

STUDIES ON MALARIA PARASITES OF RODENTS

by

R. KILLICK-KENDRICK

A thesis submitted for the degree of Doctor of Philosophy
of the University of London

Department of Zoology and Applied Entomology
Imperial College of Science and Technology

January 1972

ABSTRACT

This work is in five parts: in the first there is a general introduction and an historical account of the discovery of malaria parasites of rodents and the elucidation of their life-cycles.

In Part II the complete life-cycle of Plasmodium berghei from Nigeria is described, and its distribution examined. Important faunal barriers exist between Nigeria and the localities of named subspecies of P. berghei and because of this, and morphological differences, it is concluded that the Nigerian parasite is a new subspecies.

Part III deals with malaria of African scaly-tailed flying squirrels in the Ivory Coast. Two new species of Plasmodium are described from Anomalurus peli 5/15 of which had malaria parasites. Two out of six A. derbianus also had malaria, but parasitaemias were too low to identify the parasites. All anomalurines had pigmented spleens. No malaria parasites were found in 16 Idiurus macrotis.

In Part IV the concept of the protozoan species and subspecies, and the taxonomy and origins of murine malaria parasites are discussed. It is concluded that, with modifications, genetic definitions of species and subspecies apply well to malaria parasites, though not to protozoa in which exchange of genetic material does occur. The assumption that trypanosomatids do not conjugate is considered not to be conclusive. The taxonomic position of sub-

2

species of P. berghei is examined and from the morphology and Carter's work on electrophoretic mobilities of enzymes it is concluded that P. berghei could be divided into two species, one from the highlands of Katanga, and the other from the lowland forests. P. vinckei, on the other hand, may be a cline, or four populations deserving subspecific rank. From the phylogenetic relationships and evolution of rodent hosts, it is postulated that, as murine rodents acquired arboreal habits, murine malaria parasites evolved from those of anomalurids.

Part V is a comprehensive checklist and host index of malaria parasites, haemoproteids, piroplasms and haemogregarines of rodents of the World.

In Appendix 1 parasites of flying squirrels, other than Plasmodium are considered. Descriptions are given of (i) Trypanosoma denysi in A. peli, (ii) a new trypanosome (subgenus Herpetosoma) in A. derbianus, and (iii) two new haemogregarines of I. macrotis.

Appendix 2 is work published in 1970 and 1971, offered as subsidiary matter.

ACKNOWLEDGEMENTS

I thank Prof. P.C.C. Garnham F.R.S. for advice and encouragement throughout this work; it is a privilege to have studied malaria under his supervision.

I am grateful to Prof. T.R.E. Southwood and Dr Elizabeth Canning for the facilities they have provided. I have recieved support from a grant to Dr Canning from the Medical Research Council.

For much of the experimental work mosquitoes were provided by Mr P.G. Shute O.B.E., Miss M. Maryon and Mr G.S. Gill all of whom I wish to thank.

Field work in West and Central Africa was carried out while I was acting as a Consultant for the World Health Organization. I wish to acknowledge the help received during the trip from Mr J.P. Adam in Brazzaville; Mr A.O. Lambo, Mrs. R.I. Otitoju and Dr David Happold in Nigeria; Mr and Mrs. Louis Bellier in the Ivory Coast; and Dr B.O.L. Duke in West Cameroons.

I wish to thank Miss Rosemary Church for the diligence and speed with which she typed this thesis, and Mrs. Angela Bishton for obtaining many works of reference for me. Finally, it is a pleasure to record my gratitude to my wife, without whose support this work would never have been done.

CONTENTS

Abstract	1
Acknowledgements	3
Contents	4
List of Illustrations and Tables	8

PART I

General Introduction	13
History of the Discovery of Malaria Parasites of Rodents, and the Elucidation of their Life-cycles ..	15
References in Part I	23

PART II

PLASMODIUM BERGHEI (NIGERIA)

Introduction	29
Materials and Methods	29
Life-cycle of <u>P. berghei</u> (Nigeria)	31
Sporogony	31
Effect of temperature on sporogony	35
Infectivity of sporozoites	41
Exoerythrocytic schizogony	42
Erythrocytic schizogony	48
Distribution of <u>P. berghei</u> (Nigeria)	52
Hosts	52
Limits of geographical range	54
Discussion	58
Diagnosis of <u>P. berghei</u> (Nigeria)	69
References in Part II	74

PART III

MALARIA PARASITES OF FLYING
SQUIRRELS IN THE IVORY
COAST.

Introduction		79
Materials and Methods		79
Locality		79
Flying Squirrels		80
Techniques		86
Results of a Survey		86
Malaria Parasites in <u>A. peli</u> No 70/35		91
Malaria Parasites in <u>A. peli</u> No 70/37		94
Discussion		97
References in Part III		103

PART IV

TAXONOMY OF MURINE MALARIA PARASITES

Introduction		106
The concept of protozoan species		106
The subspecies		113
Demes and clines		116
Syngens		117
Taxonomy of <u>P. berghei</u> and subspecies		119
Taxonomy of <u>P. vinckei</u> and subspecies		128
Origins of Malaria Parasites of African Rodents		130
References in Part IV		137

PART V

PROTOZOA IN THE BLOOD CELLS OF RODENTS

Introduction		144
--------------	---------	-----

An Annotated Checklist	..	..	..	..	..	..	146
Hepatozoidae	..	..	..	..	..	..	146
Plasmodiidae	..	..	..	..	..	..	159
Haemoproteidae	..	..	..	..	..	..	163
Babesiidae	..	..	..	..	..	..	166
Dactylosomidae	..	..	..	..	..	..	178
Host-Index	..	..	..	..	..	..	180
SCIUROMORPHA	..	..	..	..	..	..	180
Sciuridae	..	..	..	..	..	..	180
Sciurinae	...	..	..	..	..	..	180
Petauristinae	..	..	..	..	..	..	186
SCIUROMORPHA <u>incertae sedis</u>	..	..	..	..	..	..	187
Ctenodactylidae	..	..	..	..	..	..	187
THERIDOMYOMORPHA	..	..	..	..	..	..	188
Anomaluridae	..	..	..	..	..	..	188
Anomalurinae	..	..	..	..	..	..	188
Zenkerellinae	..	..	..	..	..	..	188
MYOMORPHA	..	..	..	..	..	..	188
Cricetidae	...	..	..	..	..	..	188
Cricetinae	..	..	..	..	..	..	188
Gerbillinae	..	..	..	..	..	..	192
Cricetomyinae	..	..	..	..	..	..	194
Muridae	..	..	..	..	..	..	194
Murinae	..	..	..	..	..	..	194
Dendromurinae	..	..	..	..	..	..	200
Otomyinae	..	..	..	..	..	..	200
Dipodae	..	..	..	..	..	..	201
Dipodinae	..	..	..	..	..	..	201
MYOMORPHA <u>incertae sedis</u>	..	..	..	..	..	..	202
Gliridae	..	..	..	..	..	..	202
Glirinae	..	..	..	..	..	..	202

LIST OF ILLUSTRATIONS AND TABLES

Plate 1.	Nigerian <u>Plasmodium berghei</u> ; 36 hr. old tissue	
	schizont	 43
Plate 2.	Nigerian <u>P. berghei</u> ; 49 hr. old tissue	
	schizonts	 45
Plate 3.	Nigerian <u>P. berghei</u> ; nuclei of infected	
	parenchymal cells	 47
Plate 4.	Nigerian <u>P. berghei</u> ; erythrocytic stages	... 49
Plate 5.	<u>Anomalurus peli</u>	 81
Plate 6.	<u>Anomalurus derbianus</u>	 83
Plate 7	Trees inhabited by <u>A. derbianus</u>	 84
Plate 8.	(i) <u>Idiurus macrotis</u>	
	(ii) Tree inhabited by <u>I. macrotis</u>	 85
Plate 9	Malaria parasites of <u>A. peli</u> No 70/35	... 93
Plate 10	Malaria parasites of <u>A. peli</u> No 70/37	... 95
Plate 11	Schizont of haemogregarine of <u>I. macrotis</u>	... 245

FIGURES

Fig 1.	Nigerian <u>P. berghei</u> ; ookinetes	 32
Fig 2.	Nigerian <u>P. berghei</u> ; pigment patterns of	
	young oocysts	 34
Fig 3.	Nigerian <u>P. berghei</u> ; drawings of the midlines	

	of sporozoites		36
Fig 4.	Nigerian <u>P. berghei</u> ; drawings of the midlines		
	of microgametes		51
Fig 5.	Map of Africa showing the distribution of murine		
	malaria parasites		56
Fig 6.	Map of West Africa showing faunal barriers	.	60
Fig 7.	Dice-Leraas diagrams of lengths of sporozoites		
	of <u>P. berghei</u> (Nigeria).		64
Fig 8.	Phylogeny of rodents showing positions of hosts		
	of malaria parasites		134
Fig 9.	<u>Trypanosoma denysi</u> in the blood of <u>A. peli</u> ...		233
Fig 10.	<u>Trypanosoma</u> sp. in the blood of <u>A. derbianus</u> .		236
Fig.11.	<u>Hepatozoon</u> sp. (short form) in the blood of		
	<u>I. macrotis</u>		240
Fig 12.	<u>Hepatozoon</u> sp. (long form) in the blood of		
	<u>I. macrotis</u>		243

TABLES

Table 1.	Effect of temperature on the sporogony of <u>P.</u>		
	<u>berghei</u> (Nigeria)		37
Table 2.	Nigerian rodents examined for malaria para-		
	sites		53

Table 3.	Measurements of sporozoites of <u>P. berghei</u> sspp.	65
Table 4.	Differences between highland and lowland populations of <u>P. berghei</u>	68
Table 5.	Results of a survey of flying squirrels in the Ivory Coast	87
Table 6.	Spleen sizes of <u>A. peli</u>	89
Table 7.	Stages of malaria parasites seen in the heart blood of infected anomalurines	90
Table 8.	Differences between three malaria parasites of anomalurines	99
Table 9.	Time-scale of the evolution of some vertebrate and invertebrate hosts of malaria parasites .	132

When a young naturalist commences the study of a group of organisms quite unknown to him, he is at first perplexed in determining what differences to consider as specific, and what as varietal; But if his observations be widely extended, he will in the end generally be able to make up his own mind: but he will succeed in this at the expense of admitting much variation - and the truth of this admission will often be disputed by other naturalists.

