerton, Davey and Bruce, 1915; "edible rat", (see 79) Malawi.

78. Babesia sp. Bruce-Chwatt and Gibson, 1955; Thryonomys swinderianus (90). Ilobi and Agege, W. Nigeria.

The piroplasm in Nigerian Thryonomys differs from the one found in the same host in Liberia (Bray 1964).

79. Babesia sp. Bruce-Chwatt and Gibson, 1955; Praomys tullbergi (73), Ilobi and Agege, W. Nigeria.
80. Babesia sp. Bruce-Chwatt and Gibson (unpublished); Stochomys longicaudatus (74), Agege, W. Nigeria.
81. Babesia sp. Bruce-Chwatt and Gibson (unpublished); Cricetomys gambianus (79) Agege, W. Nigeria.
82. Babesia sp. Gunders (Bray, 1964); Praomys tullbergi (73) Liberia.
83. Babesia sp. Landau (unpublished); Praomys jacksoni (72) Central African Republic.

It seems likely that the piroplasms of Praomys in Nigeria, Liberia, Brazzaville and the Central African Republic are all one species.

84. Babesia sp. Landau (unpublished); Hybomys univittatus (66) Central African Republic.

Fam. Dactylosomidae

85. Anthemosoma garnhami Landau, Boulard and Houin, 1969; Acomys percivali (78), Omo Valley, Ethiopia.

TABLE 10.

Genera of rodents from which blood-inhabiting Sporozoa have been recorded	<u>Hepatozoon</u>	<u>Hepatocystis</u>	<u>Plasmodium</u>	<u>Babesia</u>
SCHIUROMORPHA	7	5	2	1
Sciuridae	7	5	1	1
Sciurinae	6	4		1
Sciurini	1	1		
<u>Sciurus</u>	*	*		
Funambulini	1	1		
<u>Funambulus</u>	*			
<u>Ratufa</u>		*		
Callosciurini		2		
<u>Callosciurus</u>		*		
<u>Lariscus</u>		*		
Xerini	4			1
<u>Atlantoxerus</u>	*			
<u>Xerus</u>	*			
<u>Spermophilopsis</u>	*			
<u>Citellus</u>	*			*
Petauristinae	1	1	1	
<u>Petaurista</u>	*	*	*	

	<u>Hepatozoon</u>	<u>Hepatocystis</u>	<u>Plasmodium</u>	<u>Babesia</u>
Anomaluridae			1	
<u>Anomalurus</u>			*	
MYOMORPHA	22	1?	4	17
Cricetidae	10			6
Cricetinae	8			5
Hesperomyini	4			2
<u>Oryzomys</u>	*			
<u>Peromyscus</u>				*
<u>Akodon</u>	*			
<u>Graomys</u>	*			
<u>Holoshilus</u>	*			
<u>Neotoma</u>				*
Cricetini	1			
<u>Cricetus</u>	*			
Microtini	3			3
<u>Clethrionomys</u>	*			*
<u>Arvicola</u>				*
<u>Pitymys</u>	*			
<u>Microtus</u>	*			*

	<u>Hepatozoon</u>	<u>Hepatocystis</u>	<u>Plasmodium</u>	<u>Babesia</u>
Gerbillinae	2			1
<u>Tatera</u>	*			
<u>Taterillus</u>				*
<u>Meriones</u>	*			
Rhizomyidae		1?		
<u>Rhizomys</u>		*?		
Muridae	9		3	11
Murinae	8		3	11
<u>Apodemus</u>	*			*
<u>Thamnomys</u>			*	*
<u>Mylomys</u>	*			*
<u>Arvicanthis</u>	*			
<u>Lemniscomys</u>				*
<u>Hybomys</u>	*			*
<u>Rattus</u>	*			*
<u>Mastomys</u>				*
<u>Praomys</u>	*		*	*

	<u>Napatozoon</u>	<u>Hepatocystis</u>	<u>Plasmodium</u>	<u>Babesia</u>
<u>Stochoomys</u>				*
<u>Hylomyscus</u>				*
<u>Mus</u>	*			
<u>Leggadia</u>			*	
<u>Acomys</u> Ø				
<u>Cricetomys</u>	*			*
Dendromurinae	1			
<u>Dendromus</u>	*			
Gliridae	1			2
Glirinae	1			1
<u>Muscardinius</u>				*
<u>Eliomys</u>	*			
Graphiurinae				1
<u>Graphiurus</u>				*
Dipodae	2			
Dipodinae	2			
<u>Jaculus</u>	*			
<u>Alactagulus</u>	*			
Ø only known host of <u>Anthemiosoma</u>				

	<u>Hepatozoon</u>	<u>Hepatocystis</u>	<u>Plasmodium</u>	<u>Babesia</u>
HYSTRICOMORPHA			1	2
Hystriidae			1	
Atherurinae			1	
<u>Atherurus</u>			*	
Caviidae				1?
Cavinae				
<u>Cavia</u>				*?
Thryonomidae				1
<u>Thryonomys</u>				*
Incertae cedis				1
<u>Ctenodactylus</u>				*
TOTAL NUMBER OF GENERA:	29	6	6	23

SPOROZOA

The numbers after the names of the parasites refer to the check-list in which the identity of some of the parasites is discussed, and the localities and sources are given.

SCIUROMORPHA

Fam. Sciuridae (squirrels)

Subfam. Sciurinae (ground and tree squirrels)

Tribe Sciurini (European and American tree squirrels)

1. Sciurus carolinensis (grey squirrel)

Hepatozoon griseisciuri (16) W*

2. Sciurus griseimanus

Hepatocystis vassali vassali (56)

3. Sciurus stramineus grayanus

Hepatocystis vassali ssp. indet. (58)

4. Sciurus vittatus (red squirrel)

Hepatocystis vassali malayensis (54)

5. Sciurus vulgaris (red squirrel)

Hepatozoon sciuri W (26)

6. Sciurus sp. indet.

Hepatocystis vassali vassali (56)

Tribe Funambulini (African tree squirrels and allied Asian genera)

7. Funambulus pennanti (palm squirrel)

Hepatozoon funambuli W. (13)

* In white blood cells.

8. Ratufa bicolor gigantea (giant squirrel)

Hepatocystis vassali ratufae (55)

9. Ratufa indica

Hepatocystis vassali ratufae (55)

10. Ratufa macroura

Hepatocystis vassali ratufae (55)

11. Ratufa spp.

Hepatocystis vassali malayensis (54)

Tribe Callosciurini (Oriental tree squirrels)

Ellerman (1940) recognised 320 forms of

Callosciurus, the largest genus of this tribe.

Recognition is difficult, and it is quite possible that in some early records the host was wrongly identified.

12. Callosciurus caniceps (forest squirrel)

Hepatocystis vassali malayensis (54)

13. Callosciurus erythraeus centralis (Central Formosan red-bellied squirrel) Hepatocystis vassali yokogawai (57)

14. Callosciurus erythraeus roberti (North Formosan red-bellied squirrel) Hepatocystis vassali yokogawai (57)

15. Callosciurus erythraeus taiwanensis

Hepatocystis vassali yokogawai (57)

16. Callosciurus erythraeus ssp.indet.

Hepatocystis vassali yokogawai (57)

17. Callosciurus finlaysoni (tree squirrel)
Hepatocystis vassali vassali (56)
18. Callosciurus flavimanus
Hepatocystis vassali vassali (56)
19. Callosciurus maclellandii kongoensis (striped tree squirrel)
Hepatocystis vassali vassali (56)
20. Callosciurus maclellandii ssp. indet.
Hepatocystis vassali yokogawai (57)
21. Callosciurus nigrovittatus (black-striped squirrel)
Hepatocystis vassali malayensis (54)
22. Callosciurus notatus (common Malay plantain squirrel)
Hepatocystis vassali malayensis (54)
23. Callosciurus prevosti
Hepatocystis vassali ssp. indet. (58)
24. Callosciurus tenuis (slender squirrel)
Hepatocystis vassali malayensis (54)
25. Callosciurus vittatus (striped squirrel)
Hepatocystis vassali vassali (56)
26. Callosciurus spp.
Hepatocystis vassali malayensis (54)
27. Lariscus insignis (tree squirrel)
Hepatocystis sp. indet. (59)
This is probably H. v. malayensis (54)

Tribe Xerini (African ground squirrels)

- 28.
- Atlanoxerus getulus
- (Barbary ground squirrel)

Hepatozoon getulum (15)

- 29.
- Xerus erythropus
- (red-legged ground squirrel)

Hepatozoon sp. Léger, 1922 (3)

- 30.
- Xerus rutilus

Hepatozoon sp. Landau (36)

Tribe Xerini incertae sedis

- 31.
- Spermophilopsis leptodactylus
- (ground squirrel)

Hepatozoon sp. Latyshev, 1949 (38)

- 32.
- Citellus beecheyi
- (gopher)

Hepatozoon citellicolum (7)

- 33.
- Citellus guttatus
- (ground squirrel)

Hepatozoon mereschkowskii W. (20)

- 34.
- Citellus musicus
- (ground squirrel)

Hepatozoon mereschkowskii W. (20)

Subfam. Petauristinae (flying squirrels)

- 35.
- Petaurista inornatus
- (flying squirrel)

Hepatocystis rayi (53)

- 36.
- Petaurista magnificus
- (Himalayan flying squirrel)

Hepatocystis rayi (53)Hepatozoon sp. Dasgupta, 1965 W, (30)

- 37.
- Petaurista petaurista
- (giant flying squirrel)

Plasmodium booliati (47)

38. Petaurista petaurista grandisHepatocystis rayi (53)Plasmodium watteni (52)SCIUROMORPHA incertae sedis

Fam. Anomaluridae

Subfam. Anomalurinae

39. Anomalurus fraseri (scaly tailed squirrel)Plasmodium anomaluri (41)

This parasite, or a new species of Plasmodium or Hepatocystis may occur in West Africa. The writer found undoubted malaria pigment in sections of enlarged spleens of two Anomalurus peli and a smaller flying squirrel, possibly Anomalurops beecrofti, collected by Mr. P. Hunkeler of the Centre Suisse de Recherches Scientifique in the Ivory Coast; the collections were made at Bolo, 50 km. from Gallandra.

MYOMORPHA

Fam. Cricetidae

Subfam. Cricetinae

Tribe Hespermyini (New World mice)

40. Oryzomys capito (rice rat)Hepatozoon sp. Lainson W. (39)41. Akodon fuliginosus (vole mouse)Hepatozoon akodoni W. (1)

42. Graomys mediusHepatozoon groamysi (17)

The specific name of this haemogregarine is from an erroneous spelling of Graomys (Brumpt, 1946)

43. Holochilus brasiliensisHepatozoon sp. Lainson W. (33)

Tribe Cricentini (hamsters)

44. Cricetus cricetusHepatozoon criceti W. (8)

C. frumentarius, the name of the host of H. criceti, in the original record, is a synonym of C. cricetus (Ellerman, 1941)

Tribe Microtini (voles)

45. Clethrionomys glareolus (bank vole)Hepatozoon erhardovae W. (12)Babesia microti (64)46. Clethrionomys rutilusHepatozoon erhardovae W. (12)47. Arvicola amphibius (water vole)Babesia microti (64)

Southern (1964) discusses the naming of the four European water voles.

48. Pitymys savii (pine mouse)Hepatozoon pitymysi W. (24)

49. Microtus agrestis (short-tailed field vole)Hepatozoon microti W. (21)Babesia microti50. Microtus arvalisHepatozoon lavieri W. (13)

Another species of Hepatozoon, H. arvalis (3) was reported from an Ethiopian rodent wrongly identified as M. arvalis (Martoglio, 1913). The true identity of the host will never now be known with certainty, but it was possibly Arvicanthis niloticus (syn. A. abyssinicus), (Brumpt, 1946).

51. Microtus incertusBabesia microti (64)52. Microtus montebelloiHepatozoon arvicolae (5)

The host of this parasite found by Miyairi (1932) in Japan was given as Arvicola hatengumi. Arvicola is absent from Japan (Ellerman, 1941), and no reference to the specific epithet "hatengumi" appears in Ellerman's comprehensive lists. It seems likely that the host was M. montebelloi, the only Japanese vole Ellerman gives.

53. Microtus oeconomusHepatozoon sp. Kramptiz, 1964(32)

54. ? Microtus sp.Babesia rossica (71)

The host was described simply as "le campagnol"
(Yakimoff and Saphronowitsch 1917).

Subfam. Gerbillinae (gerbils, sand-rats, antelope rats)

55. Tatera indica (Indian gerbil)Hepatozoon gerbilli R* (14)56. Taterillus gracilis (slender gerbil)Babesia taterilli (72)

The host of this record from Mali was given as
Tatera emini, a rodent which is not found in West
Africa. The gerbil most likely to have been collected
in Mali is Taterillus gracilis (see Rosevear, 1969).

57. Meriones erythrourus (gerbil)Hepatozoon sp. Latyshev, 1949 (37)

Latyshev (1949) called the host M. libycus, a
predominantly N. African species with many forms.
Since the report was from the U.S.S.R., it seems
better to assign the host to M. erythrourus, a closely
related rodent with a wide distribution in that
country. The group is, however, difficult and the
authorities give no clear guide; possibly erythrourus
and libycus are distinct, although it has been
suggested that erythrourus and its races may represent
subspecies of libycus (Ellerman, 1940).

* In red blood cells

Fam. Rhizomyidae (bamboo rats)

58. Rhizomys sumatrensis

Hepatocystis sp. Dunn et al., 1968 (59)

Fam. Muridae

Subfam. Murinae (Old World rats and mice)

59. Apodemus flavicollis (yellow-necked mouse)

Hepatozoon sylvatici W. (27)

60. Apodemus sylvaticus (long-tailed field mouse)

Hepatozoon sylvatici W. (27)

Babesia microti (64)

61. Thamnomys rutilans (shining thicket rat)

Plasmodium berghei yoelii (45)

P. b. killicki (44)

P. berghei ssp. Killick-Kendrick et al., 1968 (46)

P. vinckei chabaudi (48)

P. vinckei ssp. Adam et al., 1966 (49)

P. vinckei ssp. Bruce-Chwatt and Gibson, 1955 (51)

62. Thamnomys surdaster (thicket rat)

Plasmodium berghei berghei (43)

? P. vinckei vinckei (61)

Babesia rodhaini (70)

When the rodent taxonomists have reached agreement,
this rodent may become known as Grammomys surdaster.

63. Mylomys dybowski (3 toed grass rat)Babesia golundae (63)Hepatozoon sp. Kleine 1910 W. (31)

The host of B. golundae from Senegal was given as Golunda campanae (Léger and Bédier, 1923), but this genus is an Indian one, and it seems probable that the infected rodent was an African groove-toothed swamp rat related to Golunda. It is most likely it was confused with an Angolan species, Pelomys campanae. Although modern authorities are not in agreement (see Rosevear 1969), Pelomys is generally considered to be absent from West Africa, where closely related forms are now placed in Mylomys. This is probably a monospecific genus, and a tentative identification of the host of B. golundae is therefore Mylomys dybowski rather than Pelomys campanae as suggested by Bray (1964). An objection to this identification is the apparent absence of Mylomys in Senegal. But determined trapping almost anywhere in Africa typically extends the known range of many species, and it is unlikely that the genus is absent from that country.

The host of Hepatozoon sp. Kleine, 1910 was misidentified as Pseudomys cunninghami. Pseudomys is an Australasian genus (Simpson, 1945), and the rodent was probably "Mylomys cuninghamei", now a synonym

of M. dybowskii (Rosevear, 1969).

64. Arvicanthis niloticus (harsh-furred rat)

Hepatozoon arvicanthis W (4)

Babesia ratti (68)

The host is referred from A. abyssinicus rubescens to A. niloticus which Rosevear (1969) suggests is the only species in the genus. Earlier, Ellerman (1941) considered there was little essential difference between the described distinct species of Arvicanthis.

Martoglio(s (1913) record of Hepatozoon arvalis (3) in a rodent in Ethiopia, mistaken for Microtus arvalis, was possibly from a misidentified Arvicanthis (Brumpt 1946).

65. Lemniscomys barbarus

Babesia arvicularis (60)

Wenyon (1909) recorded B. arvicularis in Arvicanthis zebra from the Sudan. Ellerman (1941) does not list zebra as a form of Arvicanthis, and it is probable that Wenyon's rodent was Lemniscomys barbarus zebra.

Lemniscomys is closely allied to Arvicanthis, and their separate identities were not fully appreciated until the name Lemniscomys was resurrected in 1916 (Rosevear 1969). As two forms of L. barbarus other than zebra are known from the Sudan, the record is best left with the subspecies undetermined.

66. Hybomys univittatus (one-striped rat)
Hepatozoon sp. Landau W. (35)
Babesia sp. Landau (84)
67. Hybomys trivirgatus (three-striped rat)
Hepatozoon sp. Bruce-Chwatt and Gibson (28)
68. Rattus norvegicus (brown rat)
Hepatozoon muris W. (22)
Babesia decumani (62)
Babesia muris (65)
69. Rattus rattus (black rat)
Hepatozoon muris W. (22)
70. Rattus sp. ("grey rats")
Hepatozoon epsteini (11)
71. Mastomys coucha (multi-mammate rat)
Babesia ratti (68)

The identification of this host cannot be accepted without question. The record is from the Congo and the host was given as M. ugandae, now a subspecies of M. coucha (Ellerman, 1941). Mastomys is, however, easily confused with Praomys, a common host of piroplasms (see nos. 72 and 73 below).

72. Praomys jacksoni
Plasmodium berghei berghei (43)
Babesia sp. Landau (83)
Babesia sp. Adam and Landau (73)

73. Praomys tullbergiPlasmodium vinckei ssp. indet. (51)Babesia sp. Bruce-Chwatt and Gibson, 1955 (79)Babesia sp. Gunders (82)74. Stochomys longicaudatusBabesia sp. Bruce-Chwatt and Gibson (80)75. Hylomyscus sp. indet.Babesia sp. Bafort, 1969 (75)76. Mus musculus (house mouse)Hepatozoon musculi W. (23)

An unnamed piroplasm was recorded by Bruce et.al. (1911) in a white laboratory mouse (Mus musculus) in unusual circumstances. The mouse was being used for the maintenance of a strain of spirochaetes isolated from the blood of a bushbuck. Seven days after an inoculation of infected mouse blood, a recipient mouse was found to be infected not only with the spirochaete, but also with a piroplasm which was seen on four successive days; there was then a spontaneous cure. The most likely explanation of this record is that the piroplasm was a parasite of the bushbuck and, most interestingly, that the concomitant infection with a spirochaete resulted in the piroplasm gaining a temporary foothold in an abnormal host.

77. Leggada bella (pygmy mouse)

Plasmodium berghei berghei (43)

78. Acomys percivali (spiny mouse)

Anthemiosoma garnhami (85)

79. Cricetomys gambianus (giant rat)

Hepatozoon cricetomysi R. (9)

Babesia sp. Bruce-Chwatt and Gibson (21)

Bruce et al., (1915) published a painting of a piroplasm of an "edible rat". Many African rats are eaten, but perhaps none more enjoyed than C. gambianus. Possibly Bruce's unnamed parasite from Malawi was from this rodent.

Subfam. Dendromurinae (African tree mice)

80. Dendromus ?insignis

Hepatozoon dendromysi R. (10)

Fam. Gliridae

Subfam. Glirinae (dormice)

81. Muscardinus avellanarius (European dormouse)

Babesia myoxi (66)

82. Eliomys quernicus

Hepatozoon lusitanicum (19)

This haemogregarine was reported from E. lusitanicum then thought to differ from E. quernicus but now reduced to a subspecies of the latter (Ellerman, 1941).

Subfam. Graphiurinae (African dormice)

83. Graphiurus murinusBabesia rigolleti (68)

The host of B. rigolleti was originally called Myoxus murinus, now a synonym of G. murinus (Rosevear, 1969). Bray (1964) assumed G. murinus was absent from West Africa, and inferred the host was probably G. hueti, a very large squirrel-like dormouse unlikely to have been misidentified.

Fam. Dipodidae (jerboas)

Subfam. Dipodinae

A record by Rodhain et al., (1913) of Hepatozoon sp. in the erythrocytes of a dipodine ("Jaculus .. ?johnstoni") from Sankisia in the Democratic Republic of the Congo was probably from a wrongly identified host. The distribution of Jaculus does not extend far south of the Sahara desert (Ellerman, 1940), and it is not possible now even to guess which was the rodent examined.

84. Jaculus gordoniiHepatozoon balfouri R. (6)85. Jaculus orientalis (greater Egyptian jerboa)Hepatozoon balfouri R. (6)86. Jaculus jaculus (lesser Egyptian jerboa)Hepatozoon balfouri R. (6)

87. Alactagulus pumilioHepatozoon alactaguli R. (2)

"A. acontien" in the original report (Sassuchin
1931) is now a synonym of A. pumilio (Ellerman, 1940)

HYSTRICOMORPHA

Fam. Hystricidae (Old World porcupines)

Subfam. Atherurinae (brush-tailed porcupines)

88. Atherurus africanus (brush-tailed porcupine)Plasmodium atheruri (42)

Fam. Caviidae (guinea-pigs or cavies)

Subfam. Caviinae

89. Cavia porcellus (guinea-pig)Babesia cobayae (61)

As this infection was found in a laboratory animal
in Nigeria, it cannot be considered as a truly
natural parasite of this New World rodent.

Fam. Thryonomyidae

90. Thryonomys swinderianus (cutting grass)Babesia sp. Bruce-Chwatt and Gibson, 1955 (78)Babesia sp. Bray, 1964 (76)Babesia sp. Adam and Landau (74)

HYSTRIGOMORPHA or MYOMORPHA incertae sedis

Fam. Ctenodactylidae (gundi)

91. Ctenodactylus gundi (gundi)Babesia quadrigemina (67)

ADDENDUM TO CHECK-LIST

Since the check-list was prepared and cross indexed, a number of other records have been found. These are:

1. Hepatozoon sp. Krampitz, Sachs, Schaller and Schindler, 1968
Praomys natalensis, Tororo, Uganda
2. Babesia citelli Becker and Roundabush, 1933;
Citellus tridecemlineatus, Iowa, U.S.A.
3. Babesia microti (France, 1912); Microtus pennsylvanicus,
Island of Martha's Vineyard, U.S.A. (Tyzzer, 193⁹~~2~~)
New York, U.S.A. (Kirner, Barbehenn and Travis, 1958);
Peromyscus californicus, Microtus californicus, Neotoma
lepidus California, U.S.A. (van Peenen and Duncan, 1968).
4. Babesia wrighti Tomlinson, Smith and Fogg, 1948; Citellus
variegatus buckleyi, Texas, U.S.A.
5. Babesia sp. van Peenen and Duncan, 1968; Citellus beecheyi
California, U.S.A.

SUBSIDIARY MATTER

Candidate's published work on murine malaria
parasites

In the joint publications listed below, the candidate initiated the work in papers 1, 2 and 3; collaborated in the work in papers 4 and 5; and played a minor role in the work in papers 6 and 7.

1. KILLICK-KENDRICK, R., and WARREN, M. (1968).
Primary exoerythrocytic schizonts of a
mammalian Plasmodium as a source of
gametocytes. Nature, Lond., 220, 91.
2. KILLICK-KENDRICK, R., SHUTE, G.T., and LAMBO, A.O.
(1968). Malaria parasites of Thamnomys
rutilans (Rodentia, Muridae) in Nigeria.
Bull. Wld. Hlth. Org., 38, 822.
3. WARHURST, D.C., and KILLICK-KENDRICK, R. (1967).
Spontaneous resistance to chloroquine in
a strain of rodent malaria (Plasmodium
berghei yoelii). Nature, Lond., 213, 1048.
4. LANDAU, I., and KILLICK-KENDRICK, R. (1966).
Note préliminaire sur le cycle évolutif
des deux Plasmodium du rongeur Thamnomys
rutilans de la République Centrafricaine.
C.R. Acad. Sci. Paris, 262, 1113.
5. LANDAU, I., and KILLICK-KENDRICK, R. (1966).
Rodent Plasmodia of the République
Centrafricaine: the sporogony and tissue
stages of Plasmodium chabaudi and P.
berghei yoelii. Trans. R. Soc. Trop. Med.
Hyg., 60, 633.

6. GARNHAM, P.C.C., LANDAU, I., KILLICK-KENDRICK, R., and ADAM, J.P. (1967). Répartition et caractères différentiels des Plasmodiums de muridés. Bull. Soc. Path. exot., 60, 118.
7. GARNHAM, P.C.C., BIRD, R.G., BAKER, J.R. and KILLICK-KENDRICK, R. (1969) Electron microscope studies on the motile stages of malaria parasites. VII: The fine structure of merozoites of exoerythrocytic schizonts of Plasmodium berghei yoelii. Trans. R. Soc. Trop. Med. Hyg., 63, 328.

Notes on Laboratory Demonstrations at Meetings of the Royal Society of Tropical Medicine and Hygiene.

- GARNHAM, P.C.C., LANDAU, I., and KILLICK-KENDRICK, R. (1966). Primary exoerythrocytic schizonts of three rodent malaria parasites. Trans. R. Soc. Trop. Med. Hyg., 60, 4.
- WÉRY, M., and KILLICK-KENDRICK, R., (1967). Tissue schizonts of P. berghei yoelii in the livers of new-born rats. Ibid., 61, 6.
- GARNHAM, P.C.C., BIRD, R.G., BAKER, J.R., and KILLICK-KENDRICK, R. (1967). The fine-structure of mature exoerythrocytic schizonts of Plasmodium berghei yoelii. Ibid., 61, 447
- WÉRY, M., and KILLICK-KENDRICK, R. (1967). Differences in the numbers of tissue schizonts of Plasmodium berghei yoelii in the livers of individual mice, and a comparison of two routes of inoculation of sporozoites. Ibid., 61, 447.
- WÉRY, M., KILLICK-KENDRICK, R., and BRAY, R.S. (1968) Sporozoite-induced infections of Plasmodium berghei yoelii in rabbits and a guinea-pig. Ibid., 62, 1.

KILLICK-KENDRICK, R., SHUTE, G.T., LAMBO, A., and GUY, M.W. (1968). Rodent plasmodia of Nigeria. Ibid., 62, 2.

KILLICK-KENDRICK, R., and WARREN, M. (1968). Production of gametocytes directly from primary exoerythrocytic schizonts of P. berghei. Ibid., 62, 461

Primary Exoerythrocytic Schizonts of a Mammalian *Plasmodium* as a Source of Gametocytes

IN infections of mammalian malaria induced by sporozoites, the first indication of infection in the vertebrate host is the presence of exoerythrocytic schizonts in the parenchymal cells of the liver. After growth for 2-15 days, the time varying according to the species of parasite, each tissue schizont produces several thousand merozoites which, when released into the blood, invade erythrocytes. It is generally assumed that there are then one or more schizogonic cycles in the red blood cells before some of the merozoites from the erythrocytic schizonts grow into sexual forms, the gametocytes.

The experiments reported here were designed to discover whether or not gametocytes of the murine parasite, *Plasmodium berghei yoelii* Landau and Killick-Kendrick, 1966, ever develop directly from merozoites produced by the exoerythrocytic schizonts, with no intervening schizogony in the blood.

Subinoculations of heart blood from mice at timed intervals after the intravenous inoculation of sporozoites of *P.b. yoelii* have shown that the very earliest time at which the blood is infective is 43 h^{1,2}; at this time, the most precocious of the tissue schizonts may rupture, releasing merozoites into the blood. Because *P. berghei* has a quotidian periodicity³, erythrocytic schizonts derived from the first merozoites released from the liver would be expected to mature in the blood about 24 h later. If the period of development of the gametocytes is the same as that of the erythrocytic asexual forms, fully grown gametocytes originating from merozoites produced by the earliest generation of erythrocytic schizonts would be in the blood after another 24 h, that is, 91 h after the inoculation of sporozoites. Unless their growth takes less than 24 h, mature gametocytes found before this time can have arisen only from merozoites produced by primary exoerythrocytic schizonts in the liver. Our aim, therefore, was to demonstrate their presence in the blood of mice within 91 h of the intravenous inoculation of sporozoites.

About 400,000 sporozoites of *P.b. yoelii* (strain 17X) were inoculated intravenously into each of two mice (23A/1 and 23A/2). Forty-six hours later, stained thin films were prepared from tail blood of the mice at intervals of 1 h and were examined for gametocytes and mature schizonts. Batches of *Anopheles stephensi* were allowed to

feed on mouse 23A/1 72-73 and 75-77 h after the injection of sporozoites. The mosquitoes were incubated at 24° C, and midguts were examined for oocysts 6 and 7 days after the blood meal.

Very small ring forms were easily found in the blood of mouse 23A/1 at 46 h and of 23A/2 at 47 h; almost all the parasites were in blue-staining immature erythrocytes, and multiple infections were common. A fully grown macrogametocyte was seen at 69 h (only 26 h (69 less 43) after the first rupture of the tissue schizonts) and an apparently mature male gametocyte was first seen at 74 h. Both batches of mosquitoes became infected. There were surprisingly large numbers of oocysts on some midguts with, for example, from five to more than three hundred on the guts of twelve out of nineteen *A. stephensi* in the first group which had taken blood meals 72-73 h after the inoculation of sporozoites—only 29-30 h after the presumed first invasion of the blood by merozoites from the fastest maturing exoerythrocytic schizonts. Sporogony proceeded normally, with sporozoites invading the salivary glands of the mosquitoes 10 days after the blood meal; the sporozoites were shown to be infective by inoculating mice.

In this first experiment, fully mature schizonts were not found in the blood until 26 h after the first rupture of tissue schizonts. In one of a second series of mice in later work, small numbers of schizonts with sixteen fully formed merozoites and a large single clump of pigment were seen in a blood film made only 22 h after the minimum time of rupture.

In the second experiment, oocysts were found on the midguts of mosquitoes which had fed at 73-74 h after the inoculation of sporozoites, but there were none in mosquitoes fed at 55-56, 61-62 or 67-68 h.

These results show that some of the merozoites produced by the primary exoerythrocytic schizonts of *P.b. yoelii* appear to grow directly into sexual forms and, moreover, that the time taken for gametocytes to grow from ring stages to maturity is approximately the same as that taken by the asexual forms in the blood. We had calculated that if gametocytes came only from blood schizonts, the earliest time that they would be mature in the blood would be $43 + 24 + 24$ h. The finding that at least some of the erythrocytic schizonts took only 22 h to reach maturity necessitates a revision of this estimate. With a period of growth of only 22 h for both asexual and sexual forms, the minimum time is 4 h less than we had originally estimated, that is, 87 h. The presence of oocysts in mosquitoes which were fed only 72-73, 73-74 and 75-77 h after the inoculation of sporozoites is evidence of the exoerythrocytic origin of the first gametocytes maturing in the blood.

In avian malaria, several workers have noticed the simultaneous appearance of gametocytes and asexual parasites in the blood of experimentally infected birds (see ref. 4), but the origin of the sexual forms has not been proved by timed feedings of mosquitoes. Adler and Tchernomoretz⁵ suppressed the erythrocytic schizogony of *P. gallinaceum* with quinine and found that when the drug was withheld, some merozoites from the tissue forms grow directly into gametocytes. These merozoites came from secondary exoerythrocytic schizonts, and it is not known if gametocytes of *P. gallinaceum* ever arise from tissue schizonts of the primary cycle which in avian malaria occupies two generations⁶.

Our findings demonstrate yet another point of similarity between the haemoproteids and the true malaria parasites, and are of interest when considering the evolution of the haemosporidia. They also raise the question of the unknown mechanisms controlling the development of merozoites into either sexual or asexual forms. The growth of gametocytes from merozoites of the tissue schizonts occurred too early in the infection for it to have been stimulated by an acquired immunity, and merozoites from either erythrocytic or oxoerythrocytic schizonts can apparently grow into sexual forms at any time of the infection in the vertebrate. The whole process of development from tissue schizont to gametocyte, with or without an intervening erythrocytic schizogony, could well be viewed as a continuing gametogony controlled by unknown factors.

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Received July 30; revised September 12, 1968.

¹ Landau, I., and Killick-Kendrick, R., *Trans. Roy. Soc. Trop. Med. Hyg.*, **60**, 633 (1966).

² Wéry, M., *Ann. Soc. Belge Méd. Trop.*, **48**, 1 (1968).

³ Garnham, P. C. C., *Malaria Parasites* (Blackwell, Oxford, 1966).

⁴ Bishop, A., *Parasitology*, **45**, 163 (1955).

⁵ Adler, S., and Tchernomoretz, I., *Ann. Trop. Med. Parasit.*, **37**, 148 (1943).

Spontaneous Resistance to Chloroquine in a Strain of Rodent Malaria (*Plasmodium berghei yoelii*)

DURING an investigation of sporozoite induced infections of *Plasmodium berghei yoelii* in mice, chloroquine was given in an attempt to eliminate the erythrocytic stages of the parasite. Unexpectedly, the parasite failed to respond to the drug.

Further tests have shown that this strain is spontaneously as resistant to chloroquine as are the highly resistant strains of *P. b. berghei* which have been artificially produced in the laboratory (ref. 2 and unpublished work of Warhurst).

The early history of the strain of *P. b. yoelii* (No. 17X) has been given by Landau and Killick-Kendrick¹. This is the type strain of *P. b. yoelii* which was isolated by Landau and Chabaud² in the République Centrafricaine in April 1965 from *Thamnomys rutilans*. The line we have investigated was derived from mouse 38L (infected blood, preserved at -70° C for 5 months) which had been infected from *Anopheles stephensi* batch 31 L (ref. 1). After two further cyclical transmissions, with three intermediate syringe passages, a sub-line was treated with chloroquine (15 mg/kg daily oral dose) on November 8, 1965, and found to be insensitive. After another 6 week period of low-temperature preservation and fourteen more cyclical transmissions, with twenty-one intermediate syringe passages in mice and rats, another sub-line of the strain was subjected to two more syringe passages in mice, and then tested for chloroquine resistance on December 12, 1966. After the strain had been through a further three passages in mice, another drug test was carried out on January 2, 1967; the last drug test described in this note was carried out after three more mouse to mouse passages on January 16, 1967. From May 1965 to December 1966 strain 17X had been through fifteen cyclical transmissions and twenty-eight syringe passages in the laboratory. Other than when sub-lines were being tested, the strain has never been exposed to antimalarial drugs in the laboratory. The action of chloroquine on this strain was compared with the action of the drug on an old laboratory strain of *P. b. berghei* (N), and on a recently isolated mosquito-transmissible strain of *P. b. berghei* (NK65) (ref. 4). Groups of five male Parkes-strain mice were used.

In the first experiment, we compared the action of chloroquine on *P. b. berghei* (strain N), and *P. b. yoelii* (strain 17X), by a method previously described³. On

December 12, 1966, mice were inoculated intravenously with infected blood, and a single dose of the drug was given intraperitoneally 2 h later. Increases in latent periods of infection were then recorded. The dose-response curves are given in Fig. 1. The prolongation of the latent period of strain *N* is linearly related to the logarithm of the dose of chloroquine, but this is not so for strain 17X. In strain *N*, a dose of 40 mg/kg caused a delay of 4.2 days, but in strain 17X a dose of 40 mg/kg caused a delay of only 1.85 days. In the second experiment, a conventional 5 day drug test² was carried out as follows. The mice were inoculated intravenously on the first day with 10^7 parasitized erythrocytes, and drug doses were given intraperitoneally on the day of infection, and for 3 subsequent days. Blood films were taken on the day after the last dose of the drug, and the parasitaemias were evaluated. The results are expressed in Fig. 2 as dose-response curves showing mean parasitaemias in the experimental groups (as a percentage of the parasitaemias in the control groups) plotted against logarithm of drug dose.

The difference in dose-response of the two strains is clear. In mice infected with strain *N*, 5 mg/kg given daily for 4 days completely prevented the appearance of parasites, while in mice infected with strain 17X a dose as high as 50 mg/kg merely reduced parasitaemia to 28 per cent of undosed controls. The dose-response line of strain 17X is relatively flat, and resembles the dose-response lines of artificially produced chloroquine-resistant strains of *P. b. berghei*².

In the comparison of the action of chloroquine on strain 17X with that on strain NK65 of *P. b. berghei* made on January 16, 1967, a conventional 5 day drug test was carried out as in the second experiment described previously. In both experiments chloroquine was given in a dose of 5 mg/kg daily for 4 days. Strain 17X developed a parasitaemia which was 40 per cent of that of undosed controls, whereas strain NK65 developed a parasitaemia which was less than 3 per cent of the control. Chloroquine-resistance is therefore a special feature of strain 17X, and not merely a characteristic of freshly isolated strains.

The response of strain 17X to other antimalarial drugs is still in the course of investigation, but preliminary observations using the 5 day test indicate that it is sensitive (possibly hypersensitive) to pyrimethamine, sulphadiazine, and proguanil. It resisted a daily oral dose of 150 mg/kg of quinine hydrochloride but not of 300 mg/kg. It also resisted a daily intraperitoneal dose of 5 mg/kg of mepacrine hydrochloride but not 10 or 100 mg/kg. It was almost completely resistant to pamaquine given orally at sub-toxic doses (20 mg/kg) but not at doses (40 mg/kg) which killed most of the mice. These results suggest that there is a slight cross-resistance to

mepacrine and quinine, but this is not as marked as that found in artificially prepared chloroquine-resistant strains of *P. berghei*^{2,6}.

The results described here show that, although it has never before been exposed to antimalarial drugs, strain 17X is as resistant as are laboratory strains of *P. berghei* which have been produced by treatment of infected animals with chloroquine. The resistance appears stable, unlike artificially produced chloroquine-resistance^{2,6}. These observations raise several interesting points.

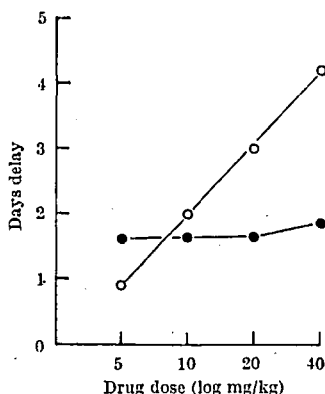


Fig. 1. Prolongation of latent period (days delay) by single doses of chloroquine in strain N (O) and strain 17X (●) of *P. berghei*.

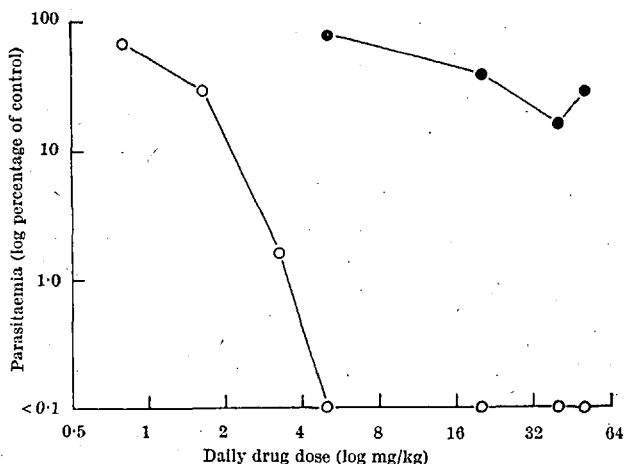


Fig. 2. Parasitaemias (as percentage of undosed control values) in mice infected with strain N (O) and strain 17X (●) of *P. berghei*, plotted against daily dose of chloroquine in the 5 day test.

(1) Chloroquine-resistance may be a feature of all recently isolated strains of *P. berghei*. Most drug tests on *P. berghei* have been carried out on old syringe-passaged laboratory strains, which do not necessarily behave as do recently isolated ones. It is well known that freshly isolated strains of *Trypanosoma rhodesiense* are insensitive to doses of arsenical drugs which will eliminate old laboratory strains. On the other hand, our experience with strain NK65 showed that *P. b. berghei* is not similarly resistant, even when freshly isolated and still infective to mosquitoes. (2) Chloroquine-resistance is perhaps restricted to the *yoelii* sub-species of *P. berghei*. We cannot be sure this is so, however, until other isolates of the sub-species have been investigated. (3) Because a spontaneously chloroquine-resistant strain of *P. berghei* has been found in nature, there seems to be no reason why the same should not apply to *P. falciparum*, which causes malignant tertian malaria of man. In South East Asia and South America, strains of *P. falciparum* have been found which are resistant to chloroquine⁷, while in Africa, where chloroquine has also been widely used as an antimalarial, there is no authenticated report of chloroquine-resistance. It seems likely that in *P. falciparum*, as in *P. berghei*, chloroquine-resistant strains may sometimes occur spontaneously.

We thank Mme. Irène Landau and Professor M. Yoeli for supplying strains of *P. berghei*; Dr. F. Hawking, Professor P. C. C. Garnham, Dr. J. Williamson and Dr. R. S. Bray for suggestions. Mr. R. Folwell gave technical assistance. This work was supported by a grant from the World Health Organization.

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Received February 1, 1967.

¹ Landau, I., and Killick-Kendrick, R., *Trans. Roy. Soc. Trop. Med. Hyg.*, **60** (5), 633 (1966).

² Hawking, F., *Amer. J. Trop. Med. Hyg.*, **15**, 287 (1966).

³ Landau, I., and Chabaud, A. G., *C. R. Acad. Sci., Paris*, **261**, 230 (1965).

⁴ Yoeli, M., and Most, H., *Amer. J. Trop. Med. Hyg.*, **14**, 700 (1965).

⁵ Warhurst, D. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **60** (1), 6 (1966).

⁶ Peters, W., *Exp. Parasitol.*, **17** (1), 80 (1965).

⁷ Resistance of malaria parasites to drugs, *Tech. Rep. Ser. WHO*, **296** (1965).

Malaria Parasites of *Thamnomys rutilans* (Rodentia, Muridae) in Nigeria *

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For the past twenty years, the malaria parasites of the African Muridae have been much used in the laboratory as research tools in studies on the biology, immunology and chemotherapy of the genus *Plasmodium*. Of all the mammalian malaria parasites, those of murine rodents are the most easily maintained in the laboratory and, with recent findings on the optimum conditions for cyclical transmission through laboratory-bred *Anopheles*, their value is now much enhanced.^{a-d}

Malaria parasites of murine rodents are known only from Africa, and have been found in four places: Katanga, in the Democratic Republic of the Congo; Western Nigeria; the Central African Republic; and near Brazzaville in the Republic of Congo.^e Least is known of the parasites from Nigeria. Bruce-Chwatt & Gibson^f examined more than 1500 murine rodents trapped near Ilobi in Western Nigeria, including 1038 *Praomys*, 384 *Lophuromys*, 63 *Lemniscomys*, 51 *Stochomys*, 21 *Malacomys*, 8 *Arvicanthis*, 6 *Hybomys*, 1 *Dasymys* and 1 *Cricetomys*,^g and found only 1 *Praomys tullbergi* infected with a malaria parasite similar to *P. vinckei* Rodhain, 1952.^h The strain was not established in the laboratory and a determined effort to find another infected rodent met with no success.^f

In this note, we report the rediscovery of Bruce-Chwatt & Gibson's parasite in Nigeria, and the new

finding of a *P. berghei*-like parasite in the same locality.

The remarkably low incidence in the early work suggested that malaria parasites were very rare in Nigerian rodents, and further attempts to rediscover the parasite and establish it in laboratory animals seemed, at first sight, unlikely to succeed. But new information on the prevalence of malaria parasites in the thicket rat, *Thamnomys rutilans*, in the Central African Republicⁱ and the Republic of Congo (Brazzaville)^j led to the idea, later shown to be correct, that Bruce-Chwatt's apparently rare *Plasmodium* was perhaps a common parasite of *Thamnomys* in the place where the infected *Praomys* was collected. *Thamnomys* was not among the very large sample examined by Bruce-Chwatt, probably because all trapping in the early work was at ground level, and this is a rodent which is seldom caught unless traps are placed in bushes above the ground.

From 16 to 23 August 1967, during the lull in the rains, at the same time of year as when the infected *Praomys* had been caught thirteen years earlier, we collected 41 murine rodents from near Ilobi (longitude 3°03' E; latitude 6°45' N) in the Egbado Division of Western Nigeria. Except for a few caught by local children in home-made traps, the animals were caught in cage-traps,^k with double spring-operated doors, placed 2m-3m above ground. Of the three baits used—apple, banana and palm nut—the first two attracted *Thamnomys* more regularly than the last.

The trapping area chosen was in secondary forest and fallow farm-land along the edge of the Ilaro Forest Reserve, a mixed deciduous area of high forest of approximately 24 square miles (62 km²), close to the north-eastern side of the village of Ilobi.

* This work was carried out under the auspices of the World Health Organization while the senior author was serving as WHO Consultant.

^a Vanderberg, J. & Yoeli, M. (1966) *J. Parasit.*, **52**, 559.

^b Landau, I. & Killick-Kendrick, R. (1966) *Trans. roy. Soc. trop. Med. Hyg.*, **60**, 633.

^c Bafor, J. (1967) *Ann. Soc. belge Méd. trop.*, **47**, 271.

^d Wéry, M. (1968) *Ann. Soc. belge Méd. trop.*, **48**, 1.

^e Garnham, P. C. C., Landau, I., Killick-Kendrick, R. & Adam, J. P. (1967) *Bull. Soc. Path. exot.*, **60**, 118.

^f Bruce-Chwatt, L. J. & Gibson, F. D. (1955) *Trans. roy. Soc. trop. Med. Hyg.*, **49**, 9.

^g Bruce-Chwatt, L. J.—personal communication.

^h Rodhain, J. (1952). *Ann. Soc. belge Méd. trop.*, **32**, 275.

ⁱ Landau, I. & Chabaud, A. G. (1965) *C. R. Acad. Sci (Paris)*, **261**, 230.

^j Adam, J. P., Landau, I. & Chabaud, A. G. (1966) *C.R. Acad. Sci (Paris)*, **263**, 140.

^k Manufrance, St. Etienne, France.

Nests of *Thamnomys rutilans*, built in bushes or banana trees 2 m–3 m from the ground, were easily found in the open forest area, which had a canopy only 6 m high interspersed with emergent trees. No nests were found in the neighbouring reserve, where the trees formed a much higher closed canopy about 30 m from the ground. In contrast to the trapping locality, the near-by forest reserve was completely undisturbed, protected by regulations strictly prohibiting hunting or wood-gathering. This wooded area lies in the High Forest Zone at the western edge of the southern Nigerian secondary forest region, and extends in patches throughout much of Western Nigeria.¹

The Egbado Division lies at an altitude of 70 m–130 m. Annual rainfall is 90 cm–140 cm (35 in–55 in) distributed over about 90 days. The rains set in gradually from April, reach a first peak in June or July, fall off during August (the time of year at which we collected animals), and rise to a second peak in September; very little rain falls from the beginning of December to the end of February. At the near-by town of Abeokuta, the mean daily maximum temperature is 31°C (87°F), and the mean daily minimum 22°C (72°F); the average relative humidity throughout the year is 80%.^m

Preliminary identifications of our rodents were made using a key to the Nigerian species based on external characters;ⁿ skulls from samples of each rodent were later sent to Mr D. R. Rosevear at the British Museum (Natural History), London, who kindly examined them and confirmed our identifications. The 41 rodents collected were 20 *Praomys tullbergi*, 18 *Thamnomys rutilans*, 2 *Stochomys longicaudatus* and 1 *Malacomys edwardsi*. They were taken to the laboratory where 0.6 ml–1.0 ml samples of blood were taken by heart puncture under ether anaesthesia using heparin as an anticoagulant; the samples were routinely inoculated intraperitoneally into 1 or 2 white mice, blood films from which were examined once or twice between the fifth and ninth days after inoculation. As the blood samples were taken, thick and thin blood films were prepared with blood from the tail of each wild rodent. These were stained in Giemsa's stain at pH 7.1; a buffer solution of high salt concentration was used when staining

the thick films to overcome the difficulty, often encountered with rodent blood, of obtaining good lysis.

Six of the 18 *Thamnomys* were infected with malaria parasites, which, in each instance, were easily found in the thick blood films; 4 of the 6 had parasitaemias sufficiently high for parasites to be seen in thin films. Prepatent periods in mice inoculated with blood from infected *Thamnomys* varied from 2 to 6 days, although in 2 groups there were individual mice which never apparently became infected. Parasitaemias rose rapidly and generally became high; in stained thin blood films there were usually 4–25 infected erythrocytes per microscope field ($\times 100$ objective) within 7–11 days. After this peak, the numbers of parasites slowly fell and eventually disappeared 2–3 weeks after inoculation.

No infections were found in the other 35 rodents. Prolonged searches of thick blood films from all except the 6 infected animals were made with negative results, and the inoculation of blood into white mice revealed no subpatent infections.

One *Thamnomys* was found to be infected with 2 species of *Plasmodium*, one of which we think is the same as the *P. vinckei*-like parasite found by Bruce-Chwatt, while the other has blood stages indistinguishable from *P. berghei berghei* Vincke and Lips, 1948, from Katanga, and *P. b. yoelii* Landau and Killick-Kendrick, 1966, from the Central African Republic. This *P. berghei*-like parasite (strain N67) has since been separated from the other by cyclical passage in white mice through laboratory-bred *Anopheles stephensi*, and its life-cycle is at present being studied. A final identification awaits the completion of those studies, but morphological differences already seen in the sporogonic stages,^o notably the unusually long sporozoites, suggest that this is a new subspecies of *P. berghei* closely resembling the "*Plasmodium* sp. *proche berghei*" found by Adam et al.¹ near Brazzaville.

The other 5 *Thamnomys* with malaria had un-mixed infections of Bruce-Chwatt's *P. vinckei*-like parasite, 2 strains of which (No. N48 and N54) are now established in the laboratory. At first sight, this *Plasmodium* more closely resembles *P. vinckei vinckei* Rodhain, 1952, from Katanga than the "*Plasmodium* sp. *proche vinckei*" from Brazzaville,¹ or *P. v. chabaudi* Landau, 1965, from the Central African Republic. Like *P. v. vinckei*, the Nigerian parasite causes no reddening of infected erythrocytes

¹ Rosevear, D. F. (1953) *Checklist and atlas of Nigerian mammals*, London, Crown Agents for the Colonies.

^m Bruce-Chwatt, L. J., Archibald, H. M., Elliot, R., Fitzjohn, R. A. & Balogun, I. A. (1955) *Information Bulletin No. 3*, Malaria Service, Department of Medical Services, Nigeria.

ⁿ Rosevear, D. F. (1950) *Nigerian Field*, 15, 148.

^o Killick-Kendrick, R., Shute, G. T., Lambo, A., & Guy, M. W. (1968) *Trans. roy. Soc. trop. Med. Hyg.*, 62, 2.

such as is seen in *P. v. chabaudi* and the strains from Brazzaville; in other ways, however, the morphology of the blood stages, and the infectivity to laboratory animals, of all 4 parasites is very much alike.^c

The single infected *Praomys tullbergi* found by Bruce-Chwatt & Gibson^f is now thought to have been an accidental host of a common parasite of *Thamnomys rutilans* in the same locality. It is not known, however, why *Praomys* is so seldom infected. This species is sometimes taken in traps set above ground and therefore shares, at least partially, the habitat of *Thamnomys*. Moreover, it is susceptible to the *P. vinckei*-like parasite. Possibly the as yet unknown mosquito host has marked feeding preferences, and only rarely feeds on rodents other than *Thamnomys*. In Malaya, *Anopheles hackeri* has been found to feed readily on the monkey *Macaca irus* when placed as a "sentinel" in the forest, while the closely related monkey *M. mulatta*, put in the same place, is remarkably unattractive to the mosquito.^g Another explanation may be differences in periods of activity between *Praomys* and *Thamnomys* in relation to the peak feeding of the unknown mosquito host. This could result in a low *Praomys*-mosquito contact, whereas one can predict a high degree of contact between the mosquito and *Thamnomys*—probably occurring several metres above ground.

We made no determined effort to discover the natural vector of the parasites at Ilobi. In the area in and around the near-by town of Ilaro, Bruce-Chwatt et al.^m recorded 9 species of *Anopheles* other than *A. gambiae* and *A. funestus*, the main mosquito hosts of malaria of man in the locality. Adult mosquitos of 6 of these 9 were among out-of-door catches using man as bait (*A. pharoensis*, *A. hargreavesi*, *A. squamosus*, *A. nili*, *A. paludis* and *A. hancocki*). The other 3 species (*A. cinctus*, *A. coustani*

and *A. obscurus*) were never in the catches, and were found only as larvae; possibly one of these mosquitos will be found to be the natural vector of the malaria parasites of rodents in western Nigeria.