Charles Darwin, 1859

PART I
GENERAL INTRODUCTION AND
HISTORICAL REVIEW

GENERAL INTRODUCTION

The present work represents a continuation of a study of malaria parasites of rodents begun in 1965. Since that time results of much of the work have been published including accounts of (i) field studies on the parasites (Killick-Kendrick, et al., 1968; Killick-Kendrick & Bellier, 1971); (ii) the collection of strains in the field and their cyclical maintenance in the laboratory (Killick-Kendrick, 1971; (iii) life-cycles in the laboratory-bred hosts (Landau & Killick-Kendrick, 1966a,b; Killick-Kendrick & Warren, 1968; Killick-Kendrick, 1970b); (iv) the distribution and differential characters of murine malaria parasites (Garnham et al., 1967); (v) naturally occurring chloroquine resistance (Warhurst and Killick-Kendrick, 1967); and (vi) the fine structure of primary exoerythrocytic schizonts (Garnham et al., 1969). In addition, a study of the Nigerian murine malaria parasites was presented early in 1970 in a thesis for the M.Phil. degree.

Because so much has already been written, it seems unnecessary in the present work to repeat all the methods used in the study of the life-cycles of murine malaria parasites. At the risk of repetition on historical account is, however, presented to serve as a background.

The aspects studied in the present work are confined to the morphology, life-cycles, zoogeography and systematics of malaria parasites of rodents. The fine structure of the parasites and pathology, immunology and chemotherapy are not considered.

Sections II and V are a departure from the conventional presentation. They are revised and extended versions of parts of the writer's M.Phil. thesis, and are written as self-contained parts in a form suitable for submission for publication.

THE HISTORY OF THE DISCOVERY OF
MALARIA PARASITES OF AFRICAN RODENTS
AND THE ELUCIDATION OF THEIR LIFE-
CYCLES

The story of the discovery of the first true malaria parasites of rodents by the late Prof. I.H. Vincke is now almost a legend (van den Berghe, 1954; Garnham, 1966; Bafort, 1971). In 1943, while studying sylvatic mosquitoes in the gallery forests of the Katangan highlands, Belgian workers found sporozoites in the salivary glands of many specimens of Anopheles dureni (later recognized as a subspecies, A.d. millicampi; see Gillies and de Meillon, 1968). The identity of the vertebrate host of the parasite remained unknown until the results of precipitin tests on blood-meals of wild-caught mosquitoes focussed attention on the rodents of the galleries.

In February, 1948, a patent infection of malaria was found in the blood of a thick-knee-rat, Grammomys surdaster, and the first strain of murine malaria parasites was established in laboratory rodents by the inoculation of blood from the wild rat. Vincke and Lips (1948) described the parasite and named it Plasmodium berghei. Blood from freshly engorged A.d. millicampi was found to give rise to infections in mice (Vincke and Lips, 1948, 1950) and after a further period of study it was finally proved that sporozoites in

the glands of the wild-caught mosquitoes were those of the parasite of the thicket-rat (see Bafort, 1971). Two other sylvatic rodents, Praomys jacksoni and Leggada bella, were also found to be natural hosts of P. berghei (Vincke, 1954).

During this work Vincke recognized a second species of parasite in the blood of a mouse which had been inoculated with sporozoites from a wild-caught specimen of A. d. millecampsi. Rodhain (1952) named this new parasite P. vinckei in honour of its discoverer.

There followed a twelve-year period of intense activity during which malariologists in many parts of the world endeavoured to establish regular cyclical transmissions of P. berghei in the laboratory. One objective was to demonstrate the tissue schizogony so that the relationships of murine malaria parasites to other species could be more clearly understood. The inoculation of sporozoites from wild-caught A. d. millecampsi into laboratory rodents had shown that the period of pre-erythrocytic development of P. berghei was only 2 - 3 days (Vincke and Lips, 1950; Vincke and Peters, 1953), and this remarkably short time led to the suggestion, later found to be wrong, that the exoerythrocytic development of the murine malaria parasites was perhaps more similar to that of avian parasites than of malaria parasites of primates (which were known to have a tissue schizogony of never less than $5\frac{1}{2}$ days).

Yoeli and Wall (1951, 1952) were the first to achieve cyclical transmission of P. berghei in the laboratory, but the infectivity of the sporozoites was too low for the demonstration of the complete

life-cycle. Perez-Reyes (1953) and Yoeli et al. (1964) met with similar partial success, but most workers (e.g. Box et al., 1953; Bray, 1954; Celaya et al., 1956; Jadin et al., 1959; Michiels, 1963) found that even when sporogony seemed healthy, sporozoites produced in laboratory-bred mosquitoes were never infective.

Throughout the 1950's it was generally assumed that the distribution of malaria parasites of African rodents was restricted to the forest galleries of the Katangan highlands, and that this limited biotope had resulted in fixed characters which hindered the establishment of the parasites in mosquitoes other than the natural invertebrate host, A. d. millescampsi (which could not be bred in the laboratory). A clue that the range of the parasites was wider than supposed was largely overlooked. In 1954, Bruce-Chwatt and Gibson (1955) found a P. vinckei-like parasite in a rodent, the identity of which is now in doubt, in Western Nigeria. Unfortunately, the parasite was not established in the laboratory, and in spite of a determined campaign to find it again, the efforts of Bruce-Chwatt and Gibson were unsuccessful.

A few years later two more malaria parasites of African rodents were discovered. Within a short time of each other, Languillon (1957) in the Cameroons and van den Berghe et al. (1958) in Congo-Kinshasa discovered P. atheruri of the brush-tailed porcupine, Atherurus africanus. The natural vector was found to be a cavernicolous anopheline, A. smithi vanthieli in the Congo and was probably A. smithi rageai in the East Cameroons (Mouchet et al., 1957).

P. atheruri was later found in the Congo-Brazzaville (Landau et al., 1969), where the vector is thought to be A. caroni (Adam, personal communication).

In 1958 Pringle (1960) found a new parasite in the blood of a single specimen of the anomalurine flying squirrel Anomalurus derbianus (= A. fraseri); he described it and named it P. anomaluri. Neither P. anomaluri nor P. atheruri were established in the laboratory, and their discovery contributed nothing to the attempts to demonstrate life-cycles in laboratory-bred hosts.

Two discoveries in 1964 resulted in rapid advances. Firstly, persistent attempts by Prof. Meir Yoeli to discover the factors limiting the infectivity of sporozoites of P. berghei in the laboratory bore fruit and, secondly, Dr Irène Landau discovered a locality in the Central African Republic (C.A.R.) where specimens of a thick-knee rat different from the one in Katanga were commonly infected with new subspecies of P. berghei and P. vinckei.

Yoeli visited Katanga and, in collaboration with Belgian workers, studied the conditions in the forest galleries. They were struck by the low temperature of the biotope, noted earlier by Vincke (1954). Wild-caught A. d. millecampsi with natural infections of P. berghei were flown to Yoeli's laboratory in New York where the effect of low temperatures on the sporogony of freshly isolated strains of P. berghei in laboratory-bred mosquitoes was investigated. This led to the discovery that if the temperature during sporogony was kept below 21°C, sporozoites were constantly infective to laboratory rodents *

* (overleaf) (Vanderberg and Yoeli, 1964).

As soon as this was known, Yoeli was able to demonstrate the complete life-cycle of P. berghei, and to describe the exoerythrocytic forms of a malaria parasite of rodents for the first time (Yoeli and Most, 1965). The tissue schizonts were, like those of the parasites of primates, in the parenchymal cells of the liver. Their rate of growth was found to be remarkably rapid, resulting in the release of merozoites into the blood only 2 days after the inoculation of sporozoites.

The second event in 1964, which was to have far-reaching consequences, was the discovery by Mme. Landau of P. chabaudi in a thicket rat, Thamnomys rutilans, from the Central African Republic (Landau, 1965). This parasite was recognized as being closely related to P. vinckei (see Landau and Killick-Kendrick, 1966b), and was later placed as a subspecies, P.v. chabaudi (see Bafort, 1968). In May, 1965, an investigation of the locality in which the infected rat had been collected revealed a second parasite in the same host, resembling P. berghei from Katanga (Landau & Chabaud, 1965).