The distribution of the Nigerian parasites in the rodent host is similar to that of the murine malaria parasites of the Central African Republic and the area near Brazzaville in the Republic of the Congo. In all 3 places, the *P. vinckei*-group is much more common than the *P. berghei*-group, and many infected rodents have patent parasitaemias. *Thamnomys rutilans* is the principal vertebrate host, and other rodents appear to be only seldom infected. In Katanga, the distribution is different; *P. b. berghei* is by far the commoner parasite, *P. v. vinckei* having been found only twice, both times in the invertebrate host, *A. durenii*.^{c, h} *P. b. berghei* has been found in 3 rodents, *Thamnomys surdaster*, *Praomys jacksoni* and *Leggada bella* but, unlike infections in the other 3 places, parasitaemias are usually subpatent.^q Vertebrate hosts of *P. v. vinckei* are unknown but, because the 2 parasites have a common mosquito vector, the rodent hosts of *P. v. vinckei* are probably the same as those of *P. b. berghei*.

The preliminary work in Nigeria has revealed an interesting enzootic in a place where there are good opportunities of studying the epizootiology of two malaria parasites. The establishment of the parasites in the laboratory has made available new strains geographically separate from others already known.

* * *

We wish to thank Professor P. C. C. Garnham, Dr L. J. Bruce-Chwatt and Dr Irène Landau for help in planning the work; Dr F. R. S. Kellett for kindly providing laboratory accommodation and transport; and Mr M. W. Guy for establishing the strains in the laboratory.

^p Warren, McW.—personal communication.

^q Vincke, I. H. (1954) *Indian J. Malar.*, 8, 245.

PARASITOLOGIE. — *Note préliminaire sur le cycle évolutif des deux Plasmodium du Rongeur Thamnomys rutilans de la République Centrafricaine* ⁽¹⁾. Note (*) de Mme IRÈNE LANDAU et M. ROBERT KILLICK-KENDRICK, transmise par M. Pierre-P. Grassé.

Les deux *Plasmodium* infectant les *Thamnomys rutilans* capturés à la station expérimentale de La Maboké (République Centrafricaine) ont été transmis par *Anopheles stephensi* à des Rongeurs de laboratoire. Cette transmission a permis l'étude de leur sporogonie et de leur schizogonie pré-érythrocytaire éclairant ainsi certains aspects de leurs rapports avec les deux autres *Plasmodium* connus chez les Rongeurs, *Plasmodium berghei* et *P. vinckei*.

L'un des hématozoaires de la République Centrafricaine, *P. chabaudi*, a déjà été décrit comme espèce nouvelle ⁽²⁾, en se basant sur l'étude morphologique de ses formes sanguines.

L'autre, dans le sang, semble indistinguishable de *P. berghei* ⁽³⁾, mais l'étude de sa sporogonie et de son cycle tissulaire hépatique nous conduit à le considérer comme une nouvelle sous-espèce de ce *Plasmodium*. Nous la nommons *Plasmodium berghei yoelii* n. subsp. en hommage au Professeur M. Yoeli.

Dans cette Note préliminaire, nous rapportons brièvement la sporogonie de *P. chabaudi* et de *P. berghei yoelii* chez *A. stephensi* et leur schizogonie pré-érythrocytaire dans le foie de Souris et de Rats blancs.

1. *Plasmodium chabaudi*. — *Sporogonie* : A des températures comprises entre 23 et 26°, la durée de la sporogonie est de 11 à 12 jours, les sporozoïtes faisant alors leur première apparition dans les glandes salivaires. Le diamètre des oocystes mûrs atteint 80 μ et la longueur moyenne des sporozoïtes, dans des frottis séchés à l'air et fixés à l'alcool méthylique, est de 13,2 μ .

Schizogonie pré-érythrocytaire : Sa durée a été déterminée en injectant du sang de Souris blanche, ayant reçu une inoculation unique de sporozoïtes, à d'autres Souris : le sang s'est avéré non infectant 52 h après l'injection de sporozoïtes et infectant à 53 h. Les foies de Souris blanches inoculées avec des sporozoïtes ont été prélevés par biopsie ou autopsie 28, 42, 50, 53 et 54 h après l'injection, puis examinés histologiquement : les schizontes pré-érythrocytaires ont été trouvés dans des cellules du parenchyme hépatique. Des parasites ont été également observés dans le foie d'un rat inoculé de la même façon, bien qu'aucune infection apparente n'ait pu être décelée dans son sang.

Le plus jeune schizonte qui ait été observé est âgé de 28 h; il mesure $9\ \mu$ de diamètre et contient 20 noyaux. En dehors d'une augmentation de la taille et d'un nombre plus élevé de noyaux, il existe peu de différences morphologiques entre les schizontes à 42 et 50 h. Leur diamètre moyen est de $20\ \mu$ à 42 h, et de $29\ \mu$ à 50 h.

Les schizontes pré-érythrocytaires immatures de *P. chabaudi* sont caractérisés par : un cytoplasme abondant, réticulé, avec de nombreuses petites vacuoles aux pourtours indistincts, plus nombreuses à la périphérie et donnant à l'ensemble un aspect spongieux; des agrégations cytoplasmiques d'une basophilie intense, dispersées dans le schizonte; des noyaux irréguliers formés de grains chromatiniens de forme et taille variables, se distinguant difficilement du cytoplasme très dense.

A 53 et 54 h, le diamètre moyen est de $36,9\ \mu$; on note une plus grande variété de formes. De nombreux schizontes sont encore immatures et la formation de mérozoïtes débute à leur périphérie. Un arrangement des noyaux et du cytoplasme en pseudo-cytomères peut être rencontré. Les schizontes mûrs conservent souvent quelques agrégations cytoplasmiques. Ils contiennent 18 000 à 20 000 mérozoïtes. Lorsqu'ils se rompent, ils sont rapidement envahis par les phagocytes et l'on trouve des mérozoïtes dans les capillaires avoisinants. Quelques formes se rompent avant d'avoir atteint leur pleine maturité.

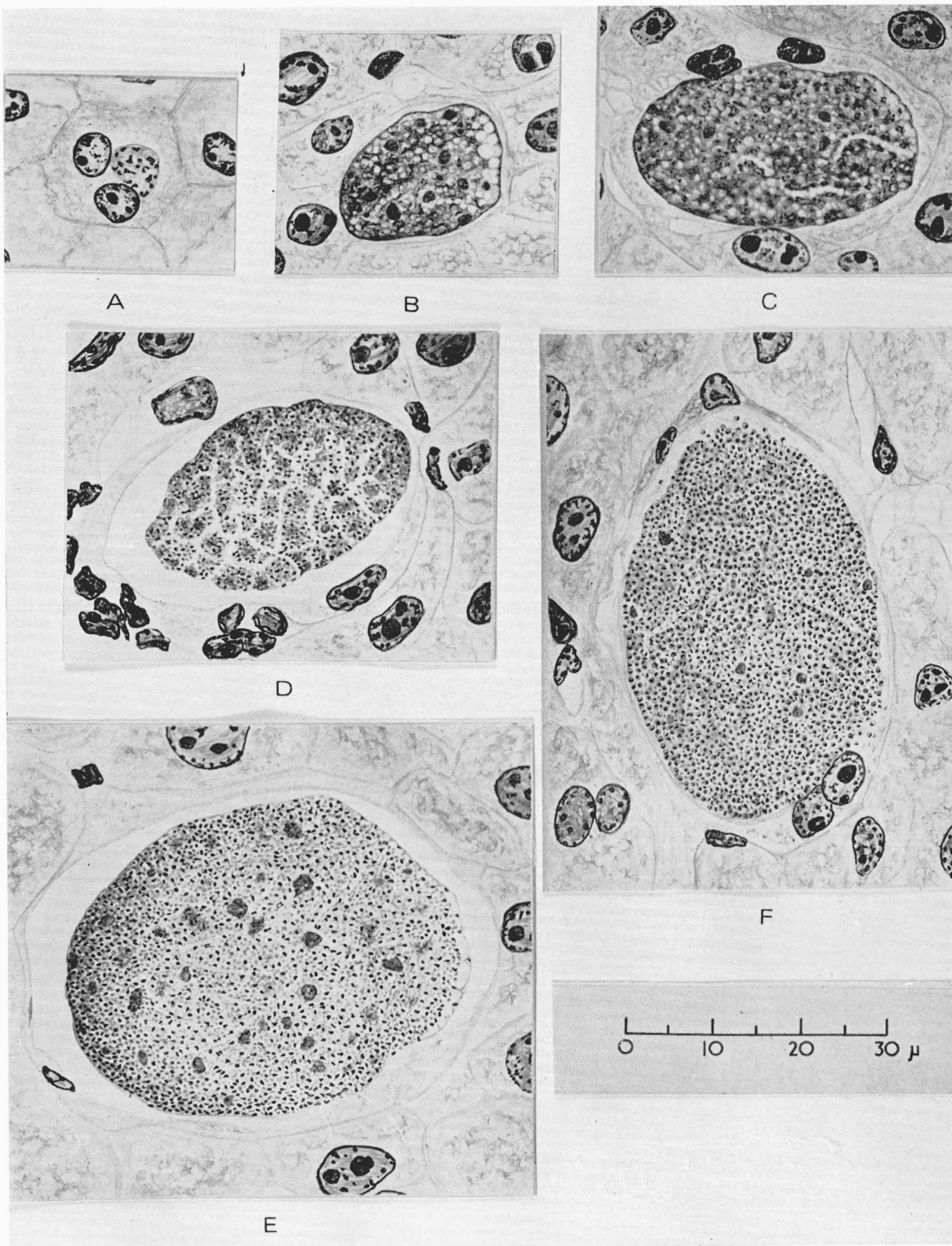
2. *Plasmodium berghei yoelii*. — *Sporogonie* : Elle est de 9 à 10 jours à des températures variant entre 23 et 26°. Les oocystes mûrs atteignent $82\ \mu$ et la taille moyenne des sporozoïtes est de $13,8\ \mu$.

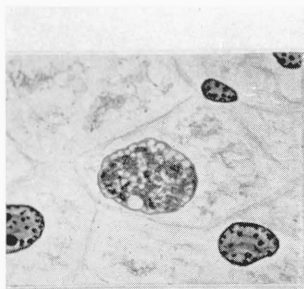
Schizogonie pré-érythrocytaire : Dans les mêmes conditions expérimentales que pour *P. chabaudi*, la durée de la schizogonie pré-érythrocytaire est de moins de 45 h. Les sporozoïtes obtenus ont une grande viabilité, car l'injection à un rat de 20 glandes salivaires infectées a permis d'obtenir 40 schizontes dans des coupes de foie ayant une surface de $0,5\ \text{cm}^2$ ($4\ \mu$ d'épaisseur). Les formes immatures présentent le même aspect spongieux et réticulé parsemé d'agrégations basophiles que celui décrit pour *P. chabaudi*. Les schizontes pré-érythrocytaires de *P. berghei yoelii* mesurent $8,1\ \mu$ à 25 h, $13,8\ \mu$ à 30 h, $20,6\ \mu$ à 36 h, $31,7\ \mu$ à 45 h et $37,3\ \mu$ à 50 h.

EXPLICATION DES PLANCHES.

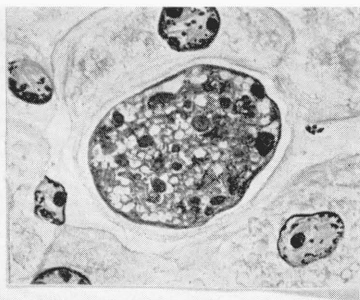
Planche I.

Plasmodium chabaudi : schizontes pré-érythrocytaires dans le foie de Souris blanches. A. 28 h (après hydrolyse selon la technique de Wolcott); B. 42 h; C. 50 h; D. 54 h : Formation de type pseudo-cytomères; E. 54 h : Stade précédant la formation des mérozoïtes; F. 53 h : mûr.

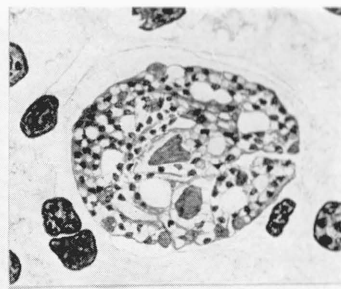




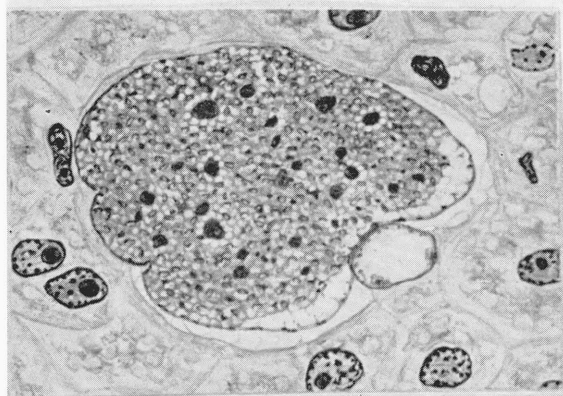
A



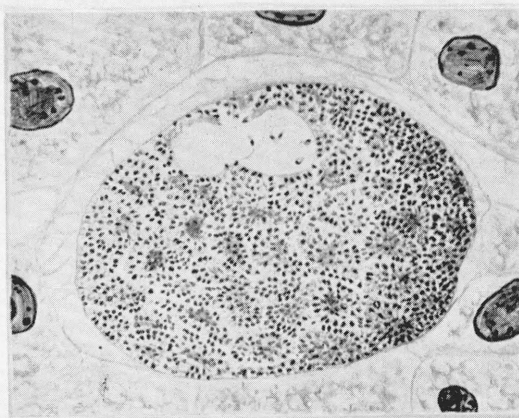
B



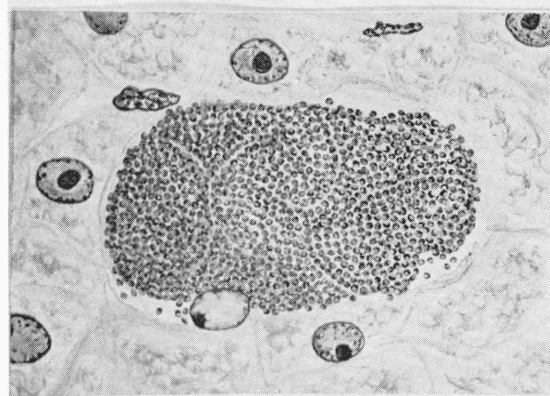
C



D



E



F

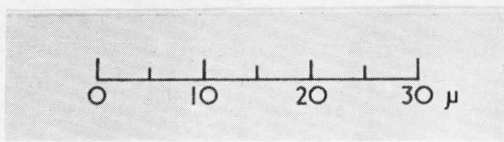


Planche II.

Plasmodium berghei yoelii : schizontes pré-érythrocytaires dans le foie des Rats blancs.
A. 25 h; B. 36 h; C. 45 h; D. 50 h; E. 50 h; F. 50 h : mûr.

Le nombre des mérozoïtes dans les schizontes mûrs est de 8 000 en moyenne, et ceux-ci ne contiennent plus d'agréations cytoplasmiques. Certains schizontes ont également des pseudo-cytomères.

Ce matériel a pu être comparé aux stades équivalents de *P. berghei* du Katanga, obtenus par P. C. C. Garnham et R. Killick-Kendrick, dans des conditions identiques, aux mêmes heures et en utilisant les mêmes procédés de fixation et de coloration. Ce matériel a été mis à notre disposition et il nous a ainsi été possible de comparer de façon précise les deux sous-espèces. La morphologie des formes sanguines est presque identique chez *Plasmodium berghei berghei* et *Plasmodium berghei yoelii*, mais la comparaison des sporogonies et des cycles pré-érythrocytaires a révélé des différences importantes. Chez *A. stephensi*, c'est essentiellement le diamètre plus élevé des oocystes mûrs (80 μ pour *P. b. yoelii*, 40 μ pour *P. b. berghei*); dans le foie des rats, c'est surtout la plus grande taille et le nombre plus élevé des noyaux des schizontes pré-érythrocytaires de *P. berghei yoelii* à tous les stades étudiés. Enfin, chez *A. stephensi* entre 23 et 26°, seule la forme de R. C. A. produit un grand nombre de sporozoïtes infectants.

La coexistence chez le rongeur *T. rutilans* de deux espèces différentes de *Plasmodium* est un phénomène comparable à celui déjà connu au Katanga où un rongeur très proche, *T. surdaster*, est l'hôte de *P. berghei berghei* et l'hôte présumé de *P. vinckei*. Comme nous venons de le voir, l'un des parasites de R. C. A. peut être considéré comme une sous-espèce de *P. berghei* du Katanga.

En ce qui concerne le statut de *P. chabaudi*, il n'est pas possible actuellement de faire une comparaison avec *P. vinckei* autrement que sur les formes sanguines d'une souche profondément remaniée par de nombreux passages de sang. Nous avons donc été astreints à considérer l'espèce comme nouvelle, mais ce que nous savons des rapports entre *P. berghei berghei* et *P. b. yoelii* laisse soupçonner une double vicariance. Il deviendrait alors très satisfaisant, si le cycle de *P. vinckei* se révélait proche de celui de *P. chabaudi*, de considérer ce dernier comme une sous-espèce de *P. vinckei*.

Bien qu'ils aient été plus particulièrement étudiés en helminthologie (*), les exemples de vicariance sont extrêmement courants en parasitologie, et les deux parasites de la République Centrafricaine nous paraissent en être un nouvel exemple.

D'un point de vue pratique, ces deux *Plasmodium* présentent un intérêt considérable, car ils ont l'avantage sur *P. berghei* et *P. vinckei* d'être d'un maniement bien plus facile et, sur les *Plasmodium* de Singes, de pouvoir être l'objet d'expériences nombreuses et répétées. Ils représentent actuellement le meilleur instrument de travail dans l'étude des formes tissulaires des *Plasmodium* de Mammifères.

(*) Séance du 21 février 1966.

(¹) Travail effectué grâce à une subvention de l'Organisation Mondiale de la Santé.

(²) I. LANDAU, *Comptes rendus*, 260, 1965, p. 3758.

(³) I. LANDAU et A. G. CHABAUD, *Comptes rendus*, 260, 1965, p. 230.

(⁴) A. J. PETTER, *Mém. Mus. Nat. Hist. Nat.*, série A, Zoologie, 39, p. 1 (sous presse).

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Reprinted from

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.
Vol. 60. No. 5. pp. 633-649, 1966.

Rodent Plasmodia of the Republique Centra-
fricaine: the Sporogony and Tissue Stages of
Plasmodium Chabaudi and *P. Berghei Yoelii*

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**RODENT PLASMODIA OF THE RÉPUBLIQUE CENTRAFRICAINE:
THE SPOROGONY AND TISSUE STAGES OF PLASMODIUM CHABAUDI
AND P. BERGHEI YOELII**

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The value of the malaria parasites of rodents as research tools has been limited for many years by the great difficulty of obtaining viable sporozoites in laboratory-bred mosquitoes. Until recently, only 2 species, both from Katanga in Central Africa, were available. The sporogony of one, *Plasmodium vinckei* Rodhain, 1952, remains incompletely known and, since the only strain discovered is now much altered by continual syringe passage, it is possible that transmission in the laboratory and the demonstration of the tissue forms of this species must await its rediscovery.

Many strains of the other species, *P. berghei berghei* Vincke and Lips, 1948, have been isolated, and its sporogony has been studied by many workers. Until a year ago, however, the transmission of this species in the laboratory had only seldom been achieved (YOELI and WALL, 1951, 1952; PEREZ-REYES, 1953; YOELI et al., 1964), and even when oöcyst growth appeared normal, sporozoites produced in laboratory mosquitoes were usually not infective to rodents (e.g. BOX et al., 1953; BRAY, 1954; CELAYA et al., 1956; MICHELIS, 1963).

Because of the apparent impossibility of obtaining numbers of viable sporozoites in the laboratory, the primary exo-erythrocytic schizonts of *P.b. berghei* remained undiscovered for a long time. The problem appeared insoluble until the influence of temperature on the infectivity of the sporozoites was recognized. The temperature of the habitat of *Anopheles duren*i, the natural invertebrate host of *P.b. berghei*, is much lower than that normally employed in the study of malaria in the laboratory (VINCKE, 1954; YOELI et al., 1965), and VANDERBERG and YOELI (1965) showed that the optimum

This work was supported in part by a grant from the World Health Organization.

We have been particularly fortunate in having continual advice and encouragement from Professor P. C. C. Garnham, and one of us (I.L.) would like to thank Prof. Garnham for the kind invitation to work in his laboratory. We are indebted to Dr. R. S. Bray and Prof. A. G. Chabaud for many helpful discussions, and for critically reading the manuscript, and to Dr. J. R. Baker, Dr. R. Lainson and Dr. P. Nelson for help in mass dissections of mosquitoes. We wish also to thank Mr. P. G. Shute and Miss M. Maryon of the Malaria Reference Laboratory, Epsom, for supplying all the *A. stephensi* used in this work, and Mr. P. E. Nesbitt, Mr. A. I. Oxbrow, Mr. M. W. Guy, Mr. R. B. Arseculeratne and Miss F. Munford of the London School of Hygiene and Tropical Medicine for technical assistance.

temperature for the production of infective sporozoites of *P.b. berghei* in laboratory-bred *A. quadrimaculatus* is only 18-21°C.; at 24°C. or above, oöcysts either fail to grow, or produce non-infective sporozoites.

This observation paved the way to the first demonstration of the primary exoerythrocytic schizonts of a rodent plasmodium (YOELI and MOST, 1965a,b).

While this work was in progress, two new rodent plasmodia, *P. chabaudi* Landau, 1965 and *P.b. yoelii* Landau and Killick-Kendrick, 1966, were discovered in the République Centrafricaine, more than 2,000 km. from Katanga (LANDAU, 1965; LANDAU and CHABAUD, 1965); both are infective to laboratory rodents, and infective sporozoites of both parasites are easily obtained in *A. stephensi*. In the present paper, detailed descriptions are given of the sporogonic and tissue stages of the two new malaria parasites; a preliminary report of this work has been published elsewhere (LANDAU and KILICK-KENDRICK 1966).

Materials and methods

Strains

The strain of *P. b. yoelii* used is the type strain (No. 17X), which is one of several isolated at the Station Expérimentale de la Maboké, in the République Centrafricaine, by LANDAU and CHABAUD (1965) in April 1965, from *Thamnomys rutilans* (Peters, 1876); the particular rodent from which the strain was obtained was captured near a village between Bébé and Boukoko. Although mice inoculated with blood from the *Thamnomys* become infected with both *P. chabaudi* and *P. b. yoelii*, an unmixed line of *P. b. yoelii* arose after the first mosquito transmission in the laboratory. The history of strain 17X since its isolation is shown in Fig. 1.

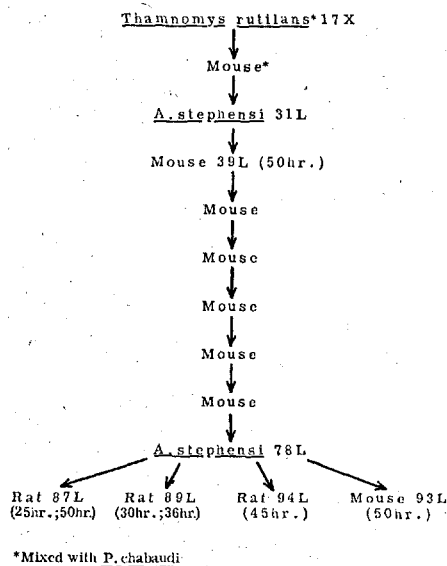


FIG. 1. Showing the history of the type strain of *P. b. yoelii*, No. 17X, from its isolation to the demonstration of the primary exoerythrocytic schizonts; the times in parenthesis indicate the age of the schizonts.

FIG. 1.

The strain of *P. chabaudi* studied (No. 54X) was isolated from another *T. rutilans* captured at the same time as the one from which strain 17X was obtained. The rodent had a patent infection when captured, and subinoculations of blood revealed no other malaria parasite. The history of the strain is shown in Fig. 2.

The strains were subinoculated in laboratory rodents by intraperitoneal or intravenous injections of heparinized blood obtained from either the tail or the retro-ocular plexus of

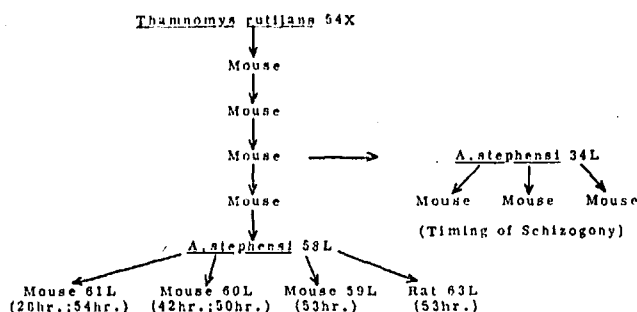


FIG. 2. Showing the history of strain 54X of *P. chabaudi* from its isolation to the demonstration of the primary exoerythrocytic schizonts; the times in parentheses indicate the age of the schizonts.

FIG. 2.

veins. At the beginning of the work, sodium citrate was sometimes employed as an anti-coagulant, but after it was found to be fatal to mice when inoculated intravenously, its use was discontinued.

Mosquitoes

A. stephensi bred at the Malaria Reference Laboratory, Epsom (SHUTE and MARYON, 1966) were obtained through the courtesy of Mr. P. G. Shute. After feeding on infected animals, mosquitoes were kept at 23°–26°C. and a relative humidity of 70–80%.

To permit the mosquitoes to feed, mice were immobilized by a method shown to us by Prof. Meir Yoeli. Thin strips of zinc oxide plaster were first attached to each leg, and the mouse was held in a supine position on a small board of soft wood; the tabs of plaster were then pinned to the board. The abdomen was shaved. Attempts to anaesthetize mice with intraperitoneal or intravenous inoculations of pentobarbitone sodium B.P. were abandoned, as infected mice often succumbed to the normal dose (0.5 mg. per 10 g. body weight), and mosquitoes seemed little attracted to anaesthetized mice, presumably because of a drop in body temperature.

The examination of stages of sporogony

Pigment patterns of oöcysts were drawn from unfixed stomachs gently flattened under a coverslip; measurements were made on similar material. As soon as mature oöcysts were seen, glands were also dissected and examined. On the day or the day after sporozoites were first found in the glands, all surviving mosquitoes were dissected to provide sporozoites for the demonstration of exo-erythrocytic schizonts. No estimation was therefore made of the sporozoite rate.

Sporozoites in Giemsa-stained smears of infected glands were measured by drawing midlines at a magnification of $\times 2,000$, and estimating the length with dividers calibrated to an equivalent of 1 μ .

The collection and inoculation of sporozoites

Laboratory rats (Wistar albinos; 50–70 g.) and mice (Theiler's original strain; 10–15 g.) were given single intravenous inoculations of suspensions of sporozoites from infected salivary glands. At dissection the glands were collected in either cold 20% rat serum in Ringer's fluid, or cold tissue culture medium 199 (MORGAN et al., 1950), which was equally suitable; the volume of fluid was kept as small as possible. The glands were broken up by repeatedly sucking them up and down a finely drawn Pasteur pipette, and the suspensions of sporozoites were then inoculated slowly into the tail veins of rats and mice.

The timing of the tissue schizogony

Blood was taken from the retro-ocular plexus of veins of rats and mice at timed intervals from the inoculation of sporozoites. Thick blood films were prepared, and the remainder of each sample was inoculated into a mouse, blood films from which were examined daily.

Biopsies and autopsies

Liver biopsies were performed on 4 rats and 3 mice by laparotomy under ether anaesthesia. After exposure by a transverse incision immediately below the ribs, part of the

frontal lobe of the liver was removed by a single cut with sharp scissors; cautery was unnecessary.

Complete livers, and in some instances other tissues, were taken post mortem. Portions of liver damaged by prior biopsies were discarded, and tissues were cut into pieces about 5 mm. thick before fixation.

Histological techniques

Pieces of liver taken at biopsy were fixed in Carnoy's fluid for 3 hours; the larger pieces and other tissues taken post mortem were fixed for 4 hours. The method of embedding used was that described by BRAY and GARNHAM (1962).

Serial sections were cut at 4μ and stained by Wolbach's (1911) Giemsa-colophonium method (SHORTT and COOPER, 1948; BRAY and GARNHAM, 1962). Since the cytoplasm of immature schizonts stains very deeply by this method, the nuclei were often difficult to see. For the study of nuclear details some sections were therefore hydrolysed in N/1 hydrochloric acid at 58°C . for 5-8 minutes (WOLCOTT, 1952), before staining by the Giemsa-colophonium method in the usual way. A few individual schizonts were examined by both staining techniques, either by removing the coverslip from Giemsa-stained sections and hydrolysing before restaining, or by staining alternate slides of a series by each of the methods.

Impression smears of liver were allowed to dry, fixed in methanol, and stained in Giemsa's stain for 1 hour.

Sporogony of P.b. yoelii

The first attempts to infect *A. stephensi* with *P.b. yoelii* were immediately successful. This mosquito was chosen because it is known to be a good laboratory host for *P.b. berghei* (YOELI, 1965). The optimum temperature was assumed to be higher than that of the nominate subspecies, because of the climatic differences between the habitats of the natural mosquito hosts.

In the first batch of mosquitoes (31L, see Fig. 1), incubated at $24-26^{\circ}\text{C}$., sporozoites were first found in the salivary glands on the 9th day. The inoculation of a single pair of glands gave rise to an infection in a mouse, and the first exo-erythrocytic schizonts of *P.b. yoelii* were found in the liver of another mouse (No. 39L) which had received glands from only 7 mosquitoes, 3-4 of which were presumed, from the oöcyst rate, to be negative.

In the second batch of mosquitoes (No. 78L), incubated at a slightly lower temperature ($23-24^{\circ}\text{C}$.), sporozoites were not seen in the glands until the 10th day. Numerous tissue schizonts were found in livers of all of three rats and one mouse inoculated with sporozoite suspensions from the glands of this batch.

In spite of slight differences in the temperatures at which the two batches of *A. stephensi* were incubated, there was no noticeable difference in the rates of oöcyst growth. On the 4th day, oöcysts measured $8-17\mu$ in diameter, and contained pigment characteristically arranged in 4 or more straight or slightly curved lines frequently crossing one another (Fig. 3).



FIG. 3. Showing the pigment pattern of 4 day-old oöcysts of *P. b. yoelii* on the stomach of an experimentally infected *A. stephensi*.

FIG. 3.

On the 7th day the first mature oöcysts, measuring $60-82\mu$, were seen; most were, however, still immature, and a noticeable feature was the very wide range of sizes, similar to that seen in *A. dureni* naturally infected with *P.b. berghei*, and in experimentally

infected *A. quadrimaculatus* (VANDERBERG and YOELI, 1965). The oöcyst rate was approximately 50% in both batches of mosquitoes (6 of 13 guts positive in batch 31L, and 6 of 12 in batch 78L); since unfed mosquitoes had not been removed, many of those which were uninfected had presumably not fed upon the mice.

Under a phase-contrast objective, and in stained films, the nuclei of sporozoites in 20% rat serum in Ringer's solution were seen to bulge out from the side of the sporozoite; they were, nevertheless, infective. This phenomenon, which was not seen in sporozoites suspended in medium 199, may represent the first stage of the emergence of a "sporoplasm" through the micropyle of the sporozoite, as suggested by GARNHAM et al. (1961). Living sporozoites examined at room temperature showed no movement other than a slight "flutter" at the extremities. The mean length of 20 sporozoites in a stained smear was 13.8μ ($9-18\mu$).

Primary exo-erythrocytic schizogony of *P.b. yoelii*

The prepatent period of sporozoite-induced infections of *P.b. yoelii* was remarkably short. Blood taken from a rat 43 hours after an intravenous inoculation of sporozoites gave rise to an infection in a mouse, and the first ring forms were seen in a thick blood film from another rat 47 hours after an inoculation of sporozoites.

Primary exo-erythrocytic schizonts were found in the livers of all the 3 rats and 2 mice given single intravenous inoculations of sporozoites; they were always in the parenchyma cells. No schizonts were seen in sections of brain, lung, spleen, kidney and bone marrow of the rat (87L) with the most heavily infected liver, killed at 50 hours. Descriptions of the schizonts seen in livers 25, 30, 36, 45 and 50 hours after intravenous inoculations of sporozoites are given below.

25-hour schizonts (Rat 87L)

1-3 schizonts were found in each section (10 sq. mm.) of a small piece of liver taken at biopsy from rat 87L 25 hours after inoculation with glands from 40 mosquitoes of batch 78L (50% oöcyst rate). The description is based on the examination of more than 30.

At 25 hours, the general appearance of the schizonts is that of small, dark-blue bodies with indistinct nuclei and a fine limiting membrane (Plate 1, Fig. 1). They measure $9.4 \times 6.9\mu$ ($10.7-7.6\mu \times 7.6-6.1\mu$) and fill only half the host cell. The cytoplasm appears as a deep blue reticulum broken by numerous very small vacuoles (0.5μ or less in diameter) most of which lie at the periphery. A few schizonts contain 1-2 larger vacuoles measuring up to 1.5μ .

In sections stained by the ordinary Giemsa-colophonium method, the nuclei are largely obscured by the heavily staining cytoplasm, but after acid hydrolysis they are seen to be round or oval, or occasionally triangular; a few, shaped like a dumb-bell, are reminiscent of cell nuclei in telophase (Plate 1, Fig. 2). The numbers of nuclei counted in serial sections of 4 schizonts ranged from 45 to 65.

30-hour schizonts (Rat 89L)

These forms were found in liver taken at biopsy from rat 89L 30 hours after the inoculation of sporozoites from glands of 25 mosquitoes from batch 78L.

Schizonts were found without difficulty, and although the piece of tissue was small, each section (25 sq. mm.) contained about 12 forms; 30 consecutive schizonts form the basis of this description.

By 30 hours the schizonts have grown to $14.7 \times 13.0\mu$ ($16.8-10.7\mu \times 13.7-10.7\mu$); they do not fill the host cell, the nucleus of which is displaced but unaltered in

appearance (Plate 1, Fig. 3). In section they are round or oval, or occasionally bean-shaped. The pellicle is fine, and is difficult to see in some schizonts.

The cytoplasm is similar to that seen 5 hours earlier, except that by this time a number of densely staining cytoplasmic flocculi have formed. The vacuoles have increased in size and number, and are still lying more at the periphery of the schizonts than in the centre.

The nuclei remain obscured by the cytoplasm, but after acid hydrolysis bizarre forms are seen (Plate 1, Fig. 3). They are much larger than at 25 hours, and appear as crowns, or as irregularly shaped masses often connected by fine strands; the impression is given of very rapid division. It was impossible to count the nuclei accurately.

36-hour schizonts (Rat 89L)

The rat which had provided the material at 30 hours was killed 6 hours later.

During this time, the schizonts grew about half as big again, and now completely filled the host cell (Plate 1, Fig. 4). Mean measurements of 10 schizonts are $22.5 \times 18.8 \mu$ (24.5 – $19.9 \mu \times 21.4$ – 16.8μ). In other respects the 36-hour schizonts do not differ in morphology from those seen 6 hours earlier. The nuclei in hydrolysed sections present the same strange appearance, and although it is still impossible to count them, they have obviously increased in number.

45-hour schizonts (Rat 94L)

Although the rat in which 45-hour schizonts were seen received glands from only 23 mosquitoes (about half of which were infected), the tissue stages were so numerous (20 per section, 25 sq. mm.) that more than 300 were found without difficulty.

At 45 hours the size of the schizonts varies much more widely than at earlier times. Mean measurements of 30 forms are $35.3 \times 28.0 \mu$ (41.3 – $30.6 \mu \times 30.6$ – 22.9μ). A few much smaller schizonts were also seen (e.g. $13.8 \times 9.2 \mu$), but since this size falls so far outside the normal range, their measurements are not included in the figures given above. In spite of their smallness, these forms are otherwise quite normal in appearance, and are indistinguishable from those seen at 30 hours.

The variation in size was accompanied by a similarly wide variation in form, ranging from the small schizonts mentioned above to a few fully mature forms, and even to ruptured schizonts invaded by phagocytes.

Most 45-hour schizonts are oval in shape, although in section some appear to be round. The smaller schizonts generally have a smooth clear-cut outline, but as they grow larger this becomes less regular. Growth of the schizont sometimes results in a pedicle pushing between the hepatic cells, like a pseudopodium of an amoeba; the examination of serial sections reveals that 3–4 of these lobose arms may be present in a single schizont. In section the false impression is often given of one or more small schizonts lying close to a larger one.

Some nuclei of host parenchyma cells stain very palely, but are otherwise unaltered.

The bewildering range of size and form makes it difficult to describe a typical 45-hour schizont, but the form illustrated in Plate 1, Fig. 5, is apparently the next growth stage from the 36-hour forms. The very small vacuoles seen at all earlier times are still present, and larger vacuoles with a clear-cut outline are seen for the first time. The cytoplasmic clumps are still a prominent feature, and are larger than those seen earlier. The small nuclei are unevenly distributed, and some parts of the cytoplasm contain none. As the schizont becomes larger, the big vacuoles increase in size but become fewer in number; they are occasionally completely surrounded by cytoplasm, but,

when followed in serial sections, most of the large vacuoles are seen to connect with the fine pellicle of the schizont.

The largest immature schizonts seen at 45 hours are indistinguishable from similar forms seen at 50 hours (Plate 1, Fig. 6). The deeply staining cytoplasmic clumps are still conspicuous, and small vacuoles persist, particularly at the periphery and around the clumps of cytoplasm. The nuclei, which at this point do not stain deeply, are partially obscured by the cytoplasm.

Mature schizonts found at 45 hours are identical with those seen at 50 hours described below.

50-hour schizonts (Mouse 39L; Mouse 93L; Rat 87L)

More than 1,000 schizonts were examined in the livers of 3 rodents 50 hours after the inoculation of sporozoites; no differences were seen between schizonts in the livers of mice and a rat. The description is based upon forms found in the liver of the most heavily infected rodent, rat 87L, the same animal from which a small piece of liver had been obtained at biopsy at 28 hours and which had received glands from 40 mosquitoes from batch 78L (50% oöcyst rate); about 40 schizonts were found in each section (35 sq. mm.).

At 50 hours most schizonts are oval or round. The outline is occasionally irregular, particularly that of large schizonts, and a few lobed forms similar to those seen at 45 hours are present. From 45 to 50 hours the mean diameter of the schizonts increases by only 5-6 μ ; mean measurements of 30 consecutive schizonts are $43.3 \times 31.3 \mu$ (55.5 - $36.7 \mu \times 36.7$ - 22.9μ). The measurements of 3 very small forms not exceeding 15 μ in diameter are not included in these figures.

Most of the schizonts seen at 50 hours fall into one of two groups easily recognized by different staining reactions. Most of the large, yet immature, schizonts stain deep blue and stand out against the paler-staining parenchyma cells (Plate 1, Fig. 6), whereas mature schizonts containing merozoites stain light purple and are immediately recognizable even at a low magnification (Plate 1, Fig. 9).

The basophilic aggregations of cytoplasm seen in younger schizonts are still present in immature schizonts at 50 hours (Plate 1, Fig. 6).

A few immature schizonts display a separation of the cytoplasm into irregularly shaped masses, or pseudocytomeres (GARNHAM, 1951), which remain connected to one another. These appear to have shrunk away from the host cell (Plate 1, Fig. 7), an appearance which is apparently associated with the cytoplasmic separation since it is not seen in other schizonts, without pseudocytomeres, lying nearby. Only few of these forms are present, and this suggests that either this represents a fleeting pattern in development, or all schizonts do not grow in the same way.

At a point when nuclear division is apparently complete, but merozoites are not yet formed, "cracks" are sometimes seen in the schizont, giving a mosaic-like appearance (Plate 1, Fig. 8). When hydrolysed, the nuclei of such schizonts are found to be arranged in a pattern of circles, with a suggestion of a palisade of nuclei at the periphery (Plate 2, Fig. 4).

Partially mature schizonts similar to those described for *P. cynomolgi* and *P. ovale* (BRAY, 1957; BRAY et al., 1963) are commonly seen. In these, merozoites form first at the periphery of the schizont and lie around a central schizogonic mass.

Merozoites are evenly distributed in fully mature schizonts (Plate 1, Fig. 9), and the basophilic aggregations, a marked feature of immature stages, are absent; occasionally, large vacuoles persist. In stained liver smears containing ruptured schizonts, merozoites appear as round or pyriform compact bodies, with an elongate or round nucleus lying to

one side. The number of nuclei in hydrolysed serial sections of mature 50-hour schizonts ranges from 7,500 to 8,000.

In general, the response of the rodent hosts to the presence of the tissue forms is no different from the response in primate malaria (SHORTT and GARNHAM, 1948; GARNHAM and BRAY, 1956; BRAY et al., 1963), and once merozoites are released, phagocytes quickly enter (Plate 2, Fig. 3). As described by YOELI and MOST (1965b) in studies on *P.b. berghei*, the place occupied by a schizont may become completely filled by leucocytes.

Some schizonts with nuclei arranged in conspicuous patterns are very different from those described above; most fall into one of two groups. Firstly, schizonts exhibiting pseudocytomeres, in which all the cytoplasm is clumped into masses staining very deep blue (Plate 2, Fig. 1). After hydrolysis, the nuclei are seen to be distributed at the periphery of the cytoplasmic aggregations (Plate 2, Fig. 2). Phagocytes are attracted to some such forms, and this suggests that they may be dying.

Secondly, schizonts with unusually few nuclei arranged in circles, and in a palisade. The cytoplasm of these forms stains deep blue and the nuclei pale pink. After hydrolysis, the comparatively large nuclei stain deep purple, and parts of the schizont are seen to contain no nuclei (Plate 2, Fig. 5). Phagocytes are usually gathered close to these forms. Since rupturing schizonts with many more nuclei sometimes show a similar pattern (Plate 2, Fig. 3), this form may be a degenerating "skeleton" of a partially mature schizont from which merozoites have been released.

Among the very small forms seen at 50 hours, were 2 with only 4 or 5 large nuclei formed by beads of chromatin arranged in circles or semicircles, and with a pale-staining vacuolated cytoplasm (Plate 2, Fig. 7). No phagocytes were found near either of these schizonts, and we believe that they are simply slowly growing forms.

At 15 and 21 days after the inoculation of sporozoites, no schizonts were found in the livers of two rodents, which had previously undergone biopsies, and had been found to be infected.

Sporogony of *P. chabaudi*

A. stephensi were easily infected with *P. chabaudi*. Sporogony was studied in two batches of mosquitoes, and sporozoites from both groups gave rise to primary exo-erythrocytic schizonts in the livers of laboratory rodents.

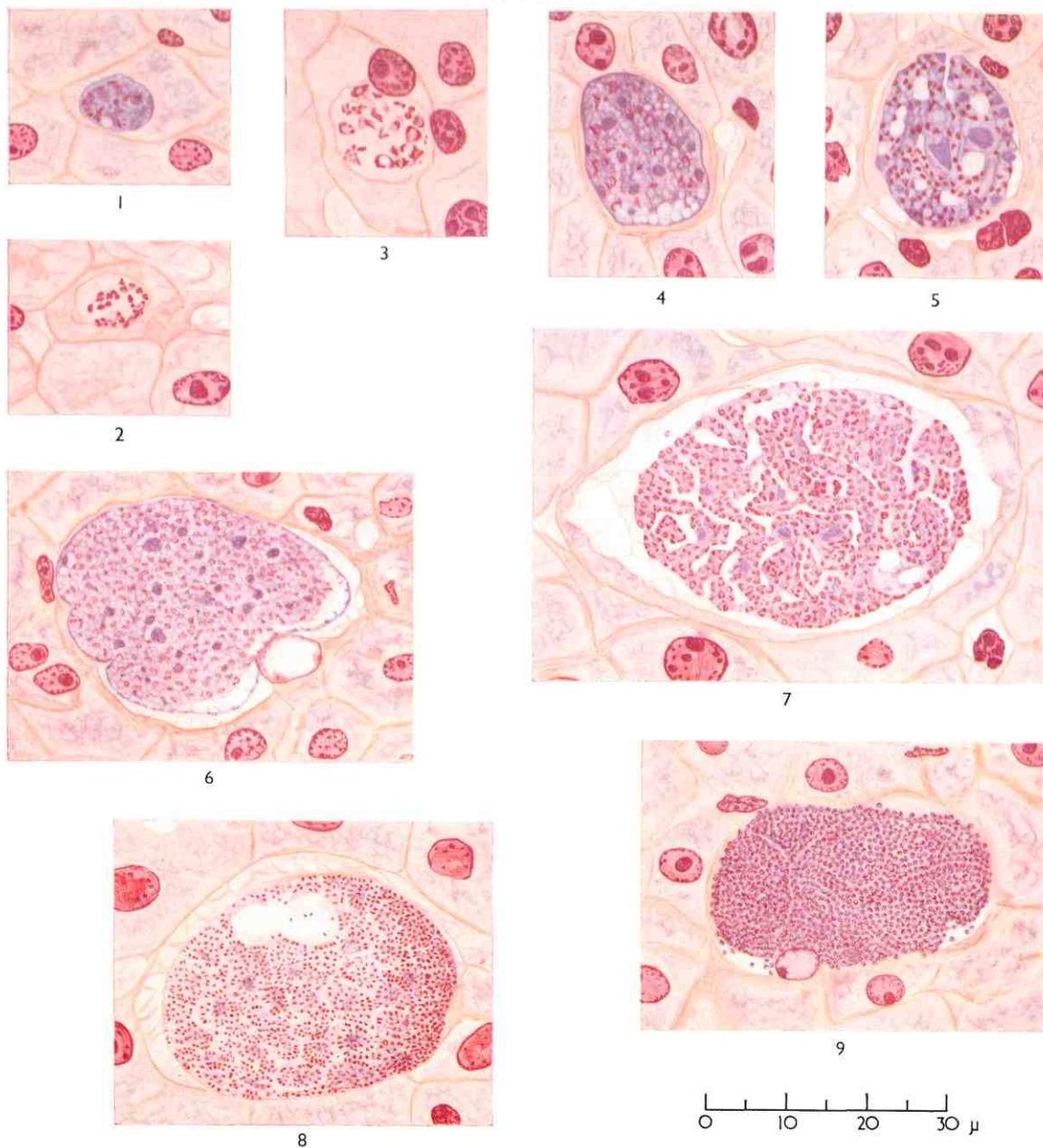
At 25°C. sporozoites were first seen in the salivary glands on the 11th day. At 4 days, oöcysts measured 8-12 μ ; unlike the pigment of 4-day oöcysts of *P.b. yoelii* (Fig. 3), this pigment is in large, almost black grains tending to lie in a loose clump or, occasionally, forming short lines crossing one another. On the 6th day, the largest oöcyst measured 17 μ . A wide range of oöcyst size was recorded on the 10th day (45-74 μ), and almost fully formed sporozoites were present in the largest oöcysts. Fully mature oöcysts on the 11th day measured up to 80 μ .

In saline preparations of crushed glands sporozoites measured 15 μ ; in stained smears the mean length of 20 sporozoites was 13.2 μ (10.0-15.5 μ). As with *P.b. yoelii*, sporozoites suspended in 20% rat serum in Ringer's solution showed marked bulging of their nuclei; but were infective to mice. This phenomenon was not seen when sporozoites were suspended in culture medium 199.

There was a difference between the oöcyst rate and numbers of oöcysts in the two batches of mosquitoes. In the first batch, No. 34L, approximately 120 out of 200 mosquitoes fed on a mouse 7 days after it had been inoculated intraperitoneally with infected blood. 5-30 oöcysts were seen on the midguts of 4 of 14 mosquitoes dissected (28%).

PLATES 1-4. Primary exo-erythrocytic schizonts of *P. b. yoelii* and *P. chabaudi* in 4 μ sections of liver from experimentally infected rats (R) and mice (M). Carnoy-fixed material. Giemsa-colophonium stain. Magnification $\times 1,200$.

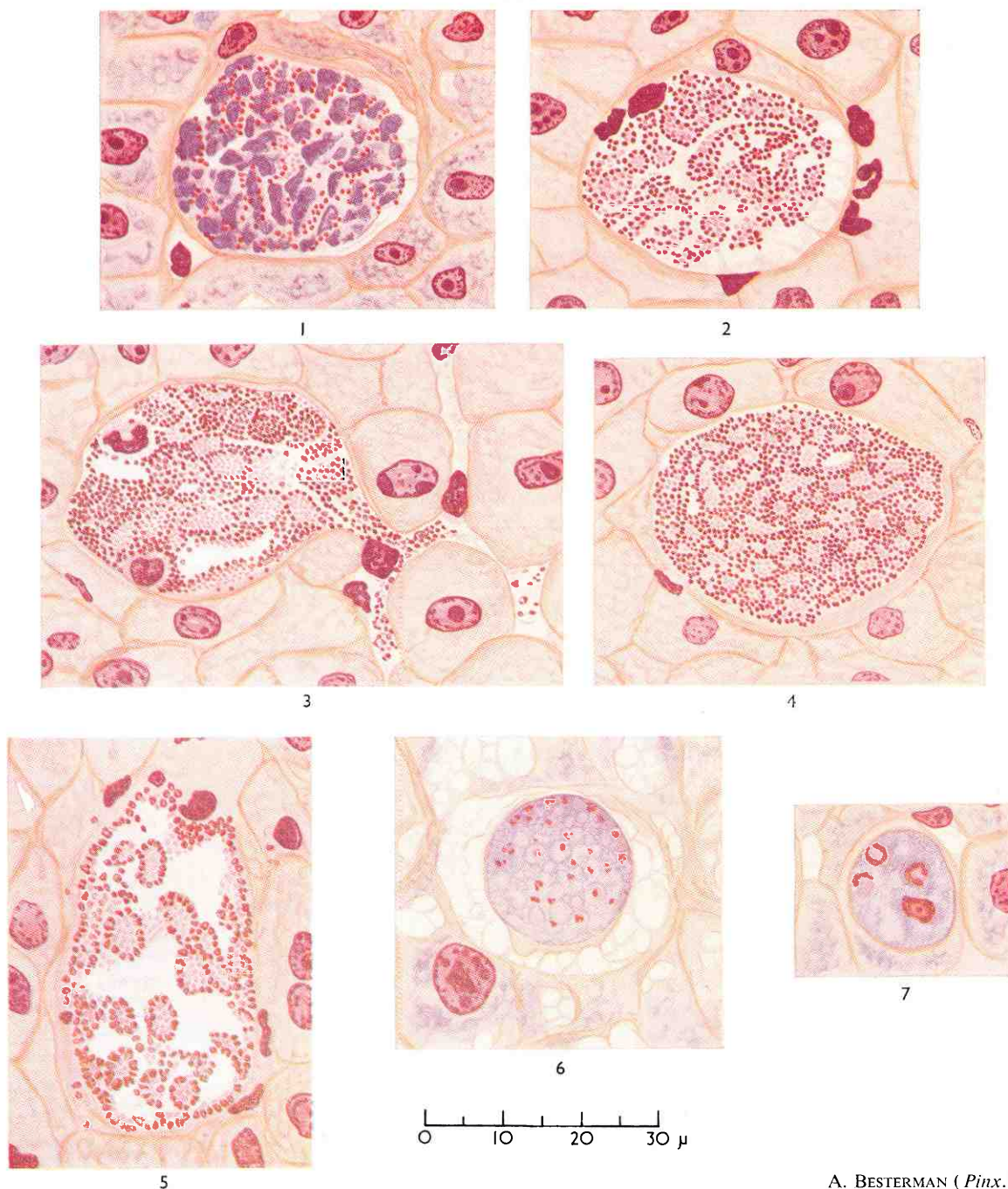
PLATE 1



A. BESTERMAN (*Pinx.*)

PLATE 1. *P. b. yoelii*. Fig. 1. 25 hours (R). Fig. 2. 25 hours. Hydrolysed. Note number of nuclei (R). Fig. 3. 30 hours. Hydrolysed. Note shapes of nuclei (R). Fig. 4. 36 hours. Note basophilic aggregations of cytoplasm and peripheral vacuoles (R). Fig. 5. 45 hours (R). Fig. 6. 50 hours (R). Fig. 7. 50 hours. Note pseudocytomere formation and contraction away from host cell (M). Fig. 8. 50 hours. Almost mature. Note nuclear pattern (R). Fig. 9. 50 hours. Mature (M).