The two new parasites from the C.A.R. were found to produce infective sporozoites in laboratory mosquitoes at higher temperatures than P. berghei from Katanga (Wéry, 1968), and complete life-cycles of both were obtained in A. stephensi (at 24°C) and in white rats

* Bafort (1971, pp 55 & 76) does not believe this is typical of P. berghei from Katanga. His conclusion is based on the unsound supposition that an old resurrected strain of P. b. berghei (K173) is typical of those occurring in Nature, and on the mistaken idea that the wider tolerance to high temperatures of the sympatric parasite P. v. vinckei must necessarily apply to P. b. berghei.

and mice at the very first attempt (Landau and Killick-Kendrick, 1966b). The demonstration of the tissue forms of P. v. chabaudi was the first of the species P. vinckei.

The second, P. berghei-like parasite of the C.A.R. was named P. berghei yoelii, in honour of the discoverer of the life-cycle of P. berghei from Katanga (Landau and Killick-Kendrick, 1966a); the latter parasite was then designated as the nominate subspecies, P. berghei berghei. It is curious to note that had Prof. Yoeli's work been delayed, the Anglo-French workers may well have cheated him of the justly deserved priority of discovery of the tissue schizogony; they were working with less difficult parasites.

The comparative ease with which P. b. yoelii was cyclically transmitted in the laboratory enabled the writer and Dr McWilson Warren (1968) to demonstrate an unusual facet of the life-cycle of the species; it was shown that some merozoites from the exoerythrocytic schizonts developed directly into gametocytes with no intervening schizogony in the blood.

During Mme. Landau's study of malaria of rodents of the C.A.R. she found a number of schizonts in the livers of wild-caught Thamnomys, the morphology of which was very different from that of the primary exoerythrocytic forms of P. b. yoelii and P. v. chabaudi. (Landau and Chabaud, 1965; Landau and Killick-Kendrick, 1966b). It was later shown that these forms grew very slowly (Landau, Chabaud et al., 1968) and that their numbers were increased in the livers of laboratory-bred African rodents if the animals were

subjected to low temperatures after the inoculation of sporozoites (Landau, in press). The slow-growing schizonts appear to arise directly from sporozoites and not to be a second generation of tissue forms.

The discovery of the new enzootic locality in the C.A.R. was followed by new field work in other parts of Africa. In Brazzaville, Mme. Landau found yet another locality where, again, 2 parasites were found in the thick-knee rat, Thamnomys rutilans (Adam et al., 1966). The life-cycles of both have been described and one has been named P. b. killicki and the other P. v. lentum (Landau, Michel et al., 1968, 1970).

Early in 1967, Bafort isolated a strain of P. v. vinckei from the salivary glands of a wild A. d. millescampsi collected in the type locality in Congo-Kinshasa. This was only the second occasion that this parasite had been found, and the new isolate enabled Bafort (1967, 1968) to demonstrate the sporogony and tissue schizogony of P. v. vinckei for the first time. In spite of the intensive work in Katanga, P. v. vinckei has never yet been found in a wild rodent.

As a direct result of Mme. Landau's field work, the writer went to Nigeria in 1967 to look for the almost-forgotten P. vinckei-like parasite of Bruce-Chwatt and Gibson. The French workers' results in the C.A.R. and Congo-Brazzaville strongly suggested that the rodent most likely to be a host of malaria parasites in Nigeria was Thamnomys rutilans, a species which had not been examined in the early work in that locality. A P. vinckei-like parasite, believed

to be the same as the one seen by Bruce-Chwatt and Gibson (1955) was found in six of these thicket-rats, and a second P. berghei-like parasite was also discovered in the same host (Killick-Kendrick et al., 1968). An account of the life-cycle of the second parasite forms part II of the present work. The sporogony of the P. vinckei-like parasite has been described elsewhere (Killick-Kendrick, 1970 a, b). Bafort (in press) has successfully transmitted a strain through laboratory-bred A. stephensi and has found the first tissue schizont of this parasite.

In 1970 an Anglo-French field project led to the discovery of malaria parasites in two anomalurine flying squirrels (Anomalurus peli and A. derbianus) in the Ivory Coast (Killick-Kendrick and Bellier, 1971); the parasites, which are described in part III of the present work, were thought to be different from P. anomaluri from Tanzania. The appearance of the spleens of the specimens of anomalurines examined in the Ivory Coast suggested that all had had malaria, and it now seems as though malaria parasites of flying squirrels in Africa may be very widespread.

REFERENCES TO PART I

- BAFORT, J. (1967). La transmission cyclique de Plasmodium vinckei. Annls Soc. belge Med. trop., 47, 271-276.
- BAFORT, J. (1968). Primary exo-erythrocytic forms of Plasmodium vinckei. Nature, London, 217, 1264-1265.
- BAFORT, J.M. (1971) The biology of rodent malaria with particular reference to Plasmodium vinckei Rodhain, 1952. Annls Soc. belge Méd. trop., 51, 1-204.
- BAFORT, J. (in press). Le cycle biologique de Plasmodium vinckei de Nigeria. Proc. 1st European Multicolloquium in Parasitology, Rennes, 1971.
- van den BERGHE, L. (1954). The history of the discovery of Plasmodium berghei. Indian J. Malar., 8, 241-243.
- van den BERGHE, L., PEEL, E., CHARDOME, M., & LAMBRECHT, F. L. (1958). Le cycle asexué de Plasmodium atheruri n. sp. du porc-épic Atherurus africanus centralis au Congo belge. Annls. Soc. belge Med. trop., 38, 971-976.
- BOX, E.D., CELAYA, B.I., & GINGRICH, W.D. (1953). Development of Plasmodium berghei in Anopheles quadrimaculatus. Am. J. trop. Med. Hyg., 2, 624-627.
- BRAY, R.S. (1954). The mosquito transmission of Plasmodium berghei. Indian J. Malar., 8, 263-274.
- BRUCE-CHWATT, L.J. & GIBSON, F.D. (1955). A Plasmodium from a Nigerian rodent. Trans. R. Soc. trop. Med. Hyg. 49, 9.
- CELAYA, B.I., BOX, E.D. & GINGRICH, W.D. (1956). Infectivity of Plasmodium berghei for Anopheles quadrimaculatus and other mosquitoes. Am. J. trop. Med. Hyg., 5, 168-182.
- GARNHAM, P.C.C., (1966). Malaria parasites. Oxford: Blackwell.
- GARNHAM, P.C.C., BIRD, R.G., BAKER, J.R., & 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, 328-332.
- GARNHAM, P.C.C., LANDAU, I., KILLICK-KENDRICK, R., & ADAM, J.P. (1967). Répartition et caractères différentiels des Plasmodiums des muridés. Bull. Soc. Path. exot., 60, 118-127.
- GILLIES, M.T., & de MEILLON, B. (1968). The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region). 2 ed., S. African Institute for Medical Research.
- JADIN, J., YOELI, M., & PIERREUX, G. (1959). Réapparition du processus d'extraflagellation chez une souche de Plasmodium berghei régulièrement entretenue par passage mécanique. Annls Soc. belge Méd. trop., 39, 847-850.
- KILLICK-KENDRICK, R. (1970a). Studies on the life-cycles of murine malaria parasites from Nigeria. M. Phil. thesis, University of London.
- KILLICK-KENDRICK, R. (1970b). The life-cycles of two murine malaria parasites from Nigeria. J. Parasit., Suppl., 56, No. 4, Sect II, Part 2, 435-436.
- KILLICK-KENDRICK, R. (1971). The collection of strains of murine malaria parasites, and their maintenance in the laboratory by cyclical passage. In Isolation and Maintenance of Parasites in vivo, A.E.R. Taylor & R. Muller (Eds), Symp. Brit. Soc. Parasit., 9, 39-64.
- KILLICK-KENDRICK, R., & BELLIER, L. (1971). Blood parasites of scaly-tailed flying squirrels in the Ivory Coast. Trans. R. Soc. trop. Med. Hyg., 65, 430-431.
- KILLICK-KENDRICK, R., SHUTE, G.T., & LAMBO, A.O. (1968). Malaria parasites of Thamnomys rutilans (Rodentia: Muridae) in Nigeria. Bull. Wld Hlth Org., 38, 822-824.
- KILLICK-KENDRICK, R., & WARREN, M. (1968). Primary exoerythrocytic schizonts as a source of gametocytes. Nature, London, 220, 191-192.

- LANDAU, I. (1965). Description de Plasmodium chabaudi n.sp. parasite de rongeurs africains. C. r. hebd. Séanc. Acad. Sci., Paris, 260, 3758-3761.
- LANDAU, I., 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, 8-9.
- LANDAU, I., & CHABAUD, A.G. (1965). Infection naturelle par deux Plasmodium du rongeur Thamnomys rutilans en République Centre Africaine. C.r. hebd. Séanc. Acad. Sci., Paris, 261, 230-232.
- LANDAU, I., CHABAUD, A.G., 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, 63-72.
- LANDAU, I., & 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, 113-116.
- LANDAU, I., & KILLICK-KENDRICK, R. (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, 633-649.
- LANDAU, I., & MICHEL, J.C. (1970). Reproduction expérimentale de schizontes hépatiques retardées de Plasmodium berghei yoelii dans le foie d'un Steatomys (Dendromurinae). C. r. hebd. Séanc. Acad. Sci. Paris, 271, 1627-1629.
- LANDAU, I., MICHEL, J.C., & ADAM, J.P. (1968). Cycle biologique au laboratoire de Plasmodium berghei killicki n. subsp. Annls Parasit. hum. comp., 43, 545-550.
- LANDAU, I., MICHEL, J.C., ADAM, J.P. & BOULARD, Y. (1970). The life-cycle of Plasmodium vinckei lentum n. subsp. in the laboratory: comments on the nomenclature of the murine malaria parasites. Ann. trop. Med. Parasit., 64, 315-323.