PLATE 2



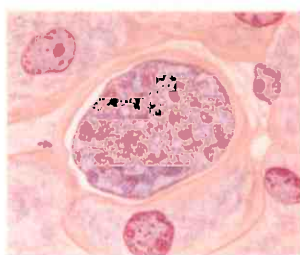
A. BESTERMAN (Pinx.)

PLATE 2. *P. b. yoelii*. 50-hour schizonts (except fig. 6). Fig. 1. Note pseudocytomeres (R). Fig. 2. A section of the same schizont as Fig. 1. Hydrolysed. Note pattern of nuclei and presence of phagocytes (R). Fig. 3. A rupturing schizont. Hydrolysed. Note phagocytes (R). Fig. 4. An almost mature schizont. Hydrolysed. Note pattern of nuclei (R). Fig. 5. Possibly the remains of a partially mature schizont after release of merozoites. Hydrolysed. Note phagocytes (R). Fig. 6. A schizont of uncertain identity in the liver of *T. rutilans* naturally infected with *P. b. yoelii* and *P. chabaudi*. Note small number of nuclei, staining reaction of cytoplasm and obvious limiting membrane. Fig. 7. An unusually small schizont (at 50 hours). Note beaded nuclei (R).

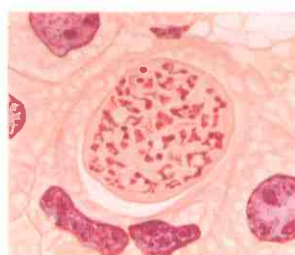
PLATE 3



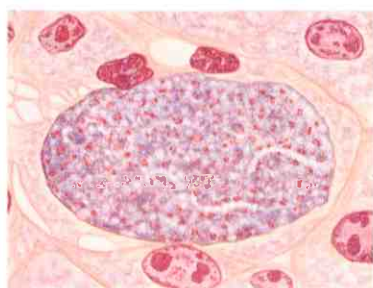
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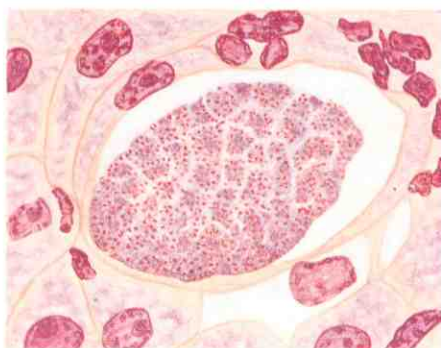
2



3



4



5



6

0 10 20 30 μ



7

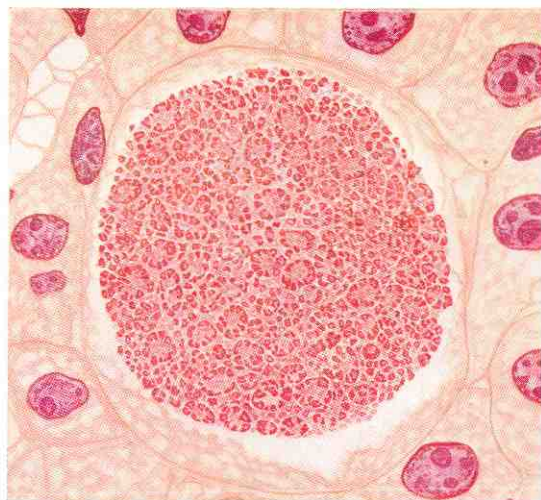
A. BESTERMAN (Pinx.)

PLATE 3. *P. chabaudi*. Fig. 1. 28 hours. Hydrolysed. Note number of nuclei (M). Fig. 2. 42 hours. Note basophilic aggregations of cytoplasm and obscured nuclei (M). Fig. 3. 42 hours. Hydrolysed. Note shapes of nuclei (M). Fig. 4. 50 hours (M). Fig. 5. 54 hours. Note pseudocytomere formation and contraction away from host cell (M). Fig. 6. 54 hours. Almost mature (M). Fig. 7. 53 hours. Mature (M).

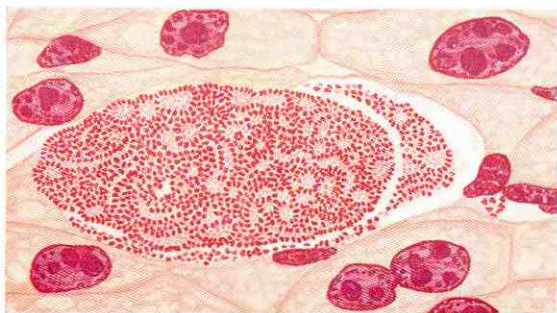
PLATE 4



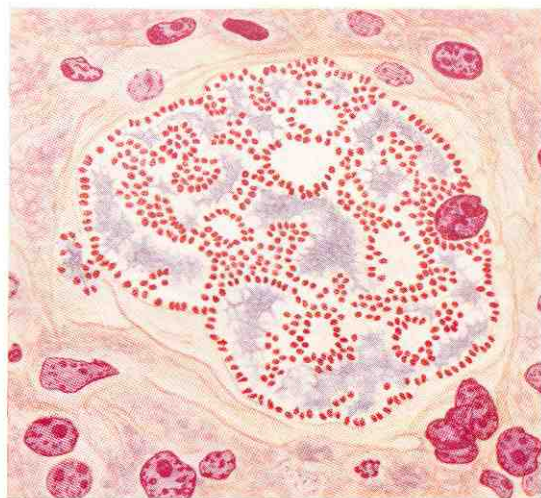
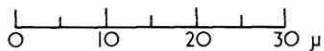
1



2



3



4

A. BESTERMAN (Pinx.)

PLATE 4. *P. chabaudi*. Fig. 1. 53 hours. Partially mature. Note free merozoites and phagocytes (M). Fig. 2. 53 hours. Hydrolysed. Last division of nuclei (M). Fig. 3. 53 hours. Hydrolysed. Note released merozoites at right, polar, sleeve-like lobe and pattern of nuclei (M). Fig. 4. 53 hours. Unusual form. Note number, size and arrangement of nuclei ; also phagocytes (R).

In the second batch, No. 58L, about 95% of the mosquitoes fed on a mouse 6 days after inoculation. The oöcyst rate was 80% (11 of 14) and 50-200 oöcysts were seen on each infected gut. Because of a drop in temperature from 25° to 23°C. on the 10th and 11th days, the oöcyst growth in this batch was slightly delayed. Very few sporozoites were present in the glands on the 11th day but the glands of one of two mosquitoes dissected on the 12th day were heavily infected.

Primary exo-erythrocytic schizogony of *P. chabaudi*

Subinoculation of blood from mice given intravenous inoculations of sporozoites from the glands of infected mosquitoes (batch 34L) showed that the earliest primary exo-erythrocytic schizonts of *P. chabaudi* mature in 52-53 hours.

Schizonts were found in sections of liver, taken at biopsy or autopsy, from a series of mice 28, 42, 50, 53 and 54 hours, after single intravenous inoculations of sporozoites from the glands of 60 mosquitoes of the second batch (58L; 80% oöcyst rate); each of 3 mice received sporozoites from the glands of 20 mosquitoes. Schizonts were also seen in sections of a piece of liver taken at biopsy from a laboratory rat 53 hours after the intravenous inoculation of sporozoites from the glands of 18 mosquitoes; no parasitaemia developed in the rat.

In many ways the primary exo-erythrocytic schizonts of *P. chabaudi* resemble those of *P.b. yoelii*. Deeply staining cytoplasmic clumps, marked nuclear patterns and a sponge-like appearance due to the presence of tiny vacuoles are common to both parasites. The main differences are in the slower growth and the larger number of merozoites of the tissue forms of *P. chabaudi*. All the schizonts were in parenchyma cells, the nuclei of which remained unaffected by the parasite.

28-hour schizont (Mouse 61L)

A single 28-hour schizont was found in 200 sections (24 sq. mm.) of a piece of mouse liver taken at biopsy.

The schizont was first seen as a densely staining blue body, 9 μ in diameter, lying within a binucleate parenchyma cell. On re-staining in Giemsa's stain after acid hydrolysis it is seen to contain approximately 20 irregularly shaped nuclei (Plate 3, Fig. 1); it fills little of the host cell, and is similar in size to the parenchyma cell nuclei.

42-hour schizonts (Mouse 60L)

About 4 schizonts were found in each section (15 sq. mm.) of a small piece of liver taken at biopsy from a mouse 42 hours after the inoculation of sporozoites; more than 50 schizonts have been examined.

In size and morphology, the 42-hour schizonts of *P. chabaudi* are similar to 36-hour forms of *P.b. yoelii*. They measure $23.1 \times 11.8 \mu$ ($26.0-19.9 \mu \times 19.9-10.5 \mu$) in diameter, and are round or oval in section, with a fine limiting membrane. Their outline is often irregular. The cytoplasm is broken by small indistinct vacuoles, and deep blue cytoplasmic aggregations are almost always a prominent feature (Plate 3, Fig. 2). In sections stained by the Giemsa-colophonium method the nuclei are partially obscured by the deep blue cytoplasm, but after hydrolysis they are revealed as irregularly shaped masses often connected to one another by fine strands of chromatin (Plate 3, Fig. 3). Because of their bizarre shapes, an accurate count of the nuclei is impossible.

50-hour schizonts (Mouse 60L)

The mouse on which a biopsy had been performed at 42 hours was killed 8 hours later.

Between 42 and 50 hours the schizonts had grown considerably; mean measurements are $36.2 \times 23.7 \mu$ ($43.7-26.2 \mu \times 31.2-18.7 \mu$).

The cytoplasm stains deep blue and is broken by numerous very small vacuoles; the cytoplasmic aggregations seen at 42 hours are still present (Plate 3, Fig. 4). The nuclei are round or oval, and are evenly distributed throughout the schizont. In sections 3μ thick they appear as groups of 3-4 small "points." Although an accurate count was not possible, the number of nuclei is obviously greater than at 42 hours.

53- and 54-hour schizonts (Rat 63L; Mouse 59L; Mouse 61L)

About 80 schizonts were studied in sections of the livers of 3 rodents 53 and 54 hours after single inoculations of sporozoites. A liver biopsy was performed at 53 hours on rat 63L; mouse 59L was killed at 53 hours and mouse 61L, which had undergone a biopsy at 28 hours, was killed at 54 hours. There are 1-4 schizonts in each section (30-60 sq. mm.) of liver from all three rodents. Except for a few unusual schizonts described below, the forms seen in the liver of the rat are indistinguishable from those in the livers of the mice.

Mean measurements of 16 53-hour schizonts are $44.2 \times 29.7 \mu$ ($53.5-33.7 \mu \times 33.7-24.5 \mu$). They are usually oval, and sleeve-like lobes at one pole are common (Plate 4, Fig. 3).

At 53 and 54 hours, nearly all the immature schizonts seen have apparently completed their last nuclear division. The nuclei are very small and numerous and, when stained by the Giemsa-colophonium method, their red colour imparts a pink hue to the pale-blue cytoplasm. Blue cytoplasmic aggregations occur in immature schizonts (Plate 3, Fig. 6) and even persist in some fully mature forms (Plate 3, Fig. 7). Some schizonts contain a large vacuole.

Merozoites form first at the periphery, and are often released before full maturity. We estimate there are 18-20,000 merozoites in serial sections of fully mature schizonts. Rupturing schizonts are very quickly invaded by leucocytes (Plate 4, Fig. 1), and the cellular response of the host is the same as that seen in infections of *P.b. berghei* (YOELI and MOST, 1965b) and *P.b. yoelii*.

A few forms show a formation of pseudocytomeres similar to that demonstrated in a schizont of *P.b. berghei* by GARNHAM et al. (1966), and by us in *P.b. yoelii* (compare Plate 1, Fig. 7 and Plate 3, Fig. 5). Such schizonts are typically contracted away from the host cell cytoplasm, and in section give the appearance of a mosaic. As with *P.b. yoelii* the scarcity of these forms suggests that this is a fleeting stage shortly before the final nuclear division.

Hydrolysis revealed 2 types of nuclear patterns. Firstly, in large immature schizonts apparently in the final nuclear division, the nuclei are large, triangular or irregular in shape, and are arranged in rosettes (Plate 4, Fig. 2). Secondly, when the last nuclear division is complete and merozoite formation is in progress, the nuclei appear as small oval or round dots arranged in circles or tubes, and tend to form a palisade at the periphery of the schizont (Plate 4, Fig. 3).

Among the 53-hour schizonts in the liver of rat 63L there were a few unusual forms which were never seen in the livers of mice (Plate 4, Fig. 4). They are round or somewhat irregular in shape, and are always large (40μ in diameter). The nuclei, which are comparatively few, are larger than those of other schizonts of *P. chabaudi*, and are round or oval. They stain bright red by the Giemsa-colophonium method, and are arranged in roughly circular groups, and in a palisade at the periphery of the schizont with the longitudinal axes of the nuclei pointing towards the centre. A loose basophilic reticulum lies in clumps between the groups of nuclei. Phagocytes are usually found lying against

the schizont, and in some instances, one or two seem to be just within the pellicle. Because of the host's reaction to these unusual schizonts, we believe them to be abnormal; possibly they are the remains of a ruptured, partially mature form and may be the equivalent in *P. chabaudi* to somewhat similar forms of *P.b. yoelii* occasionally seen at 50 hours (Plate 2, Fig. 5).

Schizonts in the livers of wild-caught *Thamnomys rutilans*

LANDAU and CHABAUD (1965) briefly describe some schizonts found in the parenchyma cells of the liver of a *T. rutilans*, from the République Centrafricaine, killed 10 days after capture. The rodent was infected with both *P. chabaudi* and *P.b. yoelii*, and the schizonts were thought to be secondary exo-erythrocytic forms of one or other of these parasites. Since this discovery one of us (I.L.) has found similar schizonts in the liver of a *Thamnomys* which had been in captivity for 8 months, and which was also infected with both plasmodia.

Schizonts from both rodents are round or oval, and have a conspicuous limiting membrane $0.5\ \mu$ thick which is PAS-negative. The forms found in the first *Thamnomys* measure approximately $25\ \mu$ (LANDAU and CHABAUD, 1965), whereas those in the second rodent are larger; most are about $30\ \mu$ in diameter, and one, the largest found, is $48 \times 35\ \mu$. The cytoplasm, which is vacuolated, stains pale blue by the Giemsa-coloophonium method, and contains a number of very small PAS-positive granules. The nuclei, which are few (e.g. 150 nuclei in serial sections of a schizont $30\ \mu$ in diameter), are round or irregularly shaped masses, and stain deep red (Plate 2, Fig. 6). The nuclei of some host cells are markedly enlarged. Mature schizonts containing merozoites have not been found.

Discussion

The blood stages of *P.b. berghei* and *P.b. yoelii* appear to be morphologically indistinguishable, and the separation into subspecies is based upon differences in the sporogony and primary exo-erythrocytic schizogony (LANDAU and KILLICK-KENDRICK, 1966). The important criteria are among those used to separate 3 subspecies of *P. cynomolgi* (GARNHAM, 1959; DISSANAIKE et al., 1965).

In the sporogony, an obvious morphological difference is in the diameter of the mature oöcyst. Those of *P.b. berghei*, grow to approximately $40\ \mu$ (RODHAIN et al., 1955; YOELI and MOST, 1960), whereas those of *P.b. yoelii* reach $60-80\ \mu$. A second difference is in the temperatures at which infective sporozoites are produced. The optimum temperature for the sporogony of the nominate subspecies is unusually low ($19-21^{\circ}\text{C}.$), and if sporozoites are produced in oöcysts at $24^{\circ}\text{C}.$ or above, they fail to reach the salivary glands of experimentally infected mosquitoes, and are uninfective when taken from crushed oöcysts (VANDERBERG and YOELI, 1965). In contrast, infective sporozoites of *P.b. yoelii* are easily obtained in the glands of mosquitoes incubated at $25^{\circ}\text{C}.$ This difference is presumably related to the temperature at which sporogony of the two parasites occurs in nature.

While the present work was in progress, Professor Garnham and one of us (R. K.-K.) obtained a series of schizonts of *P.b. berghei* in the livers of laboratory rats 30, 36, $45\frac{1}{2}$ and $50\frac{1}{2}$ hours after single intravenous inoculations of sporozoites from the glands of experimentally infected *A. stephensi* incubated at $19-20^{\circ}\text{C}.$ Professor Garnham very kindly made this material available to us, and we are thus able to compare the tissue

stages of *P.b. yoelii* and *P.b. berghei* in two series of rat livers obtained in similar conditions.*

The most obvious difference is in size. Measurements of tissue forms of both parasites are shown in Table I, and in Fig. 4 the means of the diameters are plotted

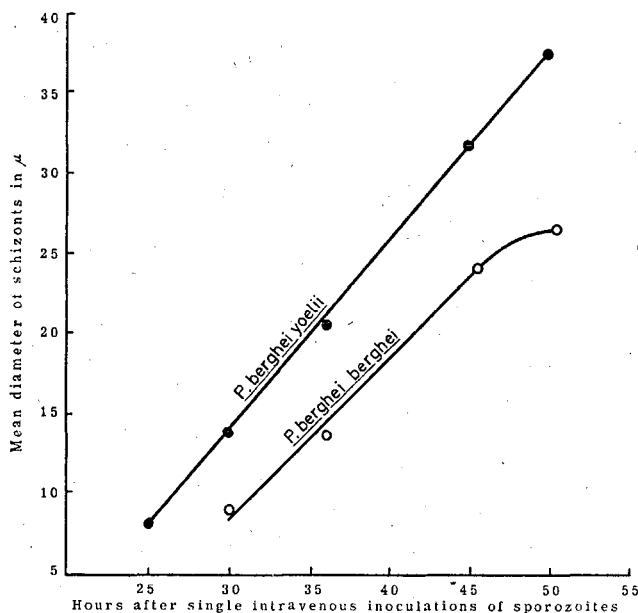


FIG. 4. Comparing the rates of growth of the primary exoerythrocytic schizonts of *P. b. yoelii* and *P. b. berghei* in the livers of laboratory rats from 25 to 50 hr. after single intravenous inoculations of sporozoites.

FIG. 4.

against the number of hours after the inoculation of sporozoites. The lines of the graph represent parts of normal growth curves, and at each point the schizonts of *P.b. yoelii* are seen to be larger than those of *P.b. berghei*.

Our measurements of tissue forms of *P.b. berghei* differ from those given by YOELI and MOST (1965b), but since nearly all the experiments reported by these workers involved more than one inoculation of sporozoites, the exact age of many of the schizonts they describe is uncertain. In the liver of their *Thamnomys* 134, however, which was killed 51 hours after the intravenous inoculation of sporozoites from crushed oöcysts and infected glands of *A. stephensi*, Yoeli and Most found schizonts with a wide range of sizes, and record a mean diameter much larger than ours. The largest of 18 schizonts was $65 \times 48 \mu$, and the smallest $36 \times 32 \mu$; the mean diameter of "nearly mature schizonts" is given as $43 \times 39 \mu$. At $50\frac{1}{2}$ hours after the inoculation of sporozoites of the same strain we found that the diameter of 40 consecutive schizonts of *P.b. berghei*, in the liver of a laboratory rat, varied from $33.7\text{--}26.0 \mu \times 27.5\text{--}19.9 \mu$, with a mean of $30.5 \times 22.4 \mu$. The difference is not easily explained, but may be due to the differ-

*Ideally the comparison should have been made between exo-erythrocytic schizonts of *P. b. yoelii* and those of the type strain of *P. b. berghei*. Unfortunately all early strains of the nominate subspecies no longer produce gametocytes, and such a comparison is now impossible. The strain studied, however, No. NK65, was isolated from the type invertebrate host, *A. dureni*, caught in the type locality (YOELI and MOST, 1965b), and among many similar isolations no subspecific differences have yet been recognized.

TABLE I. Comparison of the mean diameters of primary exo-erythrocytic schizonts of *P. b. berghei* and *P. b. yoelii* in sections of liver of rats. The figures in parentheses are the combined means of the average largest and smallest diameters

Age of schizont (hours)	<i>P. b. yoelii</i>			<i>P. b. berghei</i>		
	No. measured	Mean diameters (μ)	Range of diameters (μ)	No. measured	Mean diameters (μ)	Range of diameters (μ)
25	6	9.4 $\times$ 6.9 (8.1)	10.7 – 7.6 $\times$ 7.6 – 6.1			
30	10	14.7 $\times$ 13.0 (13.8)	16.8 – 10.7 $\times$ 13.7 – 10.7	3	10.0 $\times$ 7.9 (8.9)	12.6 – 7.9 $\times$ 7.9
36	10	22.5 $\times$ 18.8 (20.6)	24.5 – 19.9 $\times$ 21.4 – 16.8	10	16.5 $\times$ 10.7 (13.6)	19.9 – 15.3 $\times$ 12.2 – 9.2
45*	30	35.3 $\times$ 28.0 (31.7)	41.3 – 30.6 $\times$ 30.6 – 22.9	40	27.3 $\times$ 20.6 (23.9)	30.6 – 24.4 $\times$ 22.9 – 18.4
50*	30	43.3 $\times$ 31.3 (37.3)	55.5 – 36.7 $\times$ 36.7 – 22.9	40	30.5 $\times$ 22.4 (26.4)	33.7 – 26.0 $\times$ 27.5 – 19.9

*Schizonts of *P. b. berghei* were half an hour older than these times.

TABLE II. The main differences between *P. b. berghei* and *P. b. yoelii*

	Locality	Principal vertebrate host	Invertebrate host	Size of mature oöcyst	Temperature of sporogony	Mean diameter of exo-erythrocytic schizonts at 50 hours (in laboratory rats)	Approximate no. of merozoites in mature schizonts at 50 hours (in laboratory rats)
<i>P. b. berghei</i>	Katanga	<i>Thamnomys surdaster</i>	<i>Anopheles durenii</i>	35-40 μ	19-21°C. (sporozoites not infective at 24°C.)	26 μ	3,000-4,000
<i>P. b. yoelii</i>	République Centrafricaine	<i>Thamnomys rutilans</i>	Not known (No record of <i>A. durenii</i> in the locality)	60-80 μ	Sporozoites infective at 24-26°C.	37 μ	8,000

ence in rodent host. In spite of the lack of agreement in these measurements, the conclusion that the tissue forms of *P.b. yoelii* from 25 hours to maturity are larger than those of *P.b. berghei* remains valid, since it is based upon two series of schizonts obtained and measured in very similar conditions; it is an important character separating the subspecies.

There are two other differences in the morphology of the primary exo-erythrocytic schizonts. Firstly, the nuclei of large but immature schizonts of *P.b. yoelii* are arranged in a striking pattern (Plate 2, Fig. 4) which is much less well defined in tissue forms of *P.b. berghei* at a similar point of growth. Secondly, mature schizonts of *P.b. yoelii* in the livers of rats 50 hours after the inoculation of sporozoites contain approximately 8,000 merozoites, whereas, in the material examined by us, those of *P.b. berghei* at the same time, and in the same host, contain only 4,000. The main differences between the subspecies are shown in Table II.

The schizonts of *P. chabaudi* differ in a few ways from those of the two subspecies of *P. berghei*; they take a longer time (53 hours) to mature, and produce an even larger number of merozoites (18,000–20,000). In other respects, however, schizonts of *P. chabaudi* are like those of *P.b. yoelii*. Both grow larger than the tissue forms of *P.b. berghei* (37 μ in diameter compared with 26 μ), and large immature schizonts of both parasites from the République Centrafricaine exhibit nuclear patterning to the same degree. Similarly, in the sporogony, the mature oöcysts of *P. chabaudi* and *P. yoelii* reach a much greater size than those of *P.b. berghei* (80 μ compared with 40 μ) and it thus seems that the parasites from the République Centrafricaine share some characteristics not shared by the nominate subspecies of *P. berghei*.

Secondary exo-erythrocytic schizonts of rodent plasmodia have not been demonstrated in experimentally infected rodents. When the schizonts in the liver of a naturally infected *T. rutilans* captured in the République Centrafricaine, and kept in the laboratory for 10 days before examination, were first found, they were assumed to be secondary forms of one of the two malaria parasites present in the blood of the animal (LANDAU and CHABAUD, 1965). Since these forms are now known to differ morphologically from the primary exo-erythrocytic schizonts of *P.b. yoelii* and *P. chabaudi*, this identification is less certain. The only schizonts we saw in experimentally infected rodents with any similarity to the forms in the *Thamnomys* (Plate 2, Fig. 6), were 2 unusually small schizonts in the liver of the rat infected with *P.b. yoelii*, and killed at 50 hours (Plate 2, Fig. 7). Unlike what is thought to occur in the primate plasmodia, perhaps slowly growing relapse forms of rodent malaria parasites arise directly from sporozoites, or perhaps rodent plasmodia have secondary exo-erythrocytic schizonts bearing little resemblance to the primary forms.

Elsewhere we have briefly commented upon the relationships between 4 malaria parasites of African rodents, and have suggested that they represent two examples of *vicariance* (LANDAU and KILLICK-KENDRICK, 1966). In Katanga, *P.b. berghei* is a parasite of *T. surdaster*, whereas in the République Centrafricaine the closely related rodent, *T. rutilans*, is commonly infected with *P.b. yoelii*; the blood forms are very similar, but the subspecies can be differentiated by the characters of the sporogony and primary exo-erythrocytic schizogony. Similarly, *P. vinckei* occurs in Katanga, and *P. chabaudi* in the République Centrafricaine; these smaller parasites can be separated from one another by morphological differences in their blood forms, and by differences in their effect upon infected erythrocytes. More examples of *vicariance* among the malaria parasites of the African Muridae may be revealed after a study of the life-cycles of two plasmodia—one of the *berghei*-group, and the other of the *vinckei*-group—recently reported in *Thamnomys* captured near Brazzaville in the Congo Republic (ADAM et al., 1966).