- LANGUILLON, J. (1957). Note sur deux parasites sanguicoles de mammifères du Cameroun. Bull. Soc. Path. exot., 50, 362-363.
- MICHELIS, G. (1963). Observations sur la transmission cyclique du Plasmodium berghei. Annls Soc. belge Méd. trop., 43, 83-90.
- MOUCHET, J., GARIOU, J., & RIVOLA, E. (1957). Observations sur la biologie d'Anopheles smithi var. rageai Mattingley et Adam, 1954. Vecteur d'un Plasmodium de mammifère aux environs de Yaoundé (Sud Cameroun). Bull. Soc. Path. exot., 50, 157-164.
- PEREZ-REYES, R. (1953). Anopheles aztecus (Hoffman, 1935) a new definitive host for the cyclical transmission of Plasmodium berghei Vincke and Lips, 1948. J. Parasit., 39, 603-604.
- PRINGLE, G. (1960). Two new malaria parasites from East African vertebrates. Trans. R. Soc. trop. Med. Hyg., 54, 411-414.
- RODHAIN, J. (1952). Plasmodium vinckei n. sp. Un deuxième Plasmodium de rongeurs sauvages au Katanga. Annls Soc. belge Méd. trop., 32, 275-280.
- VANDERBERG, J., & YOELI, M. (1964). Some physiological and metabolic problems related to maintenance of the Plasmodium berghei cycle in Anopheles quadrimaculatus. Annls Soc. belge Méd. trop., 45, 419-426.
- VINCKE, I.H. (1954). Natural history of Plasmodium berghei. Indian J. Malar., 8, 245-256.
- VINCKE, I.H., & LIPS, M. (1948). Un nouveau Plasmodium d'un rongeur sauvage du Congo: Plasmodium berghei n. sp. Annls Soc. Belge Méd. trop., 28, 97-104.
- VINCKE, I.H. & LIPS, M. (1950). Note sur la transmission du Plasmodium berghei. Annls Soc. belge Méd. trop., 30, 1605-1611.
- VINCKE, I.H. & PEETERS, E. (1953). Observations sur la transmission de sporozoïtes d'Anopheles durenii à des rongeurs sauvages et de laboratoire. Annls Soc. belge Méd. trop., 33, 87-93.

- WARHURST, D.C., & KILLICK-KENDRICK, R. (1967). Spontaneous resistance to chloroquine in a strain of rodent malaria (Plasmodium berghei yoelii) Nature, London, 213, 1048-1049.
- WÉRY, M. (1968). Studies on the sporogony of rodent malaria parasites. Annls Soc. belge Méd. trop., 48, 1-137.
- YOELI, M., & MOST, H. (1965). Pre-erythrocytic development of Plasmodium berghei. Nature, London, 205, 715-716.
- YOELI, M., MOST, H., & BONÉ, G. (1964). Plasmodium berghei; cyclical transmission by experimentally infected Anopheles quadrimaculatus. Science, N.Y., 144, 1580-1581.
- YOELI, M., & WALL, W.J. (1951). Complete sporogonic development of Plasmodium berghei in experimentally infected Anopheles spp. Nature, London, 168, 1078.
- YOELI, M., & WALL, W.J. (1952). Cyclical transmission of Plasmodium berghei in the laboratory. Nature, London, 169, 881.

PART II

PLASMODIUM BERGHEI (NIGERIA)

THE LIFE-CYCLE AND DISTRIBUTION OF PLASMODIUM BERGHEI (NIGERIA)

In West and Central Africa, three populations of the murine malaria parasite Plasmodium berghei are thought to be geographically separated from one another, and have been designated as subspecies viz: P. berghei berghei Viucke & Lips, 1948 from Katanga; P. berghei yoelii Landau & Killick-Kendrick, 1966 from the Central African Republic; and P. berghei killicki Landau, Michel & Adam, 1968 from Brazzaville. Morphological differences in the sporogonic and exoerythrocytic stages of strains from different places (see Landau, Michel, Adam & Boulard, 1970) and differences in the electrophoretic mobilities of enzymes (Carter, 1970) lend support to the notion that geographical separation is leading to speciation.

In the present work, an account is given of the distribution and life-cycle of P. berghei from a fourth locality, Nigeria. This population is believed to represent another subspecies for which a new name will be proposed. To avoid premature use of the name in an unpublished document, the new subspecies is here referred to as Plasmodium berghei (Nigeria). A brief note on the life-cycle was published in an abstract of a paper given at the 2nd International Congress of Parasitology, 1970, in Washington, U.S.A. (Killick-Kendrick, 1970a).

MATERIALS AND METHODS

The strain studied, No. N67, is the only strain of P. berghei (Nigeria) at present established in the laboratory. It was isolated

in 1967 from a thickset-rat, Thamnomys rutilans, trapped near the village of Ilobi in Western State, Nigeria (Killick-Kendrick, Shute & Lambo, 1968). On first isolation by the inoculation of blood from the wild rat into white mice, the strain was mixed with a P. vinckei-like parasite which is probably the same as one isolated earlier in the same locality from a specimen of Praomys tullbergi by Bruce-Chwatt & Gibson (1955). The P. vinckei-like parasite from the Thamnomys failed to produce infective sporozoites in Anopheles stephensi, and the strain of P. berghei became separated from the other by the first cyclical passage from mice at the third blood-passage from the natural host. A detailed history of strain N67 in the laboratory from 1967 - 1969 is given elsewhere (Killick-Kendrick, 1970b).

Mice used in the present work were from a commercially available outbred line of Theiler's Original strain. They were free from infection with Eperythrozoon and were fed on a balanced cube-diet. The rats used were the Wistar strain, and were similarly maintained.

Mosquitoes were bred at 27°C by the methods given by Shute & Maryon (1966). The strain of A. stephensi was from India, and was originally established in England by Mr P.G. Shute in 1947.

The growth of oocysts was observed on freshly dissected unsquashed midguts in normal saline under a coverslip; they were measured with an ocular scale. Sporozoites in stained smears of infected salivary glands, and microgametes in blood films, were measured by first drawing midlines at a magnification of X3800 with the aid of a drawing-tube and X2.5 magnifier fitted to a "Wild" M20 microscope. The lengths

of the parasites were then obtained with dividers set against a scale, drawn from a stage micrometer, to a distance equivalent to $1\mu\text{m}$ (Bruce, Hamerton & Bateman, 1909; Hoare & Broom, 1938). When drawing midlines no selection was made other than the rejection of obscured or badly damaged parasites, and all series were of consecutively encountered sporozoites or microgametes.

Suspensions of living sporozoites in cold tissue-culture medium "199" were prepared from the salivary glands of experimentally infected A. stephensi (see Killick-Kendrick, 1971). Numbers of sporozoites were estimated from counts made in an improved Neubauer counting chamber.

The methods employed for the demonstration of tissue schizonts were described by Landau & Killick-Kendrick (1966b).

THE LIFE-CYCLE OF P. BERGHEI (NIGERIA)

Sporogony

The strain was remarkable for the ease with which A. stephensi became infected, and the constant infectivity of sporozoites to laboratory rodents.

Sporogonic stages at 24°C

Ookinetes were numerous in smears of blood-meals prepared 18-20 hrs after mosquitoes had fed. In smears stained with Giemsa's stain, they were broad (3-4 μm) leaf-like bodies with a mean length of $11.4 \pm 0.27\mu\text{m}$ (30 ookinetes). The anterior end bore a darkly staining polar cap. The nucleus lay in the posterior half of the body; by 18 hrs

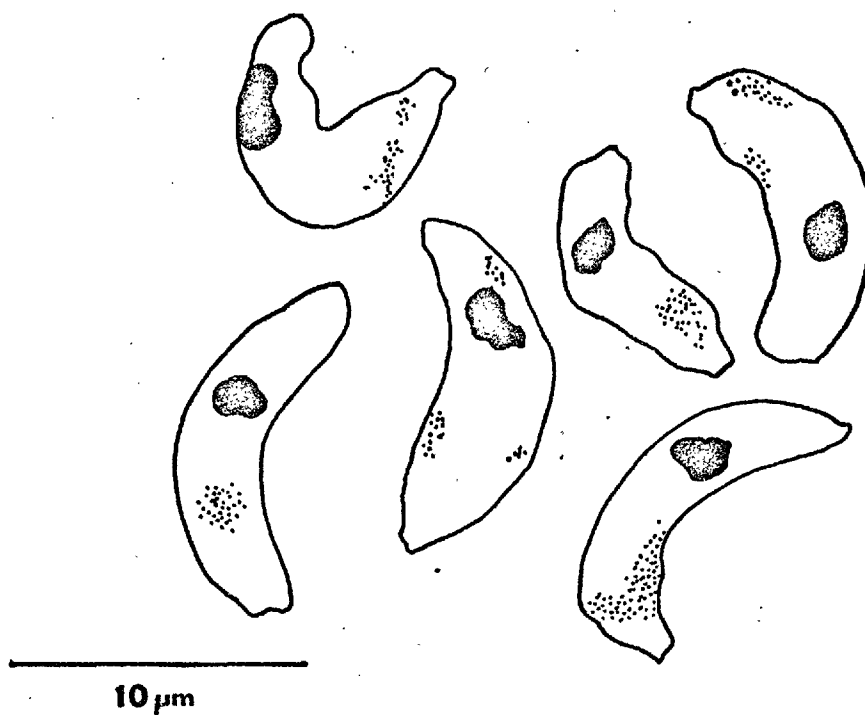


Fig. 1. Ookinetes of P. berghei (Nigeria) in a smear of a blood-meal of A. stephensi; 18 hrs (X 3,800).

the nucleus of the fertilizing microgamete had already fused with that of the macrogamete. Pigment lay scattered or in one or two masses anterior to the nucleus and not, as is more usual in other species, in a posterior position (fig. 1).

On the 4th day after feeding on mice carrying gametocytes, oocysts were numerous on the midguts of A. stephensi. The pigment in the oocysts was clearly visible and tended to lie in one place in short beaded lines, frequently crossing one another (fig. 2). The mean diameter of 12 oocysts was $19\mu\text{m}$ ($15\text{--}23\mu\text{m}$).

By day 5, pigment was no longer visible in the largest oocysts in which faint whorls were now seen. The mean diameter had now increased to $25\mu\text{m}$ ($17\text{--}32\mu\text{m}$). Growth then became slower. On day 6, the mean diameter of 25 oocysts was again $25\mu\text{m}$ ($16\text{--}34\mu\text{m}$), and pigment was still visible in the small oocysts and whorls in the large.

On day 7, a wide range of sizes was recorded, probably because some small oocysts had stopped growing. The mean diameter of the first 20 oocysts seen was $29\mu\text{m}$ ($21\text{--}39\mu\text{m}$), but a few much larger oocysts measuring $47\text{--}58\mu\text{m}$ were later encountered on the same day. Even in these very large oocysts there were 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 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 on the midguts of mosquitoes incubated at 24°C were consistent in size,

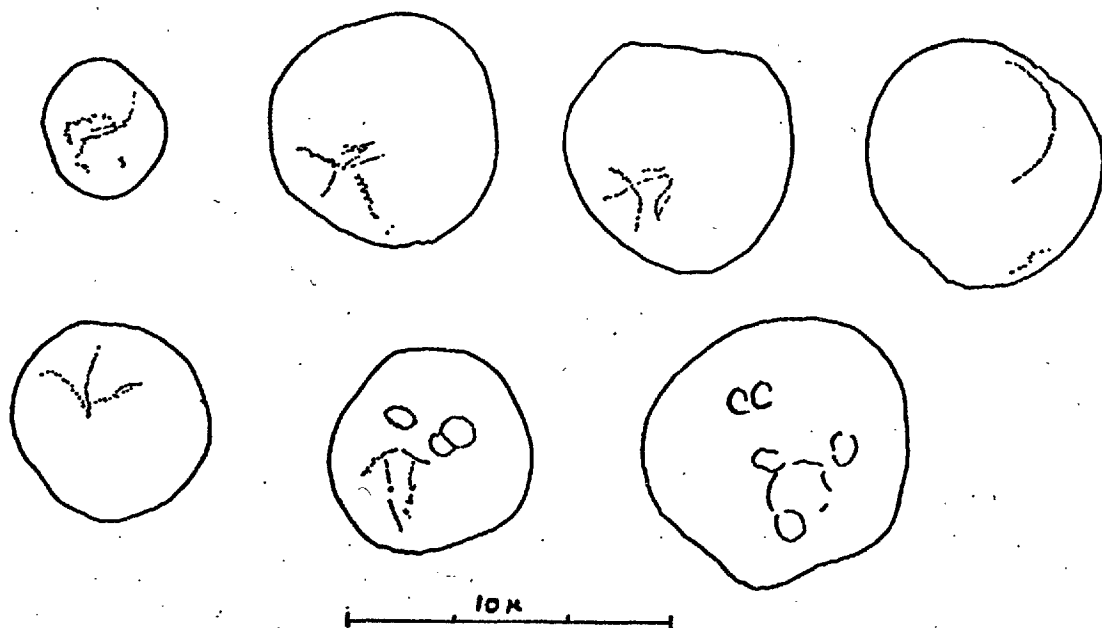


Fig. 2. Nigerian P. berghei: pigment patterns of oocysts on the midguts of A. stephensi, incubated at 24°C; days 4 and 5.

and when measured on many occasions their diameter was 50-62 μ m with a mean of 60 μ m.

Sporozoites first invaded the salivary glands of the mosquitoes on days 9-11. In fresh preparations of crushed glands they were slender, curved non-motile bodies. In a stained smear the mean length of 50 sporozoites was $16.72 \pm 0.22\mu$ m (10.0-19.5 μ m). Sporozoites in stained preparations typically lay in irregular curves (fig. 3). The nucleus was elongate and often beaded and was usually situated approximately in the centre of the body. One end of the sporozoites tended to stain more strongly than the other. From 2500 to 7000 sporozoites per mosquito were normally harvested from the salivary glands of experimentally infected A. stephensi incubated at 24°C.

Effects of high and low temperatures on the sporogony

The effects of low and high temperatures upon the growth of oocysts and the production of sporozoites were examined. The results are summarized in Table 1 .

Sporogony at 21°C In the first experiment, A. stephensi were permitted to feed on three mice three days after the inoculation of blood from another mouse with a sporozoite induced infection. Unfed female mosquitoes were removed, and the cage was incubated at 24°C for 24 hrs. It was known that sporogony was successful at this temperature, and incubation at 24°C during the formation and migration of ookinetes ensured that oocysts became established. After the first 24 hrs,

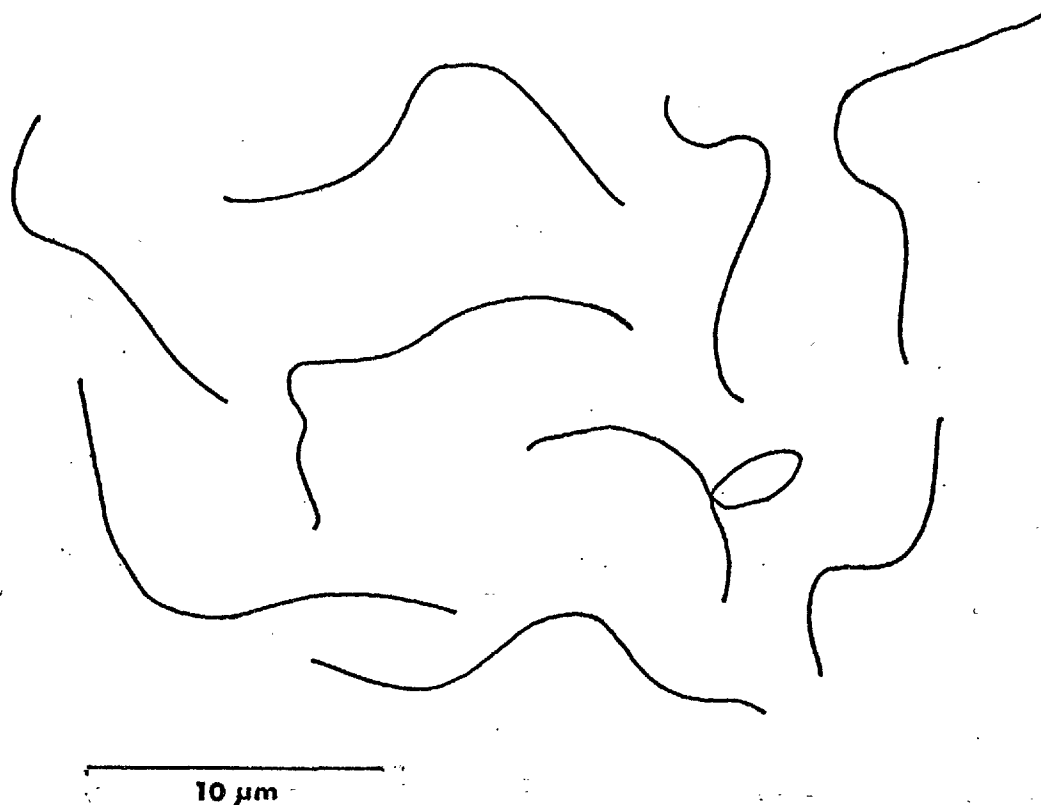


Fig. 3. Midline drawing of sporozoites of P. berghei (Nigeria) in a stained smear of salivary glands of A. stephensi (X 3,800).

TABLE 1

Sporogony of P. berghei (Nigeria) in A. stephensi at
21°C, 24°C & 28°C (First 24 hrs at 24°C)

Batch No. (1)	Temp. of sporogony °C	Infections of gut	Diameter of mature oocysts µm	Day glands first infected	Day glands harvested	Mean no. of sporozoites harvested (per mosquito)	Dose of sporozoites i.v. per mouse (2)
* 17OK/A	21	4/4 + ve > 200 oocysts	70	11 or less	12	1,500 (30)	22,000
* 17OK/B	24	5/6 + ve >200 oocysts	50	11 or less	12	2,500 (40)	22,000
** 177K/A	24	8/9 + ve 100- 250 oocysts	62	10	12	6,650 (74)	14,760
** 177K/B	28	8/8 + ve 1 - 50 oocysts	60	6	8	194 (70)	13,600

(1) Batches 17OK/A and 17OK/B were fed upon the same mice before being divided into two groups; batches 177K/A and 177K/B were similarly infected by feeding on another pair of mice.

(2) All mice became infected.

* first experiment

** second experiment

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, infection rates were found to be high in both batches, and numbers of oocysts were always more than 200 (see Table 1). 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 70µm in diameter), compared with a maximum of only 50µm on the midguts of mosquitoes at 24°C. By day 11 there were mature oocysts in both batches, and sporozoites in the salivary glands.