The new knowledge of the relationship between the two subspecies of *P. berghei* leads us to suggest that if it becomes possible to compare the sporogony and primary exo-erythrocytic schizogony of *P. chabaudi* and *P. vinckei*, any differences revealed may be so similar to those between *P.b. yoelii* and *P.b. berghei* that *P. chabaudi* may be more correctly considered as a subspecies of *P. vinckei*. We think it best, however, to make no change in the position of *P. chabaudi* until more is known of the newly isolated *vinckei*-group plasmodium from Brazzaville (ADAM et al., 1966); new light may then be thrown on the relationships of the parasites of the whole group.

We believe that *P.b. yoelii* and *P. chabaudi* will be among the most useful plasmodia available in the laboratory for studies on sporozoite-induced mammalian malaria, particularly in studies on immunity, chemoprophylaxis and chemotherapy. Because of the high incidence in wild *T. rutilans*, new strains are easily obtained, and, unlike the malaria parasites of monkeys, suitable vertebrate hosts, laboratory rats and mice, are cheap and easily housed. *A. stephensi*, a mosquito which has long been established in laboratory colonies, is a good invertebrate host* in which infective sporozoites of both parasites are readily produced; furthermore, when transmitting *P.b. yoelii* and *P. chabaudi* through mosquitoes in the laboratory there is no need to maintain a cool insectarium such as is essential for the successful sporogony of *P.b. berghei*.

Summary

The sporogony and primary exo-erythrocytic schizogony of two rodent plasmodia recently discovered in the République Centrafricaine were studied in *Anopheles stephensi*, and in laboratory rats and mice given single intravenous inoculations of sporozoites.

Oöcysts of both *Plasmodium berghei yoelii* and *P. chabaudi* grow to a diameter of 80 μ at a temperature of 25°C. Sporozoites of the former parasite enter the glands of *A. stephensi* on the 9th day, and of the latter on the 11th day. The mean length of sporozoites of both parasites is between 13 and 14 μ .

Descriptions are given of schizonts of *P.b. yoelii* in the parenchyma cells of livers of rats and mice 25, 30, 36, 45 and 50 hours after inoculation, and of *P. chabaudi* at 28, 42, 50, 53 and 54 hours. The inoculation of blood from sporozoite-infected rodents shows that the first schizonts of *P.b. yoelii* mature at 43 hours and those of *P. chabaudi* at 53 hours. Mature schizonts of both parasites grow to a mean diameter of 37 μ .

Presumed secondary exo-erythrocytic schizonts found in the livers of two *Thomomys rutilans* naturally infected with both *P.b. yoelii* and *P. chabaudi* are described; these forms differ morphologically from the primary exo-erythrocytic schizonts seen in experimentally infected rodents.

The differential characters of *P.b. yoelii* and *P.b. berghei* are reviewed, and the relationships between *P.b. berghei* and *P. vinckei* from Katanga, and *P.b. yoelii* and *P. chabaudi* from the République Centrafricaine, are discussed.

P.b. yoelii and *P. chabaudi* are easily maintained cyclically in the laboratory, and are likely to be extremely useful in studies on immunity, chemoprophylaxis and chemotherapy.

*At the time of writing, the natural invertebrate host of *P. b. yoelii* and *P. chabaudi* is unknown. *A. durenii*, the natural mosquito host of rodent plasmodia in Katanga, has not been reported from the République Centrafricaine (HAMON et al., 1956), and the only anopheline larvae found in the habitat of infected *T. rutilans* were those of *A. obscurus* and *A. paludis* (LANDAU and CHABAUD, 1965). (At first, the larvae of *A. paludis* were wrongly identified as those of *A. coustani*, a closely related species; a final identification by M. J. Hamon has since been made on mosquitoes hatched in the laboratory.)

REFERENCES

- ADAM, J. P., LANDAU, I. & CHABAUD, A. G. (1966). *C.R. Acad. Sci., Paris*, **263**, 140.
- BOX, E. D., CELAYA, B. L. & GINGRICH, W. D. (1953). *Amer. J. trop. Med. Hyg.*, **2**, 624.
- BRAY, R. S. (1954). *Indian J. Malar.*, **8**, 263.
- (1957). *Studies on the Exo-erythrocytic Cycle in Genus Plasmodium*. London: H. K. Lewis.
- , BURGESS, R. W. & BAKER, J. R. (1963). *Amer. J. trop. Med. Hyg.*, **12**, 1.
- & GARNHAM, P. C. C. (1962). *Indian J. Malar.*, **16**, 153.
- CELAYA, B. L., BOX, E. D. & GINGRICH, W. D. (1956). *Amer. J. trop. Med. Hyg.*, **5**, 168.
- DISSANAIKE, A. S., NELSON, P. & GARNHAM, P. C. C. (1965). *Ceylon J. med. Sci.*, **14**, 1.
- GARNHAM, P. C. C. (1951). *Trans. R. Soc. trop. Med. Hyg.*, **45**, 45.
- (1959). *Riv. Parassit.*, **20**, 273.
- , BIRD, R. G., BAKER, J. R. & BRAY, R. S. (1961). *Trans. R. Soc. trop. Med. Hyg.*, **55**, 98.
- & BRAY, R. S. (1956). *Rev. bras. Malar.*, **8**, 152.
- , LANDAU, I. & KILLICK-KENDRICK, R. (1966). *Trans. R. Soc. trop. Med. Hyg.*, **60**, 4.
- HAMON, J., ADAM, J. P. & GRJEBINE, A. (1956). *Bull. Wld Hlth Org.*, **15**, 549.
- LANDAU, I. (1965). *C.R. Acad. Sci., Paris*, **260**, 3758.
- & CHABAUD, A. G. (1965). *Ibid.*, **261**, 230.
- & KILLICK-KENDRICK, R. (1966). *Ibid.*, **262**, 1113.
- MICHIELS, G. (1963). *Ann. Soc. belge Méd. trop.*, **43**, 83.
- MORGAN, J. F., MORTON, H. J. & PARKER, R. C. (1950). *Proc. Soc. exp. Biol. Med.*, **16**, 118.
- PEREZ-REYES, R. (1953). *J. Parasit.*, **39**, 603.
- RODHAIN, J., WANSON, M. & VINCKE, I. H. (1955). *Ann. Soc. belge Méd. trop.*, **35**, 203.
- SHORTT, H. E. & COOPER, W. (1948). *Trans. R. Soc. trop. Med. Hyg.*, **41**, 427.
- & GARNHAM, P. C. C. (1948). *Ibid.*, **41**, 785.
- SHUTE, P. G. & MARYON, M. (1966). *Laboratory Technique for the Study of Malaria*. 2nd Ed. London: J. & A. Churchill.
- VANDERBERG, J. & YOELI, M. (1965). *Ann. Soc. belge Méd. trop.*, **45**, 419.
- VINCKE, I. H. (1954). *Indian J. Malar.*, **8**, 245.
- WOLBACH, S. B. (1911). *J. Amer. med. Ass.*, **56**, 345.
- WOLCOTT, G. B. (1952). *J. Morph.*, **90**, 189.
- YOELI, M. (1965). *Trans. R. Soc. trop. Med. Hyg.*, **59**, 255.
- & MOST, H. (1960). *Ibid.*, **54**, 549.
- & ——— (1965a). *Nature, Lond.*, **205**, 715.
- & ——— (1965b). *Amer. J. trop. Med. Hyg.*, **14**, 700.
- , ——— & BONE, G. (1964). *Science*, **144**, 1580.
- , ——— & ——— (1965). *Ann. Soc. belge Méd. trop.*, **45**, 267.
- & WALL, W. J. (1951). *Nature, Lond.*, **168**, 1078.
- & ——— (1952). *Ibid.*, **169**, 881.

RÉPARTITION ET CARACTÈRES DIFFÉRENTIELS DES *PLASMODIUMS* DE MURIDÉS

Par P. C. C. GARNHAM, I. LANDAU, R. KILICK-KENDRICK et J. P. ADAM (*)

De nombreuses souches de *Plasmodium* de Rongeurs dont le comportement dans la nature et au laboratoire n'est pas identique ont été isolées récemment d'Afrique.

Bien que, pour certaines de ces souches, l'étude épidémiologique et celle des conditions optima de transmission au laboratoire soient encore incomplètes, il nous a paru nécessaire de faire dès à présent une mise au point de leur distribution géographique et de leurs principaux caractères différentiels.

Trois foyers de paludisme des Muridés sont actuellement connus en Afrique : le Katanga, la République Centrafricaine et le Congo-Brazzaville. Un quatrième foyer, encore mal connu, existe au Nigeria où BRUCE-CHWATT et GIBSON (1955) ont signalé l'infection d'un *Praomys tulbergi* par un *Plasmodium*.

Dans chaque foyer géographique, bien que les modalités de l'infection varient chez les Rongeurs dont la majorité appartient au genre *Thamnomys*, deux espèces distinctes parasitent, seules ou en association, le même hôte vertébré. L'une appartient au groupe *berghei* qui comprend actuellement *Plasmodium berghei* Vincke et Lips, 1948 au Katanga, *P. b. yoelii* Landau et Killick-Kendrick, 1966 en R. C. A. et *Plasmodium* sp. proche *berghei* à Brazzaville. L'autre est constituée par *Plasmodium vinckei* Rodhain, 1952 au Katanga, *Plasmodium chabaudi* Landau, 1965 en R. C. A., *Plasmodium* sp. proche *chabaudi* à Brazzaville. Le *Plasmodium* sp. Bruce-Chwatt et Gibson, 1955 du Nigeria se classe vraisemblablement dans ce dernier groupe.

La classification de ces *Plasmodiums* en deux groupes repose sur des différences morphologiques, d'une part (VINCKE, 1948, RODHAIN, 1952, LANDAU, 1965, LANDAU et KILICK-KENDRICK, 1965 a et b), sur des expériences d'immunité croisée (COX et VOLLER, 1966), d'autre part. Par ailleurs, les techniques d'immuno-fluorescence et d'hémagglutination (BRAY et EL NAHAL, 1966) permettent de séparer de façon très nette *P. b. berghei* et *P. b. yoelii*, d'une part, de *P. vinckei* et *P. chabaudi*, d'autre part.

(*) Séance du 8 mars 1967.

TABLEAU I
Épidémiologie.

Parasite	Distribution géographique	Hôte vertébré dans la nature	Hôte invertébré dans la nature	Végétation	Altitude
<i>P. b. berghei</i>	Katanga	<i>Thamnomys surdaster</i> <i>Praomys jacksoni</i> <i>Leggada bella</i> (taux d'infection faible)	<i>Anopheles duren</i> (taux d'infection très élevé)	Forêt-galerie	1.000 à 1.500 m.
<i>P. b. yoelii</i>	R. C. A.	<i>Thamnomys rutilans</i> (taux d'infection très élevé)	Inconnu	Forêt tropicale primaire	600 m.
<i>Plasmodium</i> sp. proche <i>berghei</i>	Congo-Brazzaville	<i>Thamnomys rutilans</i> (taux d'infection faible)	Inconnu	Forêt-galerie	340 m.
<i>P. vinckei</i>	Katanga (région de Kamena)	Inconnu (*)	<i>Anopheles duren</i>	Forêt-galerie	1.000 m.
<i>P. chabaudi</i>	R. C. A.	<i>Thamnomys rutilans</i> (taux d'infection très élevé)	Inconnu	Forêt tropicale primaire	600 m.
<i>Plasmodium</i> sp. proche <i>chabaudi</i>	Congo-Brazzaville	<i>Thamnomys rutilans</i> (taux d'infection élevé)	Inconnu	Forêt-galerie	340 m.
<i>Plasmodium</i> sp. proche <i>vinckei</i>	Nigeria	<i>Praomys tulbergi</i>	Inconnu	Forêt tropicale secondaire	150 m.

(*) *Plasmodium vinckei* n'a jamais pu être isolé à nouveau à partir des Moustiques ou des Rongeurs malgré des tentatives répétées. Son taux d'infection doit donc être très faible.

Lorsque l'on fait une étude approfondie des parasites d'un même groupe, on peut noter des différences morphologiques et biologiques. Ainsi, l'étude du cycle complet des souches de *Plasmodium berghei* du Katanga et de la République Centrafricaine a révélé des différences dans la sporogonie, la schizogonie pré-érythrocytaire et la température optima de transmission (LANDAU et KILLICK-KENDRICK, 1966) qui ont permis de les séparer en sous-espèces distinctes.

ÉPIDÉMIOLOGIE

Des différences de température viennent s'ajouter aux variations d'altitude et de végétation. Dans les hauts plateaux du Katanga, la température peut descendre très bas au cours de la saison sèche (3°3 en Juillet) selon VINCKE (1954), alors qu'elle ne descend pas au-dessous de 14° dans la forêt en R. C. A. et de 10° à Brazzaville. L'importance du rôle de la température dans la transmission des *Plasmodiums* de Rongeurs a été mise en évidence par YOELI (1965), et VANDERBERG et YOELI (1965 et 1966) qui ont démontré que le *Plasmodium b. berghei* isolé dans les hauts plateaux du Katanga ne pouvait être transmis par les Anophèles au laboratoire qu'à des températures inférieures à 22°. Les souches de la R. C. A. et de Brazzaville, par contre, peuvent être transmises entre 23 et 26°.

Par ailleurs, le taux extrêmement élevé de l'infection dans le sang des *Thamnomys rutilans* de la R. C. A. (100 0/0 des adultes parasités en mars 1965, d'après LANDAU et CHABAUD, 1965), celui également élevé de ceux du Congo-Brazzaville (40 0/0 en mars 1966, d'après ADAM, LANDAU et CHABAUD, 1966) et la mise en évidence de leur importance comme réservoir de virus est en contradiction avec les constatations des auteurs belges qui isolent plus facilement leurs souches à partir des *Anopheles durenii* que des Rongeurs rarement trouvés infectés dans la nature.

Le taux relatif d'infection dans la nature des Rongeurs par les deux espèces de *Plasmodium* pourrait également être conditionné par les températures locales et celle à laquelle se fait la transmission de chaque parasite. Ainsi, dans le cas de *Plasmodium berghei*, c'est au Katanga où les températures sont les plus basses que l'on trouve le plus fort pourcentage d'infection, suivi par la République Centrafricaine où 50 0/0 des Rongeurs parasités le sont par cette espèce. Enfin, à Brazzaville, région la plus chaude, une seule souche de *Plasmodium* sp. proche *berghei* a été isolée pour 10 souches de *Plasmodium* sp. proche *vinckei*.

Seule l'autopsie permet de différencier *Thamnomys rutilans* de

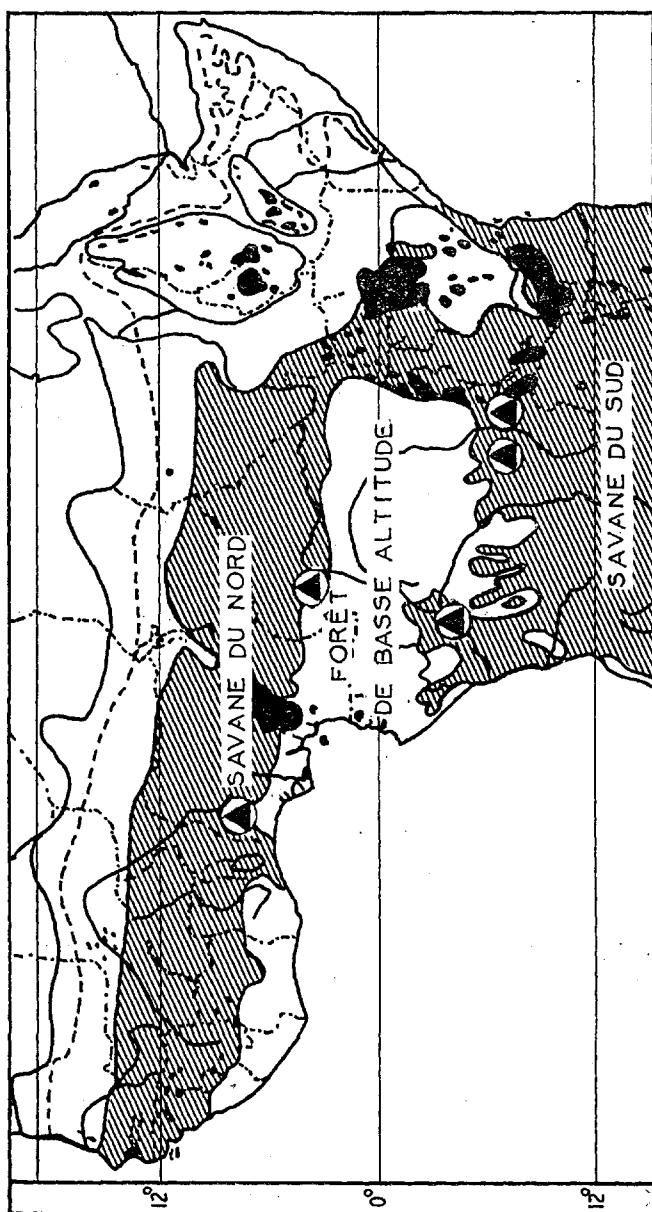


Fig. 1. — Carte des principales zones biotiques d'Afrique tropicale (d'après DAVIES, 1962), indiquant les lieux de capture de rongeurs infectés (triangles noirs).
Les taches noires correspondent aux forêts d'altitude.

T. surdaster et, comme ces deux Rongeurs coexistent souvent dans des gîtes très proches, il est parfois difficile de déterminer leur part respective dans l'épidémiologie des *Plasmodiums* de Muridés.

TABLEAU II

Schizogonie érythrocytaire des Plasmodiums de Muridés.

Parasite	Jeunes anneaux avec doubles points chromatiniens (**)	Nombre de mérozoïtes dans les schizontes mûrs (**)	Prédilection pour les réticulocytes (**)	Modifications des hématies parasitées (**)	Réceptivité des Rats et Hamsters (*)
<i>P. b. berghei</i>	Rares	8 à 14	Oui	Taille augmentée	Réceptifs
<i>P. b. yoelii</i>	Rares	8 à 16	Oui	»	Réceptifs
<i>Plasmodium</i> sp. proche <i>berghei</i> (Brazzaville)	Rares	12 à 16	Oui	»	Réceptifs
<i>P. vinckeï</i>	Fréquents	8 en moyenne	Non	Taille normale	Réfractaires
<i>P. chabaudi</i>	Fréquents	8 à 12	Non	Taille normale « rougeoiement » parfois petites « perles bleues »	Réfractaires
<i>Plasmodium</i> sp. proche <i>chabaudi</i> (Brazzaville)	Fréquents	8 à 12	Non	Taille normale « rougeoiement »	Réfractaires
<i>Plasmodium</i> sp. proche <i>vinckeï</i> (Nigeria)	Fréquents	8 à 12	Non	Taille normale	?

(*) Des souches infectant le Rat et le Hamster peuvent cependant être sélectionnées par passages à des animaux splénectomisés.
 (**) Dans le sang de la Souris blanche.

SCHIZOGONIE ÉRYTHROCYTAIRE

Dans le tableau II, nous avons comparé l'infection sanguine chez la Souris blanche des différentes souches de *Plasmodium* isolées en Afrique ainsi que la réceptivité des Rats et Hamsters.

La morphologie des *Plasmodiums* de Rongeurs dans le sang est variable dans certaines limites. Ainsi le nombre de mérozoïtes dans les rosaces mûres peut varier du simple au double selon que l'examen a lieu en début d'infection ou peu avant la crise. Les chiffres donnés dans le tableau ont été déterminés dans la mesure du possible sur des souches d'isolement récent et peu avant la crise.

La virulence des souches est également un mauvais critère de séparation des espèces. Elle dépend de nombreux facteurs tels que l'âge et la souche de Rongeurs (Most et coll., 1966, NUSSENZWEIG et coll., 1966) et de la présence dans le sang des Rongeurs d'organismes tels que *Haemobartonella* ou *Eperythrozoon* (PETERS, 1965, OTT et STAUBER, 1966).

SPOROGENIE ET SCHIZOGONIE PRÉ-ÉRYTHROCYTAIRE

Le cycle complet de trois de ces *Plasmodiums* a été étudié au laboratoire (LANDAU et KILICK-KENDRICK, 1966 a, b). Le tableau III indique les caractères différentiels de *Plasmodium b. berghei*, de *P. b. yoelii* et de *P. chabaudi*. Leur sporogonie a été étudiée chez *Anopheles stephensi*, la schizogonie pré-érythrocytaire des *Plasmodiums* du groupe *berghei* chez le Rat blanc, et celle de *Plasmodium chabaudi* chez la Souris blanche.

Les comparaisons entre les sporogonies et les schizogonies pré-érythrocytaires ont été faites sur un matériel préparé dans des conditions presque identiques, en utilisant les mêmes procédés de fixation et de coloration. En particulier, les courbes de croissance de *P. b. berghei* et de *P. b. yoelii* ont été tracées à partir de foies de Rats biopsiés ou sacrifiés à heures fixes après une inoculation unique de sporozoïtes.

Actuellement, nous pouvons donc constater que chaque souche connue peut être classée dans l'un des deux groupes, celui des *Plasmodiums* type *berghei*, ou celui des *Plasmodiums* type *vinckei*. Cependant, l'étude de la sporogonie et de la schizogonie pré-érythrocytaire de trois d'entre eux nous a permis de constater que *P. b. yoelii* et *P. chabaudi* provenant de la même région géographique et qui appartiennent à deux groupes différents possèdent en commun des caractères tels que la taille des oocytes, celle des schizontes pré-éry-

TABLEAU III

*Sporogonie et schizogonie pré-érythrocytaire
de P. b. berghei, de P. b. yoelii et de P. chabaudi.*

Parasite	Sporogonie		Schizogonie pré-érythrocytaire		
	Température nécessaire à l'obtention de sporozoïtes infectants	Taille des oocystes mûrs	Âge des premiers schizontes mûrs	Taille moyenne des schizontes	Nombre approximatif des mérozoïtes dans les schizontes mûrs
<i>P. b. berghei</i>	19 à 21°	34 à 40 μ	4 heures	26 μ à 50 heures	4.000
<i>P. b. yoelii</i>	23 à 26°	60 à 80 μ	43 heures	37 μ à 50 heures	8.000
<i>P. chabaudi</i>	23 à 26°	60 à 80 μ	53 heures	37 μ à 53 heures	18.000

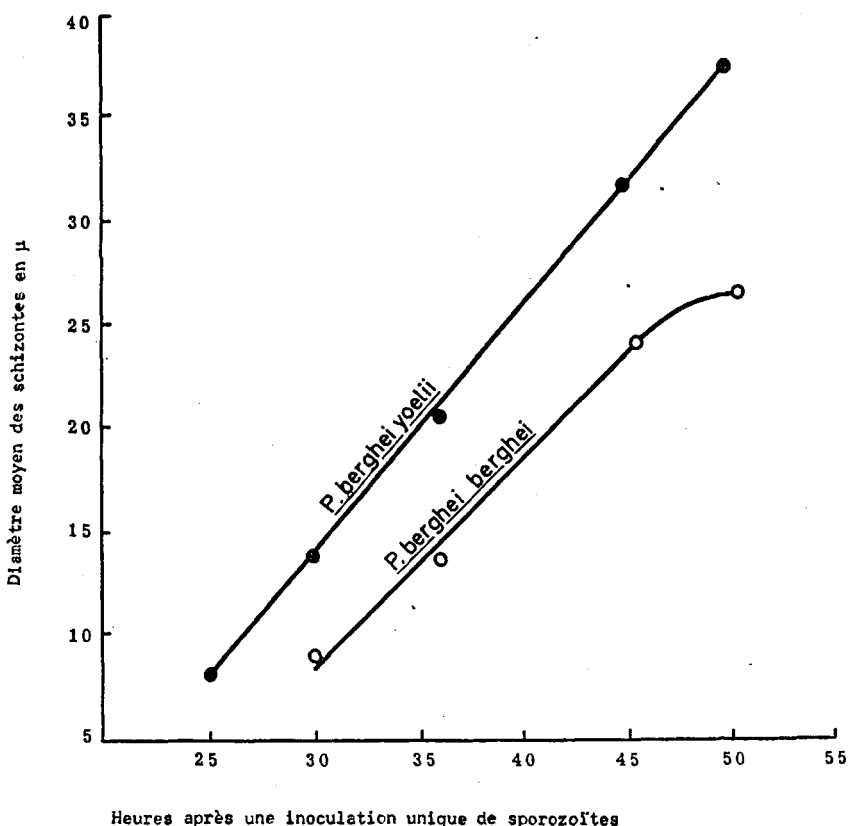


Fig. 2. — Courbe de croissance de *Plasmodium* B. Berghei et P. B. Yoelii dans le foie de rats blancs d'après LANDAU et KILLICK-KENDRICK, 1966.

throcytaires, et la température à laquelle s'effectue la transmission, qui les différencient très nettement de *P. b. berghei*.

RÉSUMÉ

1) L'identification des différents *Plasmodiums* de Muridés doit se baser sur l'étude de leur cycle biologique complet.

2) Quatre foyers ont été découverts en Afrique. Ce sont, par ordre d'altitude décroissante : le Katanga, la République Centrafricaine, le Congo-Brazzaville, le Nigeria Occidental.

3) Les espèces appartiennent à deux groupes (*berghei* et *vinckeï*) qui présentent entre eux des différences morphologiques et sérologiques nettes.

4) La différenciation des espèces et sous-espèces repose sur :
a) l'hôte vertébré, *b)* l'hôte invertébré, *c)* les formes érythrocytaires,
d) les formes exo-érythrocytaires, *e)* la sporogonie et sa température optima, *f)* la sérologie, *g)* la réceptivité des Rats et Hamsters.

SUMMARY

1. The identification of the different malaria parasites of Muridae can only be made after observations on their complete life cycles.

2. There are 4 known localities in tropical Africa where rodent malaria occurs: (i) Katanga (at a high altitude), (ii) The Central African République (at a lower altitude), (iii) Congo-Brazzaville (at a still lower altitude) and (iv) Western Nigeria (at the lowest altitude).

3. The *species* fall into two groups (*berghei* and *vinckei*) which show clear-cut morphological and serological differences.

4. Specific differentiation is made on the basis of (i) vertebrate host, (ii) invertebrate host, (iii) erythrocytic forms, (iv) exoerythrocytic forms, (v) sporogonic characters, including the effect of temperature, (vi) serology and (vii) susceptibility of rats and hamsters.