On day 12, the glands of all surviving mosquitoes were dissected and sporozoites were harvested. The mean number collected from each mosquito incubated at 21°C was 1500, and from those incubated at 24°C, 2500. Sporozoites were inoculated intravenously into mice; each of two mice were given 22000 from each batch of mosquitoes. All four mice had patent parasitaemias four days after inoculation, with 5-10 parasitized cells per field (X100 obj.) of a thin blood film.

The infectivity of the sporozoites of P. berghei (Nigeria) thus appeared to be 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.

Sporogony at 28°C A second experiment of similar design was performed

to investigate the effect of a temperature of 28°C on the sporogony in A. stephensi.

Mosquitoes were permitted to feed on two mice eight 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 hrs. 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 infection in both groups (see Table 1), 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 the 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 39µm (27-46µm), but that of 10 oocysts, all mature, in the batch at 28°C was 61µm (54-71µm). 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 no glands were infected, and there were no signs of the formation of sporozoites in the oocysts.

On day 8 there were sporozoites in the glands of 3/3 mosquitoes of the experimental batch, on the stomachs of which there were only few unruptured oocysts. Since it was then two days since the first invasion of the glands, the sporozoites from this group were harvested 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 13600 sporozoites developed a patent parasitaemia on the third day.

On day 9, mature oocysts of the control group attained the same mean diameter (60 μ m) as that reached by oocysts of the experimental group three days earlier. Sporozoites invaded the glands of the control group on day 10, and, as before, were harvested two days later. The yield was more than thirty times greater than from the experimental group, with a mean of 6650 sporozoites collected per mosquito. Three mice each inoculated intravenously with 14700 sporozoites had patent parasitaemias three days later.

The small number of oocysts on the midguts of the mosquitoes incubated at 28°C was perhaps due to interference with the penetration of ookinetes after the first 24 hrs at 24°C. In the control group, ookinete penetration and oocyst formation is presumed to have continued beyond 24 hrs. Although the higher temperature much increased the rate of growth of oocysts, their mean size when mature was the same as at 24°C (but the range of sizes was wider).

Even when the lower number of oocysts is taken into account, the higher temperature appeared to have caused a reduction in the numbers of sporozoites invading the glands; they were, nevertheless, infective to mice. The sporogony of P. berghei (Nigeria) thus seemed to be adversely affected by incubation at 28°C, but the parasite was more tolerant than P. b. berghei of the high temperature (see Yoeli, 1965; Wéry, 1968).

Infectivity of sporozoites

A. stephensi were fed on mice carrying gametocytes and were incubated for 15 days at 23-25°C. The salivary glands of 200 were then dissected, and a suspension of sporozoites was prepared in cold tissue-culture medium 199. From a count of the sporozoites, it was estimated that the suspension contained 8000 per 0.1 ml. A series of ten-fold dilutions was then prepared to provide six doses each of 8000, 800, 80 and 8 sporozoites per 0.1 ml. Throughout the dissection and the preparation of the dilutions which took 2 hrs to complete, tubes containing sporozoites were kept cool with iced water. The suspensions were well mixed and, beginning with the highest dilution, 0.1 ml doses of sporozoites were inoculated intravenously into four groups of six male mice of 15 gm body weight; the 24 inoculations took 33 minutes. Blood films from the mice were first examined four days later. The results were:

Mice	Doses	Blood films at 4 days
184K/1-6	8 sporozoites	all - ve
185K/1-6	80 sporozoites	all + ve
185K/1-6	800 sporozoites	all + ve
185K/1-6	8000 sporozoites	all + ve

The six mice given eight sporozoites 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

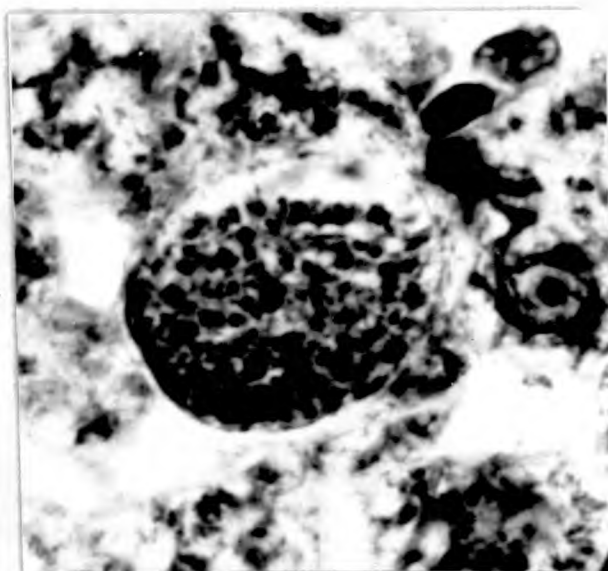
80 sporozoites. In work such as drug trials, in which it is necessary to infect many mice, doses of only a few hundred sporozoites should ensure that every mouse becomes infected. In practical terms, this means that with a yield of 2500 sporozoites per mosquito (the minimum recorded for P. berghei (Nigeria) in A. stephensi incubated at 23-25°C), as few as 10 mosquitoes would provide 50 doses of 500 sporozoites.

Primary exoerythrocytic schizogony

Tissue schizonts were obtained in the livers of white mice and rats given single intravenous inoculations of suspensions of sporozoites. The maturation time of the tissue stages was found to be approximately two days. Seven mice, given sporozoites intravenously, 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 taken 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 P. berghei (Nigeria) with those of subspecies from other localities, samples of liver were taken from white rats inoculated intravenously with sporozoites. Three rats were given inoculations of sporozoites from A. stephensi which had taken infective blood-meals from mice 11 days earlier. 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 that each rat received not less than

PLATE 1



P. berghei (Nigeria); 36 hr-old tissue schizont
in a section of liver (X 2,000).

75000 sporozoites.

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

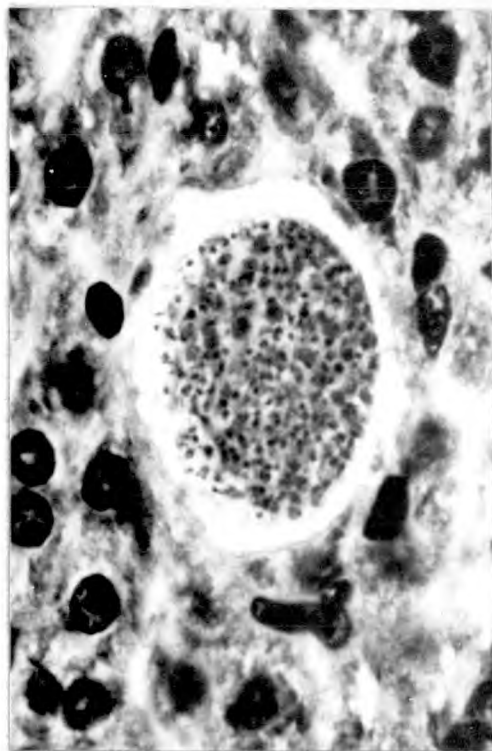
The tissue forms were very similar to those of P. b. yoelii (see Landau & Killick-Kendrick, 1966b), except that (i) the Nigerian parasite caused hypertrophy of the nuclei of some infected parenchymal cells similar to that seen in infections of P. b. killicki (see Landau et al., 1968), and (ii) mature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

*At 36 hrs the schizonts were oval in shape, and measured approximately 25 x 20µm (Plate 1, fig.i). The parasite completely filled the host-cell, the nucleus of which was pushed to one side. The nuclei of infected cells at 36 hrs were affected by the presence of the parasite, but were not enlarged. They stained lightly and the nuclear material lay clumped in a few peripheral masses; this appearance is the same as that seen in some cells infected with older schizonts of P. b. yoelii (illustrated in Plate 1, fig. 9 of Landau & Killick-Kendrick, 1966b).

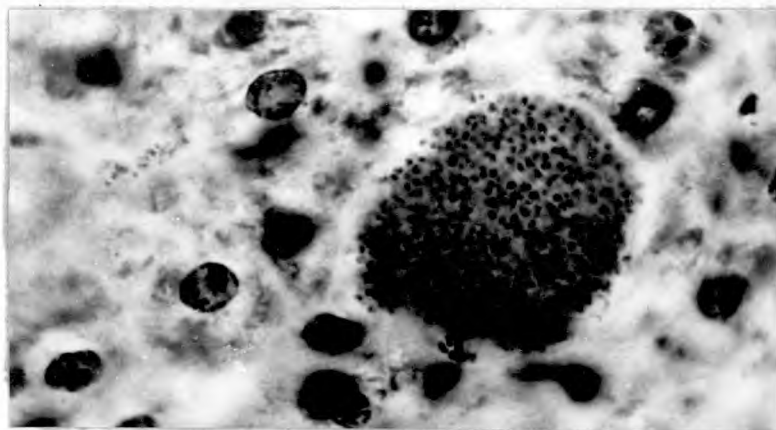
As with young tissue forms of all murine malaria parasites, the cytoplasm of the schizonts of P. berghei (Nigeria) stained a deep blue colour and obscured the nuclei of the parasite; deeply staining



(i)



(ii)



(iii)

P. berghei (Nigeria); 49 hr-old tissue schizonts in sections
of liver (X 1,500).

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 the fine limiting membrane.

The last stages of growth of the tissue forms were observed in samples of liver taken from a rat at 50 hrs and a mouse at 49 hrs. Mean diameters of mature schizonts in the rats were larger than those of P. b. yoelii in the same animal and measured 42-50 μ m (compared with 37 μ m for P. b. yoelii). In large immature forms the cytoplasmic masses seen at 36 hrs persisted; they were irregularly shaped and measured 1 - 3.5 μ m 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.

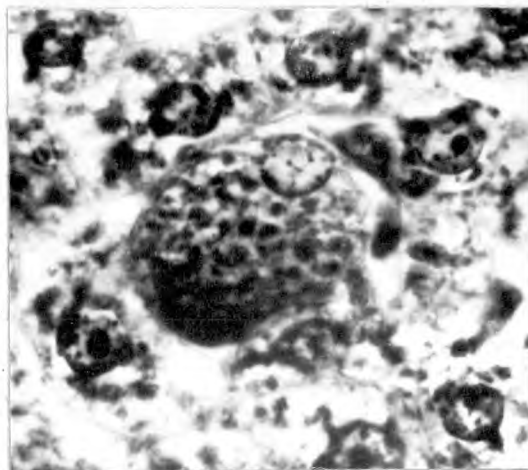
The final stages of maturation were the same as those of P. b. yoelii. The schizonts shrank a little in size, and in section appeared to lie in a large vacuole; pseudo-cytomeres arose giving a mosaic-like appearance (Plate 2, fig. i, ii) and finally numerous merozoites were formed (Plate 2, fig. iii). These were small round bodies less than 1 μ m in diameter, consisting simply of a nucleus surrounded by a fine rim of cytoplasm.

The nuclei of many infected parenchymal cells became much enlarged by 50 hrs, and measured up to 10 μ m across. The nuclear material was clumped into a few deeply staining masses, but the rest of the nucleus stained very palely (Plate 3, fig. ii).

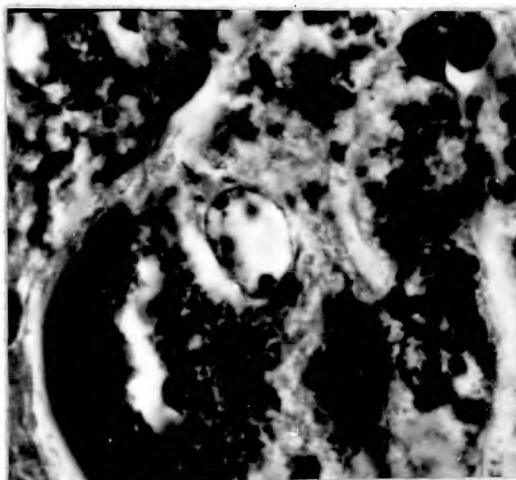
No differences were seen between schizonts in the liver of the rat and those in the mouse.

PLATE 3

Showing palely stained and enlarged nuclei of
parenchymal cells infected with primary exo-
erythrocytic schizonts of P. berghei (Nigeria)
(XI, 500)



(i) 36 hrs.



(ii) 50 hrs.

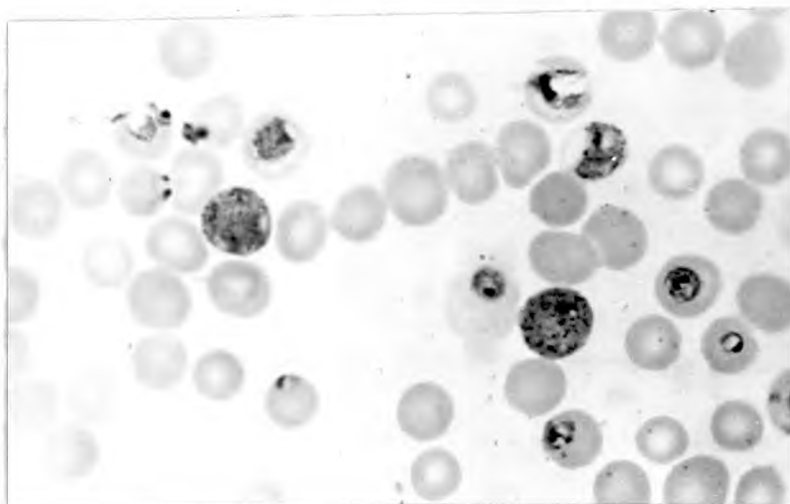
Blood stages in white mice (Plate 4)

With a few minor differences, the morphology of the blood stages of P. berghei (Nigeria) was the same as that of other subspecies of P. berghei. Polyparasitism was commonly seen, especially when parasitaemias were high. The usual predilection for immature erythrocytes was marked in many mice, but the usual reaction of the white mouse to infection (Yoeli, 1965), viz. the production of polychromatophils, was absent in about a quarter of the animals, and the 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 recovery in the second or third week of infection; mice with heavy infections in normocytes seldom survived more than two weeks.

The smallest parasites in the blood-cells of white mice were tiny compact bodies composed of a nucleus with a fine crescentic wisp of cytoplasm. They measured 1.0 - 1.2 μ m in diameter. As growth began, a central vacuole formed and the parasite then adopted the typical ring form of the genus Plasmodium; these forms occasionally 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. No stippling or enlargement of the

PLATE 4



(i)



(ii)

P. berghei (Nigeria) in the blood of a laboratory
mouse (X 1,500).

infected cell 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 grew 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.

Unusually large schizonts measuring 8.5 - 9.0 μm 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. Possibly they were aberrant forms resulting from poly-parasitism.

Gametocytes were indistinguishable from those of P. b. yoelii (see Wéry, 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 grains throughout the cytoplasm; female gametocytes seemed to contain more pigment than the males, but this appearance was perhaps due to the fineness of the pigment in the latter parasites. Some gametocytes, especially the males, contained in a large central vacuole; this may have been an indication of senility, since vacuolated forms became more numerous

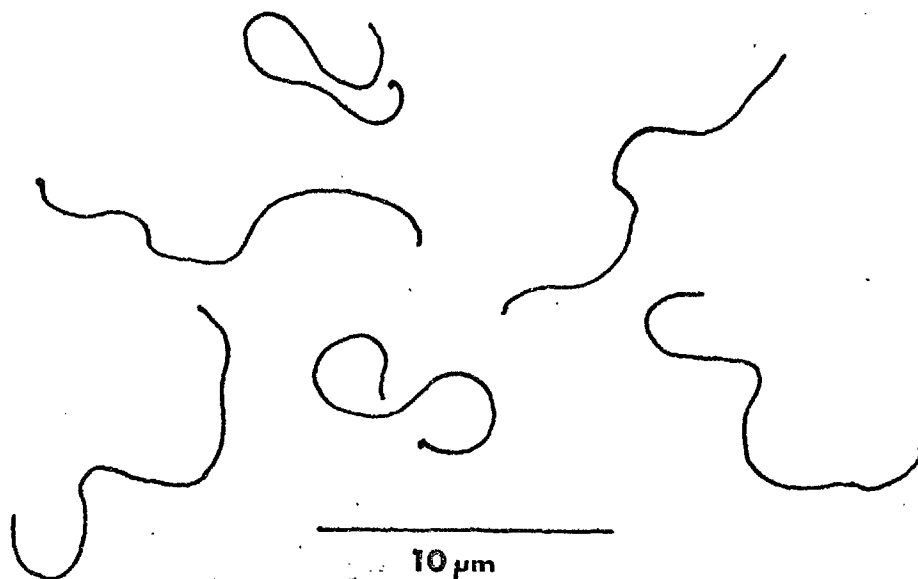


Fig. 4. Drawings of microgametes of P. berghei (Nigeria) in a stained film of blood and after exflagellation (X 3,800).

as infections progressed.

Exflagellation of male gametocytes in fresh drops of blood under a coverslip began 6 mins after withdrawal (at 16°C), and was complete within 14 mins. In stained films, microgametes were seen as slender, curved bodies with a mean length of $16.8 \pm 0.29 \mu\text{m}$ (10 microgametes). More than half bore a small knob at one end (fig 4).

THE DISTRIBUTION OF MURINE MALARIA PARASITES IN NIGERIA

Hosts

Large surveys by Bruce-Chwatt & Gibson, detailed results of which are unpublished, and later work by Killick-Kendrick et al., (1968) strongly suggest that the principal and possibly the only murine host of malaria parasites in Western Nigeria is the thick-knobbed rat, Thamnomys rutilans.

A total of 2617 specimens of nine species of murine rodents collected at Ilobi and Agege in the Western State have been examined for malaria parasites by the inoculation of blood into white mice (Table 2). Six out of 18 Thamnomys rutilans from Ilobi were infected with a P. vinckei-like parasite, and one was additionally infected with P. berghei (Nigeria). The single infected Praomys found earlier in the same place by Bruce-Chwatt and Gibson (1955) was presumably a rare accidental host of P. vinckei ssp. or, as suggested to the writer by Prof. Bruce-Chwatt, possibly a Thamnomys which

TABLE 2

Murine rodents from two localities in Western State,
Nigeria, examined for malaria parasites by the
inoculation of blood into white mice.

Rodents	Agege 1950's (1)	Ilobi 1950's (1)	Ilobi 1967 (2)	Totals
<u>Thamnomys</u> <u>rutilans</u>	0	0	18 ⁽³⁾	18
<u>Dasymus</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>trivurgatus</u>	273	6	0	279
<u>Praomys</u> <u>tullbergi</u>	549	1038 ⁽⁴⁾	20	1607
<u>Stochomys</u> <u>longicaudatus</u>	18	21	1	40
<u>Malacomys</u> <u>edwardsi</u>	18	21	1	40
<u>Lophuromys</u> <u>sikapusi</u>	108	384	0	492
Totals	1004	1572	41	2617

(1) Bruce-Chwatt and Gibson (unpublished)

(2) Killick-Kendrick et al., (1968)

(3) Six with malaria parasites

(4) One with malaria parasites (Bruce-Chwatt
and Gibson, 1955)

had been misidentified.