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BIBLIOGRAPHIE

- ADAM (J. P.), LANDAU (I.) et CHABAUD (A. G.). — Découverte dans la région de Brazzaville de Rongeurs infectés par des *Plasmodium*. *C. R. Acad. Sci. Paris*, 1966, 263, 140.
- BRAY (R. S.) et EL-NAHAL (H. M. S.). — Indirect haemagglutination test for Malarial antibody. *Nature*, 1966, 212, n° 5057, 83.
- BRUCE-CHWATT (L. J.) et GIBSON (F. D.). — A *Plasmodium* from a Nigerian rodent. *Trans. Roy. Soc. Trop. Med. et Hyg.*, 1955, 49, 9.
- COX (F. E. G.) et VOLLER (A.). — Cross immunity between the malaria parasites of rodents. *Ann. trop. Med. Parasitol.*, 60, 297.
- GARNHAM (P. C. C.), LANDAU (I.) et KILLICK-KENDRICK (R.). — Primary exo-erythrocytic schizonts of three rodents malaria parasites. *Trans. Roy. Soc. Trop. Med. et Hyg.*, 1966, 60, 4.
- LANDAU (I.). — Description de *Plasmodium chabaudi* n. sp., parasite de Rongeurs africains. *C. R. Acad. Sci. Paris*, 1965, 260, 3758.
- LANDAU (I.) et CHABAUD (A. G.). — Infection naturelle par deux *Plasmodiums* du Rongeur *Thamnomys rutilans* en République Centrafricaine. *C. R. Acad. Sci. Paris*, 1965, 260, 230.

- LANDAU (I.) et KILLICK-KENDRICK (R.). — Note préliminaire sur le cycle évolutif des deux *Plasmodiums* du Rongeur *Thamnomys rutilans* de la République Centrafricaine. *C. R. Acad. Sci. Paris*, 1966 a, 262, 1113.
- LANDAU (I.) et KILLICK-KENDRICK (R.). — Rodent Plasmodia of the République Centrafricaine. The Sporogony and Tissue-stages of *Plasmodium chabaudi* and *P. berghei yoelii*. *Trans. Roy. Soc. Trop. Med. et Hyg.*, 1966 b, 60, 633.
- MOST (H.), NUSSENZWEIG (R. S.), VANDERBERG (J.), HERMAN (R.) et YOELI (M.). — Susceptibility of genetically standardized (Jax) Mouse Strains to Sporozoite — and Blood — Induced *Plasmodium berghei* Infections. *Supplement to Military Medicine*, 1966, 131, 915.
- NUSSENZWEIG (R. S.), HERMAN (R.), VANDERBERG (J.), YOELI (M.) et MOST (H.). — Studies on Sporozoite-induced infections of Rodent Malaria (III). *Ann. J. Trop. Med. et Hyg.*, 1966, 15, 683.
- OTT (K. J.) et STAUBER (L.). — Manuscrit aimablement communiqué.
- PETERS (W.). — Competitive relationship between *Eperythrozoon coccoides* and *Plasmodium berghei* in the Mouse. *Exp. Parasitology*, 1965, 16, 158.
- RODHAIN (J.). — *Plasmodium vinckei* n. sp., un deuxième *Plasmodium* parasite de Rongeurs sauvages au Katanga. *Ann. Soc. Belge Med. Trop.*, 1952, 32, 275.
- VANDERBERG (J.) et YOELI (M.). — Some physiological and metabolic problems related to maintenance of the *Plasmodium berghei* cycle in *Anopheles quadrimaculatus*. *Ann. Soc. Belge Med. Trop.*, 1965, 45, 419.
- VANDERBERG (J.) et YOELI (M.). — Effects of temperature on sporogonic development of *Plasmodium berghei*. *J. Parasit.*, 1966, 52, 559.
- VINCKE (I. H.) et LIPS. — Un nouveau *Plasmodium* d'un Rongeur sauvage du Congo, *Plasmodium berghei* n. sp. *Ann. Soc. Belge Med. Trop.*, 1948, 28, 97.
- VINCKE (I. H.). — Natural history of *Plasmodium berghei*. *Ind. J. Malariol.*, 1954, 8, 245.
- YOELI (M.). — Studies on *Plasmodium berghei* in nature and under experimental conditions. *Trans. Roy. Soc. Trop. Med. et Hyg.*, 1965, 59, 255.
- YOELI (M.), NUSSENZWEIG (R. S.), UPMANIS (R. S.) et MOST (H.). — Resistance of *Plasmodium chabaudi* infected with mice. *Nature*, 1966, 211, n° 5044, 49.

Reprinted from
TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.
Vol. 63. No. 3. pp. 328-332, 1969.

ELECTRON MICROSCOPE STUDIES ON THE MOTILE STAGES OF MALARIA PARASITES

VII. THE FINE STRUCTURE OF THE MEROZOITES OF EXOERYTHROCYTIC SCHIZONTS OF *PLASMODIUM BERGHEI* YOELII

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COMMUNICATIONS

ELECTRON MICROSCOPE STUDIES ON THE MOTILE STAGES OF
MALARIA PARASITESVII. THE FINE STRUCTURE OF THE MEROZOITES OF
EXOERYTHROCYTIC SCHIZONTS OF *PLASMODIUM BERGHEI* YOELII

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In 1960, when we began our studies on the fine structure of malaria parasites, we intended to confine our attention to the sexual stages, where special locomotory activity of the organism occurs, and in which characteristic organelles were likely to be present. Our successive papers on this subject confirmed the correctness of this view. However, many of these structures (apical rings, conoid, paired organelles and other apical rods, subpellicular fibrils and the micropyle or cytostome) have subsequently been detected by other observers in asexual forms of the parasite, and HUFF et al. (1960) clearly demonstrated by time-lapse cine-photomicrography the remarkable motility of the exoerythrocytic merozoites escaping from the mature schizont in tissue cultures of *Plasmodium gallinaceum*. In the final development of the parasites in the blood, motility (apart from pseudopodial activity of the early trophozoite) is negligible and HEPLER et al. (1966) point out how, at this stage of the cycle, many of the characteristic organelles disappear.

The fine structure of the exoerythrocytic stages of avian species has been studied by various North American workers, chiefly from preparations in tissue culture. We thought that a comparison should be made between the avian and mammalian species, but as no culture method had yet been successfully devised for the latter, we concentrated on getting heavy infections in the liver of the vertebrate host. The use of new strains of rodent malaria parasites provided us with suitable material, in which the tissue forms were sufficiently numerous to be detectable for electron microscopy. We made no attempt to observe the early stages of exoerythrocytic schizogony but confined our attention to the mature schizonts and particularly to released merozoites. A brief account of the preliminary observations was given after a laboratory demonstration (GARNHAM et al., 1967).

Materials and methods

150 *Anopheles stephensi* which had been fed 13 days previously on a mouse infected with *P. berghei yoelii* (strain 17X), were dissected into medium 199 to produce 1.3 ml. of a suspension containing approximately 2,800,000 sporozoites per ml. (total 3.6 million). Intravenous injections of 0.65 ml. (containing about 1.8 million sporozoites) of this suspension were given to each of 2 laboratory-bred mice.

45.5 hours after infection, malaria parasites were seen in the peripheral blood in both mice. The mouse with the heaviest parasitaemia was killed by exsanguination under anaesthesia 52 hours after infection. The liver was excised at once and part put into Carnoy's fixative for light microscopy. From the remainder, a small portion was used to make impression smears for both light and electron microscopy. The rest of the liver was divided into two: one of these pieces was put into 0.9% sodium chloride solution, the other into the Rhodin and Zetterqvist buffer solution (pH 7.4). These large liver slices were immediately cut into smaller bits, 1 mm. cube; those in saline were transferred to ice-cold 1.2% potassium permanganate in 0.9% saline solution and fixed for 1 hour,

then washed in several changes of normal saline before being dehydrated and embedded in Araldite. Those in the buffer solution were fixed with ice-cold osmium tetroxide in the Rhodin and Zetterqvist buffer for one hour, half of them being prefixed for 2 hours in 3% glutaraldehyde in similar buffer.

These methods of preparation have been described in more detail in previous papers (GARNHAM, BIRD and BAKER, 1967; GARNHAM et al., 1969). Cutting, mounting and examination of sections were carried out as described in the first of this series of papers (GARNHAM, BIRD and BAKER, 1960).

Results

Examination of sections under the light microscope showed that very heavy infections of exoerythrocytic schizonts were present, e.g. 96 schizonts were seen in one section (7.3 mm.^2) from one of the mice.

Mature exoerythrocytic schizonts

LANDAU and KILLICK-KENDRICK (1966), in their description of exoerythrocytic schizogony of *Plasmodium berghei yoelii*, refer to the production of pseudocytomeres in the maturing parasite. In the present material it is apparent that some schizonts were in just this stage and the production of merozoites by budding from these structures is clearly seen in Figs. 1 and 2. The nuclei migrate to the bases of small projections from the surface of the pseudocytomeres. At the extremity of each of these projections, a second membrane develops, beneath the original surface membrane of the schizont (Fig. 3a). Subsequent changes in these two membranes are thought to give rise to the apical rings and conoid of the fully formed merozoite (Fig. 3). Just behind the conoid is the paired organelle either in the form of its electron-opaque portion or the expanded vesicular part. This arrangement clearly indicates that the anterior part of the merozoite emerges first. The cytoplasm of the pseudocytomere and of its budding merozoites contains very numerous ribosomes, but no mitochondria or centrioles were seen.

Probably the final maturation process is very rapid, for in the same material as that containing the "budding" schizonts described above, schizonts (Fig. 4) may be found of which the contents consist almost wholly of separated merozoites, and still others in which the parasite has ruptured and some merozoites can be seen in the adjacent sinusoid (Fig. 5).

Exoerythrocytic merozoites

The merozoite (Figs. 6-10 and 13) is a squat, pear-shaped object about 1.5μ in length. The anterior end is drawn out into a knob which terminates in 2 apical rings to which is attached the conoid (Figs. 8-10 and 13). The pellicle is composed of 2 unit membranes separated by an electron-transparent space, appearing at low magnification as a tripartite structure (Fig. 11). There is, in the region of the nucleus, a circular depression in the pellicle, approximately $70 \text{ m}\mu$ deep, with an average diameter of $75 \text{ m}\mu$; it is, almost certainly, a non-functioning cytostome, previously referred to as a "micro-pyle" (Figs. 12, 20). The inner electron-opaque membrane does not take part in this infolding but ends at its edge.

The nucleoplasm has at least 2 constituents. One is finely granular of medium electron-opacity and forms the ground substance, while the other is coarsely granular, heterogeneous in appearance and contains larger opaque granules of patchy distribution (Fig. 8). In the newly formed merozoites, the nucleus is nearly always immediately adjacent to the posterior, broader end.

The cytoplasm is characterized by large ribosomes, closely packed, but often seen in rows with regular spacing indicating attachment to the associated endoplasmic reticulum (Figs. 8 and 14).

A prominent feature among the ribosomes in over 25% of the merozoite sections, is an aggregation of smooth concentric membranes, varying in form and complexity but usually round or oval. This organelle lies close to the nucleus between it and the pellicle and sometimes occupies up to a quarter of the cytoplasmic area (Figs. 15 and 16).

The anterior "secretory" organelles are very prominent in all merozoites. The swollen appearance of the paired organelle is usually very marked (Fig. 7); each lobe may reach a length of $1.4-1.5 \mu$ with a width of 0.12μ , narrowing into a "duct" at the anterior extremity. Dense bodies are frequently also present (Figs. 6, 7, 8, 9), some of which may be cross sections of a highly convoluted, electron-opaque, paired organelle in a different stage of activity. Both features are often seen together, even in the budding merozoite (Fig. 2). Subpellicular microtubules were not seen, but a network of microfilaments forming a framework in the surface membrane was clearly visible at the neck of one of the flask-shaped organisms (Fig. 17).

Erythrocytic merozoites

Once the merozoite is inside an erythrocyte, many of the original organelles rapidly disappear. The organism loses one of its membranes and the paired organelles, and rounds up within the stroma of the erythrocyte in a vacuole lined by a membrane indistinguishable from the erythrocyte's own surface membrane. Multiple invasion of a corpuscle is not uncommon (Fig. 18).

A possible remnant of the apical rings is seen in one organism (Fig. 19) at the opposite pole to the aggregation of smooth membranes. In one section (Fig. 18) the latter appears to be expanding to form smooth endoplasmic reticulum.

Ribosomes are numerous and the endoplasmic reticulum is more prominent. In some sections the nucleus is lobulated as if dividing (Fig. 18). A large electron-opaque mass, similar in appearance to the erythrocytic stroma, is probably a phagotrophic vacuole (Fig. 20); the cytostome is also seen in this section.

ABBREVIATIONS USED ON MICROGRAPHS

AR	— apical rings	M	— merozoite
C	— conoid	N	— nucleus of parasite
CT	— cytostome	OM	— outer membrane of pellicle
DB	— dense body	PC	— pseudocytomere
E	— erythrocyte	PM	— parasite membrane
F	— microfilaments of surface membrane	PO	— paired organelle
HCM	— host cell membrane	PV	— phagotrophic vacuole
IM	— inner membrane of pellicle	SMO	— smooth membraned organelle
L	— leucocyte		

All figures are electron micrographs of sections through exoerythrocytic schizonts or merozoites of *P. berghei yoelii* fixed with glutaraldehyde and osmium tetroxide (except for Fig. 13).

Magnification is indicated by a line drawn on each figure.

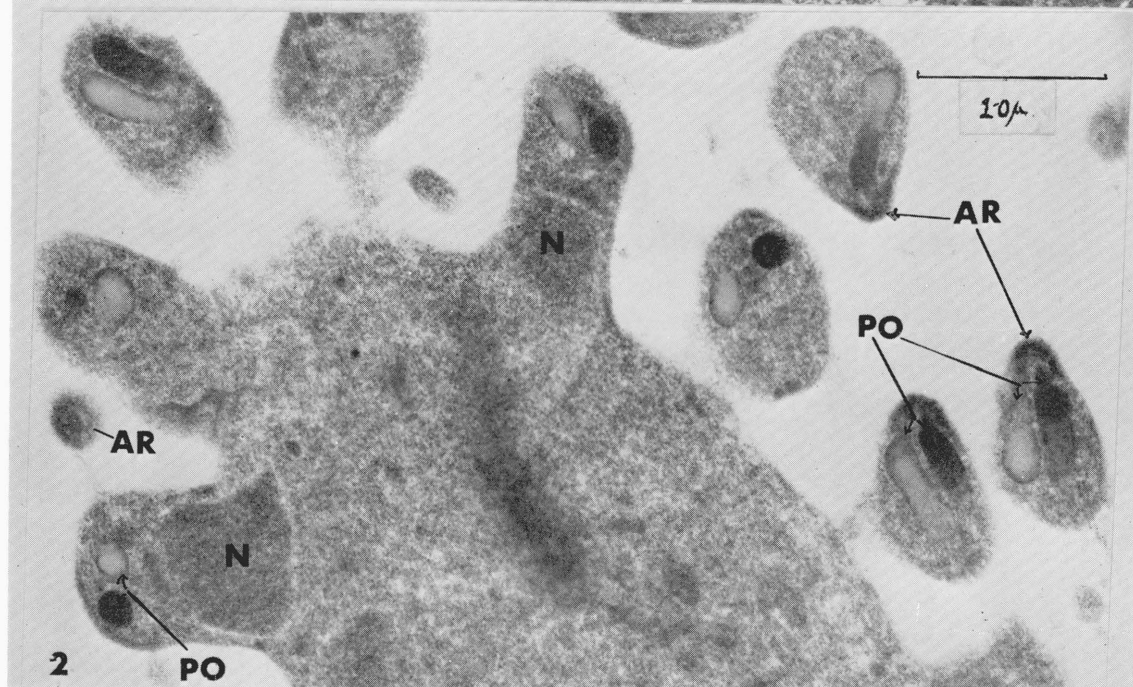
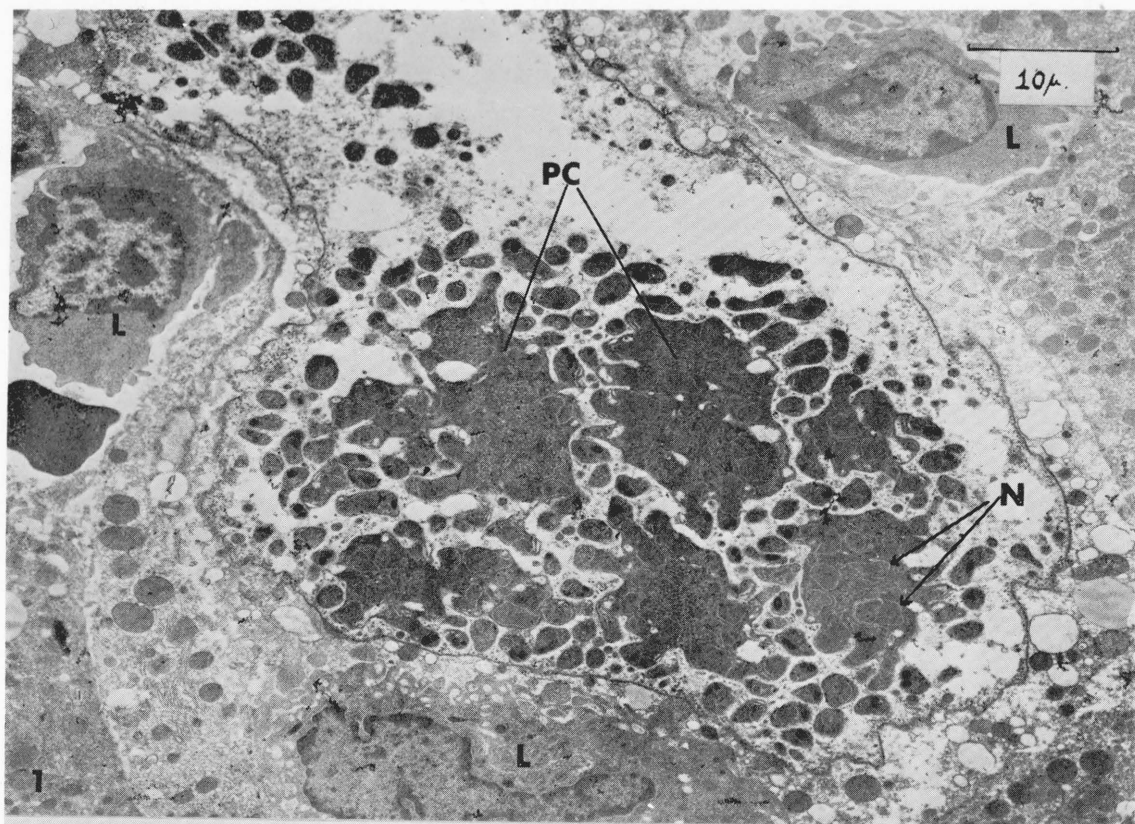


FIG. 1. Pseudocytomeres in a maturing schizont. Some indication of a host cell reaction to the parasite is shown by abnormal vacuolation of the host cell cytoplasm and the presence of 3 leucocytes. FIG. 2. Merozoites budding from a pseudocytomere.

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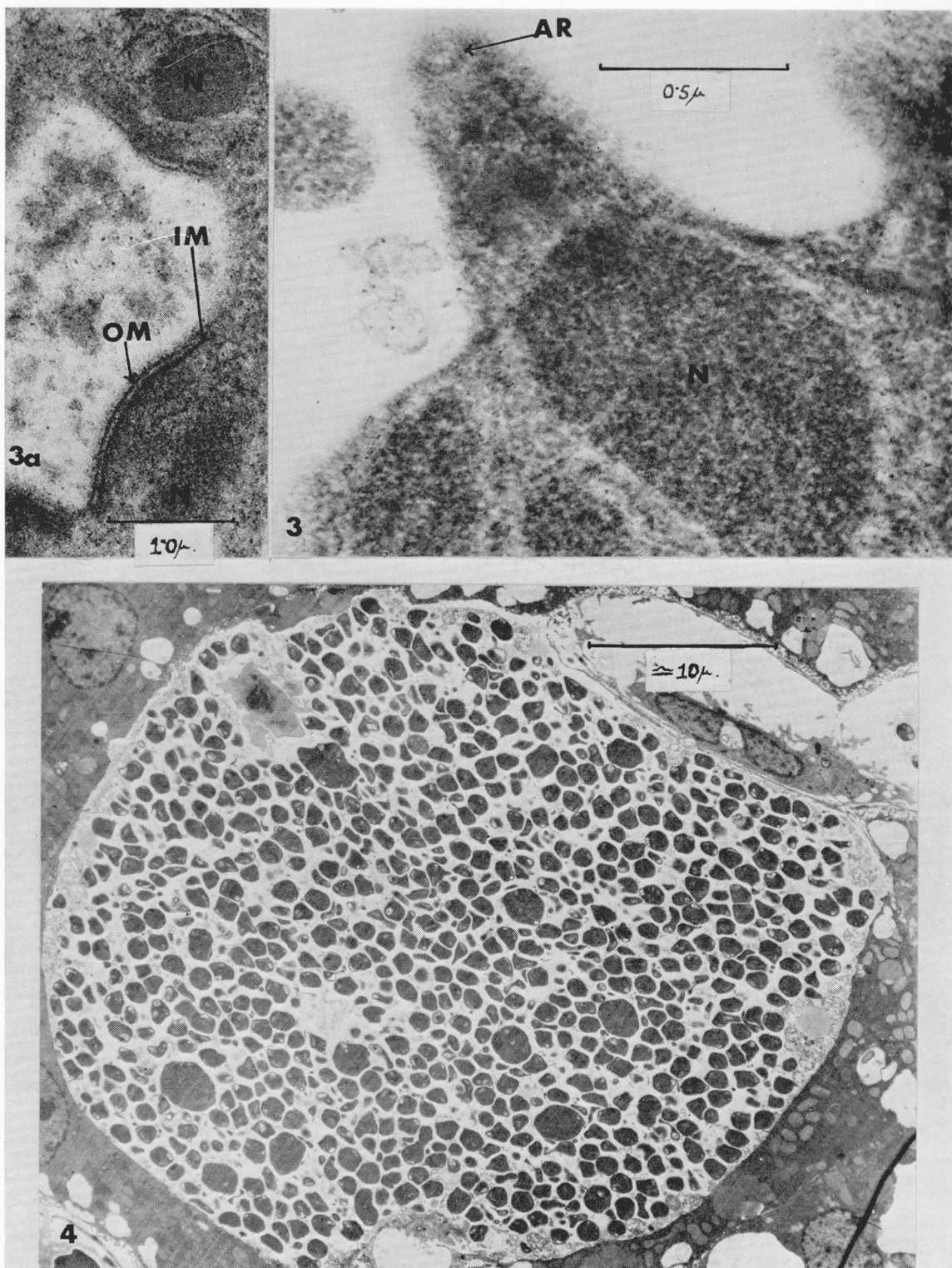


FIG. 3. An early budding merozoite, showing well developed apical rings and advanced nuclear extension. FIG. 3a. Part of a pseudocytomere showing second, inner, membrane at site of budding merozoite. FIG. 4. A mature schizont, the contents of which consist largely of separated merozoites.

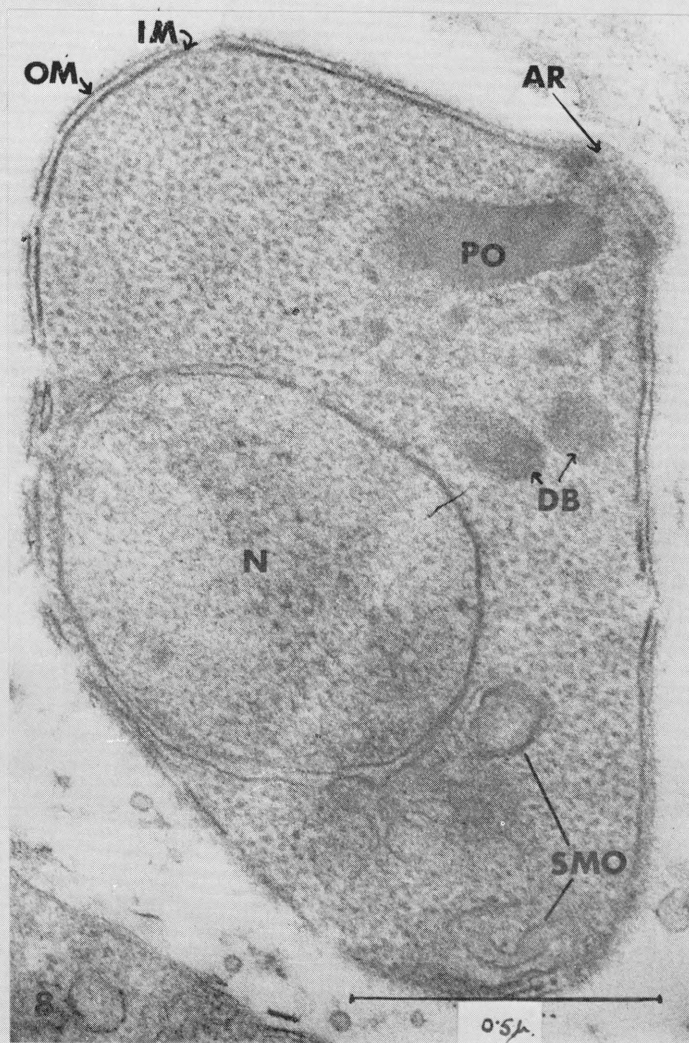
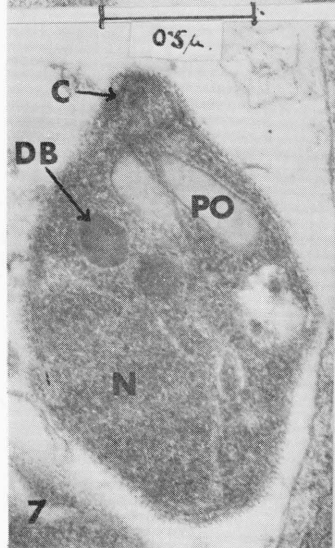
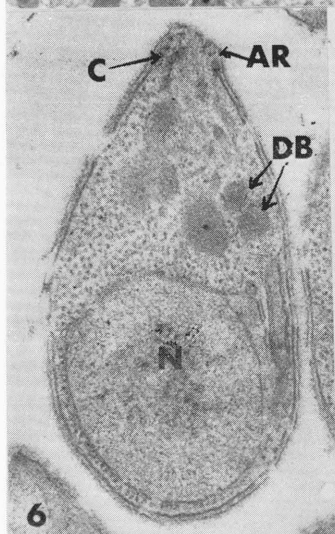
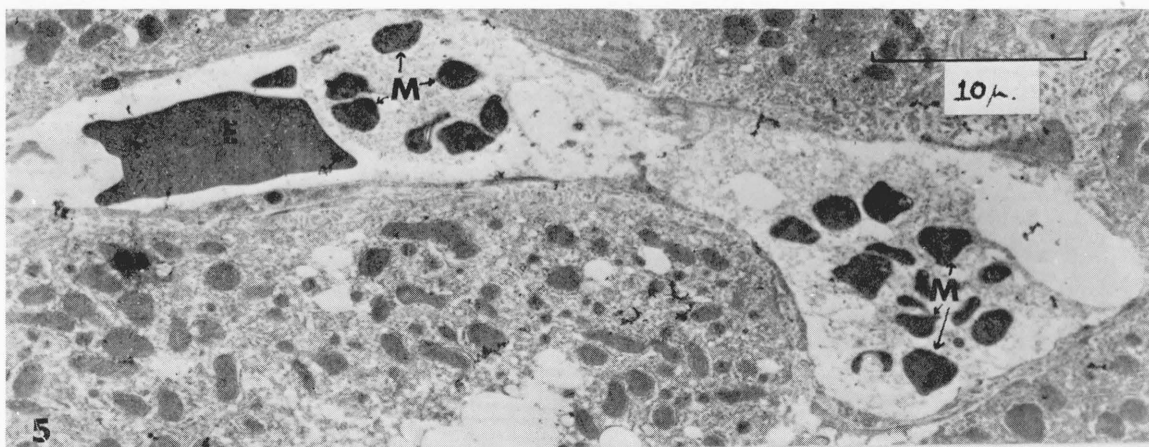
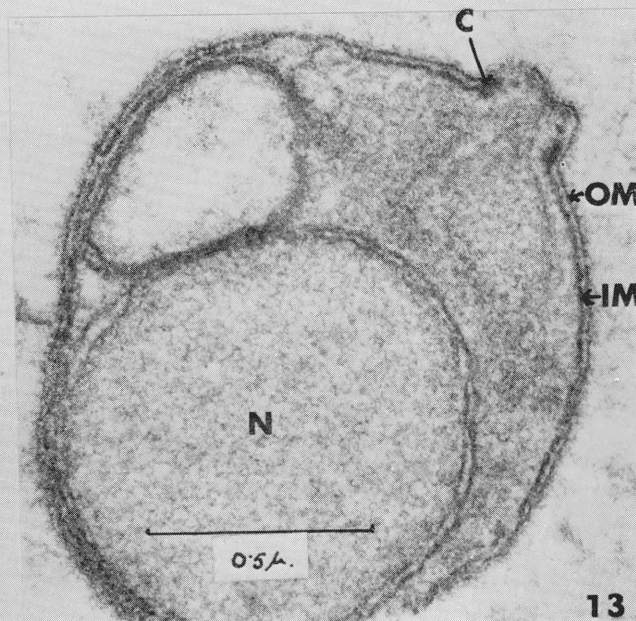
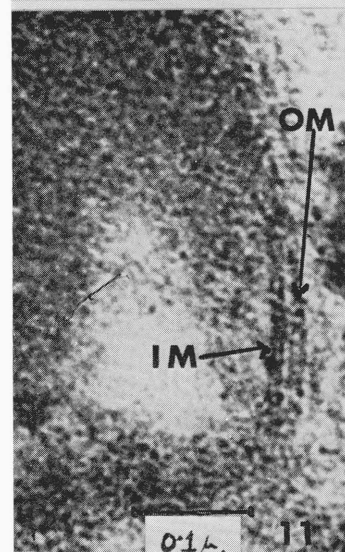
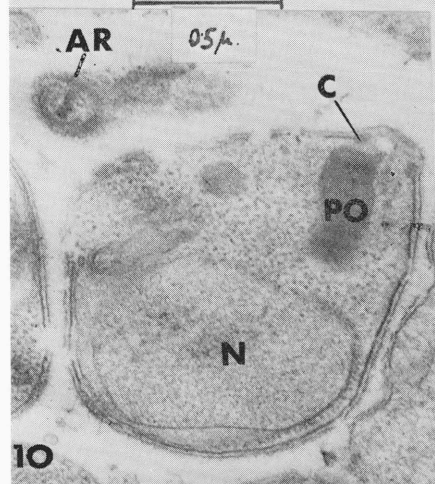
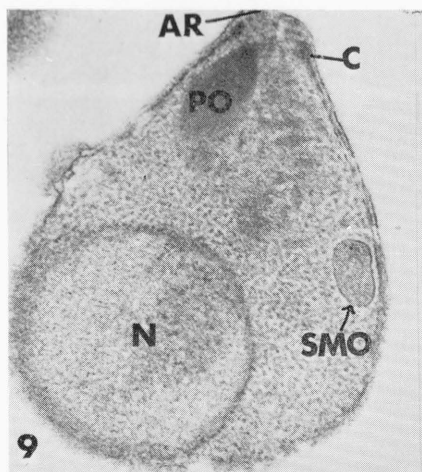


FIG. 5. Section through a sinusoid, adjacent to a ruptured schizont, containing one erythrocyte and two groups of merozoites. FIGS. 6-8. Representative sections of mature exoerythrocytic merozoites.



FIGS. 9-10. Representative sections of mature exoerythrocytic merozoites. FIG. 11. Section through the pellicle of a merozoite. FIG. 12. Section of a merozoite showing the cytosome ("micropyle"). FIG. 13. Section of a merozoite fixed with potassium permanganate, showing the conoid.

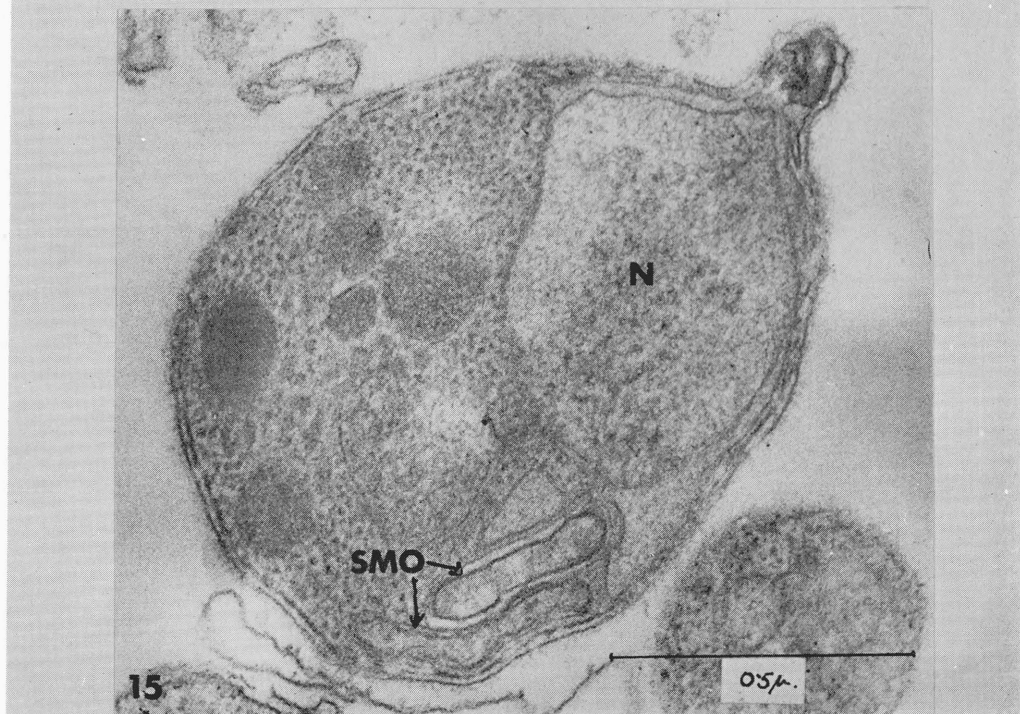
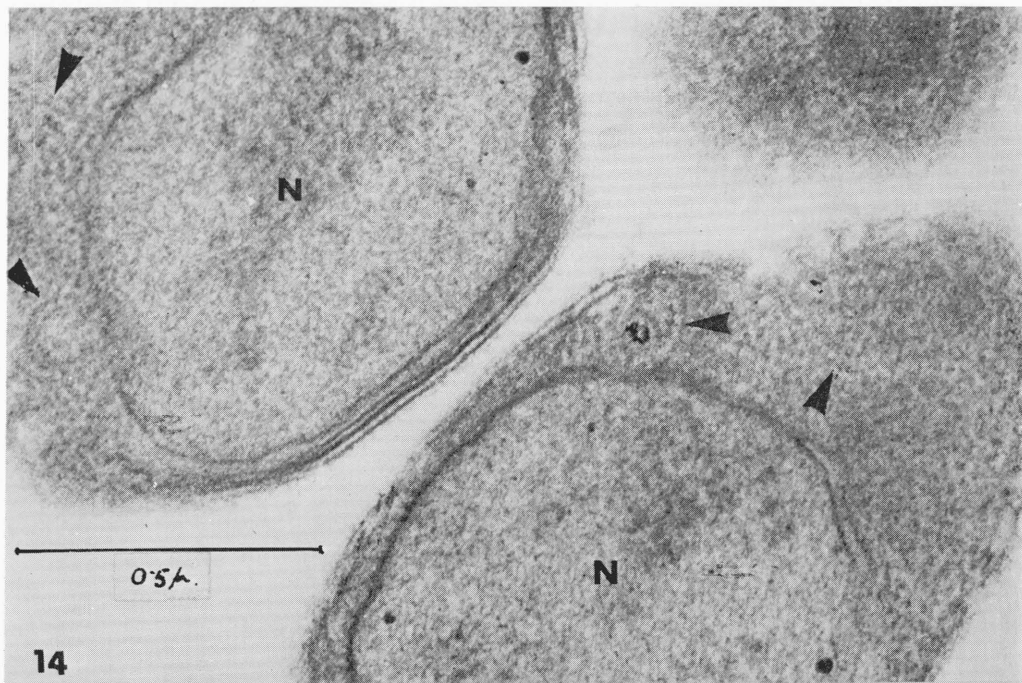


FIG. 14. Section through two merozoites showing, at arrows, ribosomes in parallel rows. FIG. 15. Section through a merozoite illustrating the aggregation of smooth membranes, close to the nucleus.

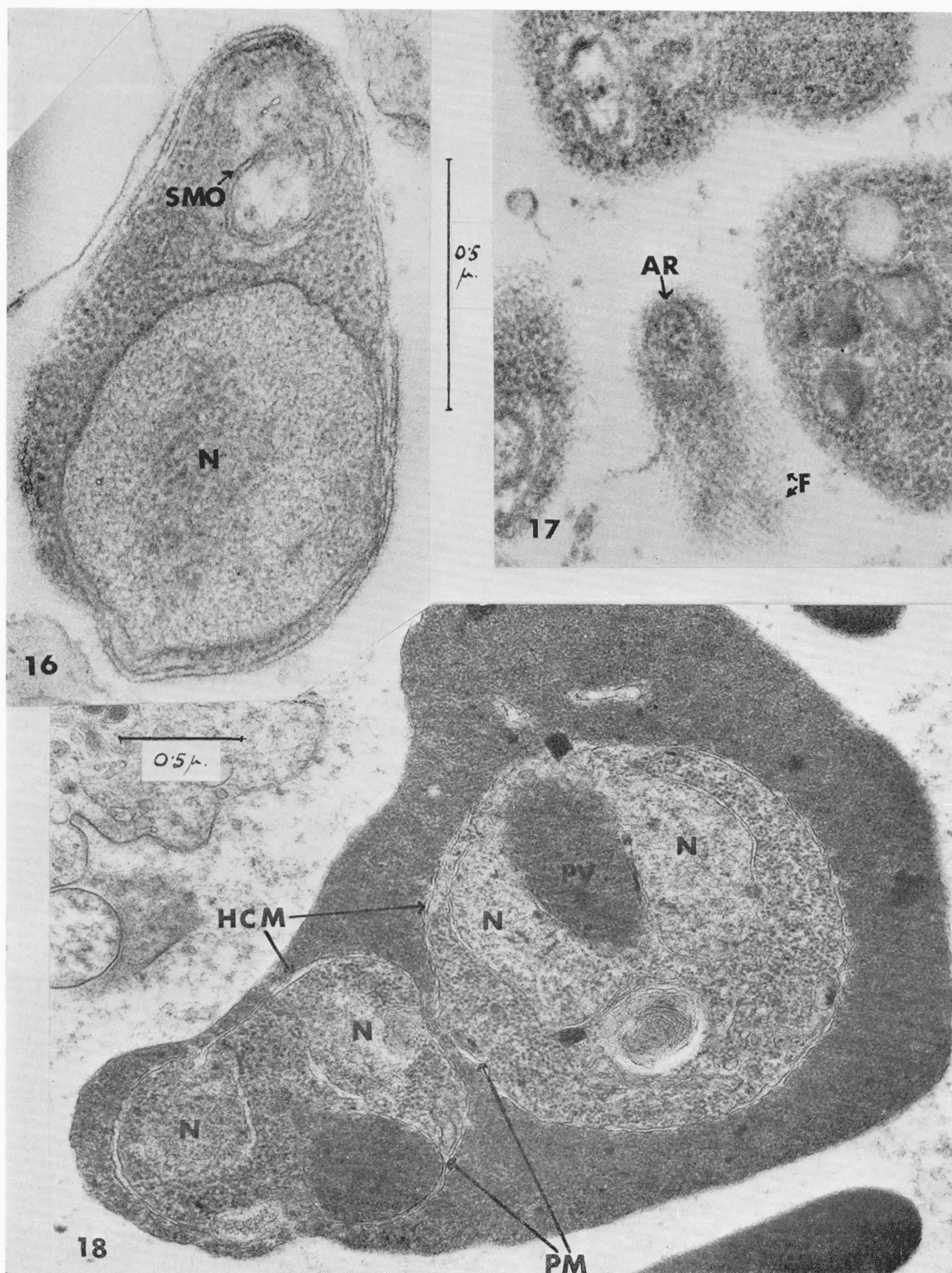


FIG. 16. Section through a merozoite illustrating the aggregation of smooth membranes, close to the nucleus. FIG. 17. Surface section of the anterior end of a merozoite showing fine microfilaments forming a framework in the surface membrane. FIG. 18. Merozoites within an erythrocyte in a liver sinusoid close to a ruptured schizont.

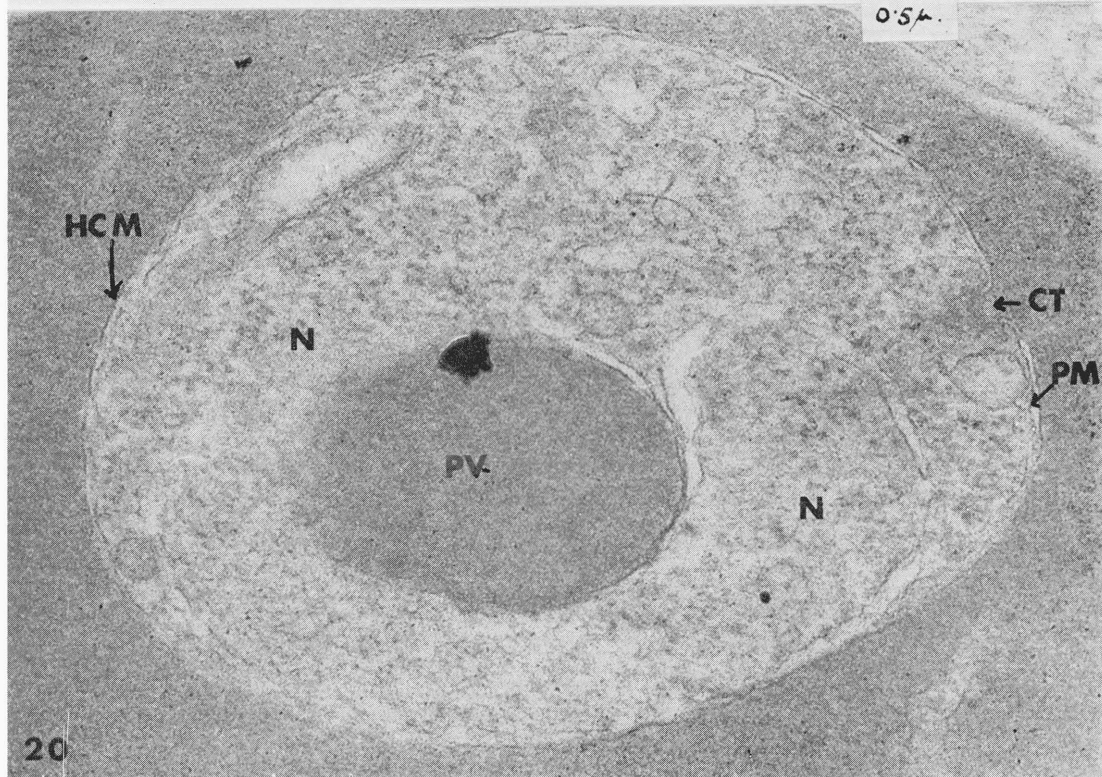
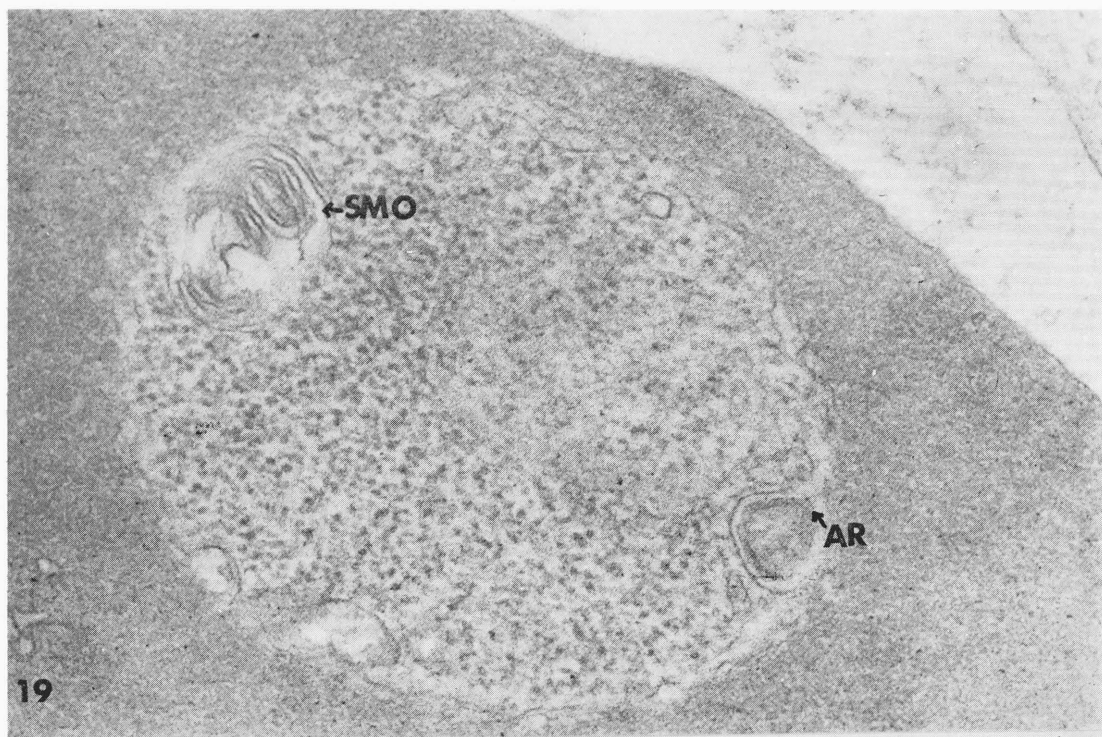


FIG. 19. Possible residual apical rings in a section through a merozoite within an erythrocyte. FIG. 20. Section of a merozoite within an erythrocyte showing phagotrophy and a cytostome.

Discussion

The exoerythrocytic merozoites which we have described resemble those of avian malaria parasites (AIKAWA, 1966; HEPLER et al., 1966) and the erythrocytic merozoites of *P. vinckei* and *P. chabaudi* (VICKERMAN and COX, 1967; SCALZI and BAHR, 1968); even the details of the budding process appear to be much the same as seen in the present work and in the description of the phenomenon in these other species. However, mitochondria, which are prominent features of the avian schizonts and merozoites, were not seen in our material, even after fixation in potassium permanganate; neither were they seen with any certainty by the above-mentioned authors in erythrocytic merozoites of *P. vinckei* and *P. berghei*. We think it possible that the round or oval organelle, composed of concentric membranes, which we saw in the merozoites of *P. b. yoelii*, may replace the more conventional mitochondrion of the avian species. RUDZINSKA and TRAGER (1959 and 1968) described "concentric-membraned organelles" in the erythrocytic forms of *P. berghei* and *P. coatneyi* respectively, which they thought were mitochondrial in function, and it may be that these organelles are characteristic of the forms of this species which develop in the vertebrate host. Conventional mitochondria have been seen in ookinetes (GARNHAM et al., 1969), oöcysts and sporozoites (VANDERBERG and RHODIN, 1967) of *P. berghei*.

The exoerythrocytic merozoites of *P. fallax* described by HEPLER et al. (1966) were more elongate than those seen in the present study; however, AIKAWA (1966) found the erythrocytic merozoites of *P. fallax*, *P. lophurae* and *P. cathemerium* to be very similar in shape to the exoerythrocytic forms of *P. berghei* described above. HEPLER et al. and Aikawa recorded the presence of subpellicular microtubules in the merozoites of these avian species of *Plasmodium* and spindle fibrils in the dividing nuclei of the schizonts. A similar spindle with fibrils about 200 Å in diameter was described by BÜTTNER (1967) in nuclear division in "Koch's blue bodies" of *Theileria parva*, and by SCALZI and BAHR (1968) in erythrocytic schizonts of *P. vinckei* and *P. chabaudi*; sub-pellicular microtubules were not, however, recorded from the two latter species. We were unable to identify either of these structures with any certainty. We have, however, observed them in the ookinete of *P. berghei* (GARNHAM et al., 1969).

In recent observations on the merozoites of *Eimeria intestinalis*, CHEISSIN (1967) described a curious type of centriole, containing a central fibril. We scrutinized our preparations for such a structure because, in the earlier stage of the same organism (the young oöcysts of *P. b. yoelii*), we had just found a similar type of centriole (GARNHAM et al., 1969). Yet in the merozoites, no centriole could be distinguished.

The cytostome or "micropyle" was seen only rarely and was less well defined than in other species. VANDERBERG et al. (1967) noticed that, in sporozoites of *P. b. berghei*, the structure was inconspicuous or apparently absent and we also failed to observe it in sporozoites of *P. b. yoelii* (unpublished observations). Without observations on more material, it would, however, be unwise to assume that the rarity of this organelle is a characteristic feature of this species of *Plasmodium*.

Summary

Maturing and mature exoerythrocytic schizonts of *Plasmodium berghei yoelii* in the livers of laboratory-bred mice have been studied by electron microscopy.

Merozoites are produced by budding from pseudocytomeres. At the tip of each bud, a thickened region of pellicle appears to give rise to the apical rings and conoid; below these structures the paired organelle is formed. As development proceeds, the nucleus enters the base of the bud. Alongside the nucleus is an aggregation of smooth

membranes. As no typical mitochondrion was seen, we suggest that this membranous structure may be its analogue.

In the pear-shaped free merozoites, there is, in addition to the above structures, a cytostome ("micropyle"), probably non-functional at this stage. The cytoplasm contains large, closely packed ribosomes; in the nucleoplasm are both fine and coarse granular elements. Subpellicular microtubules were not seen.

The development and structure of the exoerythrocytic merozoite resemble those described by other writers for avian malaria parasites, apart from the apparent absence of mitochondria and subpellicular microtubules.

After penetrating erythrocytes, the parasites lose one of their membranes, the apical rings, conoid and paired organelle; phagotrophy commences and smooth and granular endoplasmic reticulum becomes prominent. In many of these parasites the nucleus is lobulated as if dividing.

REFERENCES

- AIKAWA, M. (1966). *Amer. J. trop. Med. Hyg.*, **15**, 449-471.
 BÜTTNER, D. W. (1967). *Tropenmed. Parasit.*, **18**, 245-268.
 CHEISSIN, E. M. (1967). *Tsitologia*, **9**, 1411-1413.
 GARNHAM, P. C. C., BIRD, R. G. & BAKER, J. R. (1960). *Trans. R. Soc. trop. Med. Hyg.*, **54**, 274-278.
 ———, ——— & ——— (1967). *Ibid.*, **61**, 58-68.
 ———, ———, ——— & KILLICK-KENDRICK, R. (1967). *Ibid.*, **61**, 447.
 ———, ———, ———, DESSER, S. S. & EL-NAHAL, H. M. S. (1969). *Ibid.*, **63**, 187-194.
 HEPLER, P. K., HUFF, C. G. & SPRINZ, H. (1966). *J. Cell Biol.*, **30**, 333-358.
 HUFF, C. G., PIPKIN, A. C., WEATHERSBY, A. B. & JENSEN, D. V. (1960). *J. biophys. biochem. Cytol.*, **7**, 93-102.
 LANDAU, I. & KILLICK-KENDRICK, R. (1966). *Trans. R. Soc. trop. Med. Hyg.*, **60**, 633-649.
 RUDZINSKA, M. A. & TRAGER, W. (1959). *J. biophys. biochem. Cytol.*, **6**, 103-112.
 ——— & ——— (1968). *J. Protozool.*, **15**, 73-88.
 SCALZI, H. A. & BAHR, G. F. (1968). *J. Ultrastructure Res.*, **24**, 116-133.
 VANDERBERG, J. & RHODIN, J. (1967). *J. Cell Biol.*, **32**, C7-C10.
 ———, ——— & YOELI, M. (1967). *J. Protozool.*, **14**, 82-103.
 VICKERMAN, K. & COX, F. E. G. (1967). *Trans. R. Soc. trop. Med. Hyg.*, **61**, 303-311.