The invertebrate host, which is unknown, is probably one of three largely zoophilic species of Anopheles (cinctus, coustani and obscurus) in the enzootic locality. Oocysts which are thought not to be those of a malaria parasite of man have been recorded on the midguts of two wild-caught A. obscurus in Southern Nigeria, but in another lowland locality, Brazzaville, J.P. Adam has some evidence that the vector is A. cinctus (see Killick-Kendrick, 1971). The rarity of infections in susceptible rodents other than Thamnomys suggests that the vector lives in close contact only with the thicket-rat, and seldom feeds on other rodents.

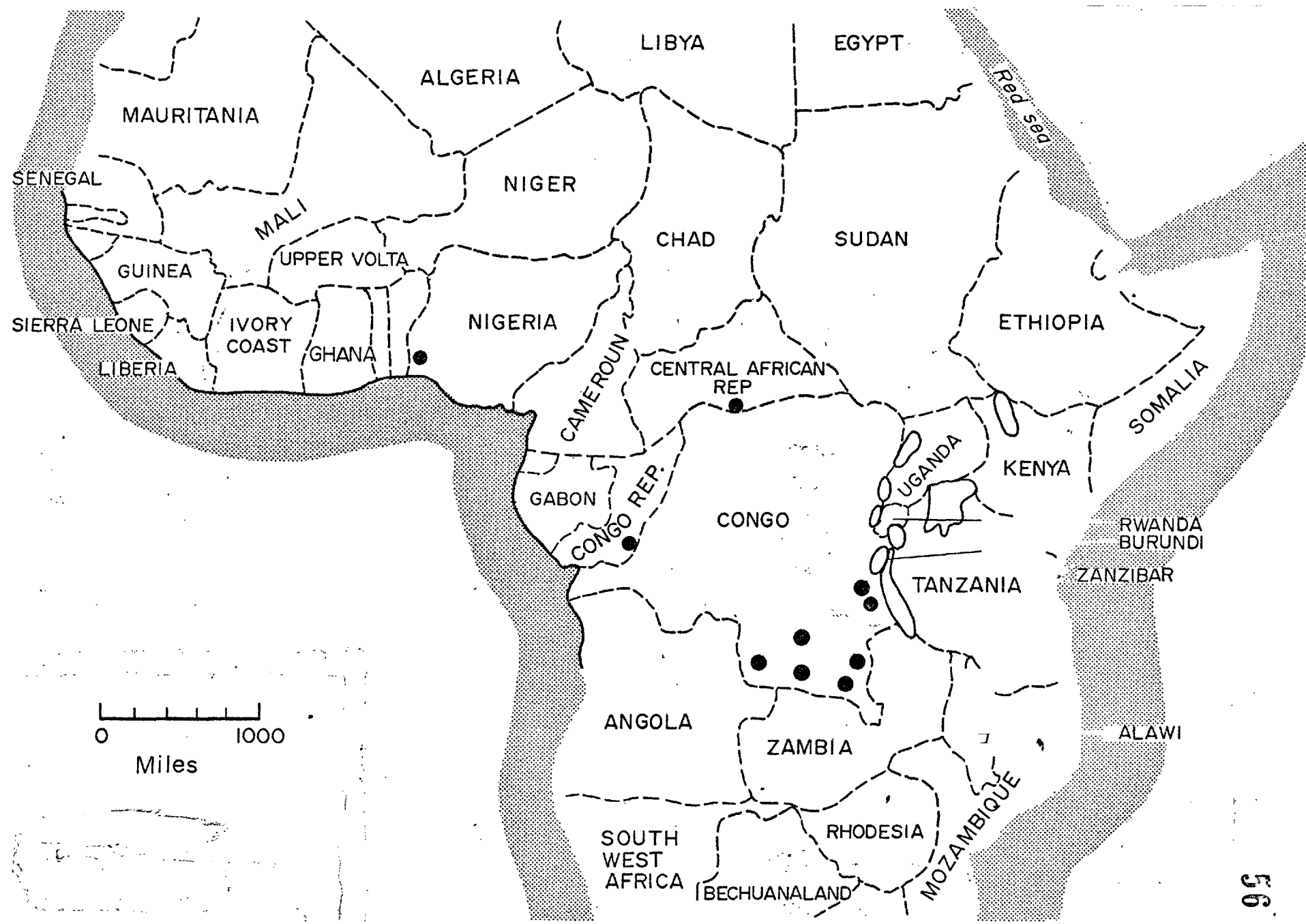
Limits of the geographical range

From the distribution of Thamnomys rutilans, the only known host of the new subspecies, in the lowland forests of West and Central Africa, the range of P. berghei (Nigeria) appears to be restricted to Western Nigeria and is thought not to overlap with that of the other subspecies of P. berghei (fig 5).

The coastal forests of West Africa are broken less than 40 miles west of Ilobi by the Dahomey Gap, an area of mixed High Forest and Savannah which, although of comparatively recent origin, forms an important barrier to some birds and mammals of the High Forest (Marchant, 1954; Booth, 1954, 1958; Rand, 1951), and from which thicket-rats appear not to have been recorded. To the north

Figure 5

Map of Africa showing where murine malaria parasites have been collected in Nigeria, the C.A.R., the Congo-Brazzaville and Katanga.



of Ilobi, the limit of the range of forest-dwelling mammals is about 40 - 50 miles away where the forest gives way to Guinea Savannah (see Rosevear, 1953). The sea forms the southern boundary.

In Nigeria to the east of Ilobi, it is assumed from the few existing records that Thamnomys rutilans occurs throughout the High Forest wherever there are suitable habitats (Rosevear, 1953). There are, however, no reports of thicket-rats from the neighbouring West Cameroons, * where rodents and other animals have been much collected since the turn of the century. To investigate the likelihood of the range of P. berghei (Nigeria) overlapping with that of P. b. yoelii or P. b. killicki, the writer carried out a search for thicket-rats on the eastern side of the Rumpi and Cameroon Mountains in October and November, 1970. By trapping, searching for nests and questioning farmers, hunters and school-children, no evidence of their presence was found. Rosevear (personal communication) points out that although there are many suitable habitats (secondary forest and fallow farmland) in this part of the country, they are of recent origin having arisen only since the Second World War when exploitation of the High Forest began on a large scale. Colonization by thicket-rats in

* In the British Museum (Natural History) there is a specimen from Eshobi which was said by Sanderson (1940) to be Thamnomys rutilans. Rosevear (1969 p. 355) has examined the specimen and, although unable to identify it, concludes it is certainly not Thamnomys.

Nigeria and Gabon has presumably been hampered by the barriers formed by the River Cross and the Cameroon Highlands in the west and the River Sanaga in the east; these are well-known limits to the ranges of other terrestrial animals (Rosevear, 1953; Booth 1954, 1958; Schøltz, 1967).

The distribution of P. berghei (Nigeria) in Nigeria must, of course, be affected as much by the range of the vector as by that of the vertebrate host. In October, 1970, Dr D.C.D. Hapold and the writer examined 19 Thamnomys rutilans collected from villages 8 miles NNE of Ibadan, Western Nigeria; no parasites were found in thick and thin blood films, and the spleens were not pigmented. Although the sample of animals is not large, it would be more than enough to reveal infections in a population of thicket-rats in any of the three lowland enzootic localities (see Killick-Kendrick, 1971). It seems as though either the conditions in the places where these Thamnomys were collected are in some way unfavourable for the transmission of murine malaria parasites, or the vector is absent.

DISCUSSION

It has been suggested that the division of P. berghei into subspecies is unsound because it is based on morphological and biological characters which are too variable and overlapping (Bafort, Timperman & Delbar, 1968; Bafort, 1970). Landau et al. (1970), however, believed that a discontinuous distribution of the non-

migratory rodent hosts has led to the isolation of populations of parasites in which speciation is occurring at the present time. Furthermore, they argue convincingly that the subspecies of murine malaria parasites from three localities are recognizable by the size of the mature oocyst, the length of the sporozoite and the rate of growth of the primary tissue stages in the liver of a standard laboratory animal.

Dissatisfaction with the concept of subspecies and its application in zoology is not new; particularly notable is the spirited attack by Wilson and Brown (1953) which stimulated a series of papers in Systematic Zoology from workers with widely differing views. Mayr (1954) believed that the dissatisfaction was usually founded on two incorrect assumptions: (i) that the subspecies was a definable, biological unit, or (ii) that it was a category like the species, but simply on a lower taxonomic level. In Mayr's opinion "the subspecies is merely a strictly utilitarian device for the pigeonholing of population samples." He had earlier described subspecies as "geographically defined aggregates of local populations which differ taxonomically from other such subdivisions of the species" (Mayr, Linsley & Usinger, 1953), and later emphasized that the geographical distribution was more important than the degree of taxonomic difference (Mayr, 1963)*.

* A working party which met in Bethesda, U.S.A. in November, 1971, under the auspices of the Fogarty International Center for Advanced Study in Health Sciences to consider the status of the lower taxa of malaria parasites defined subspecies as "infraspecific allopatric populations of organisms distinguished from each other by clearly definable biological, morphological and/or biochemical differences."

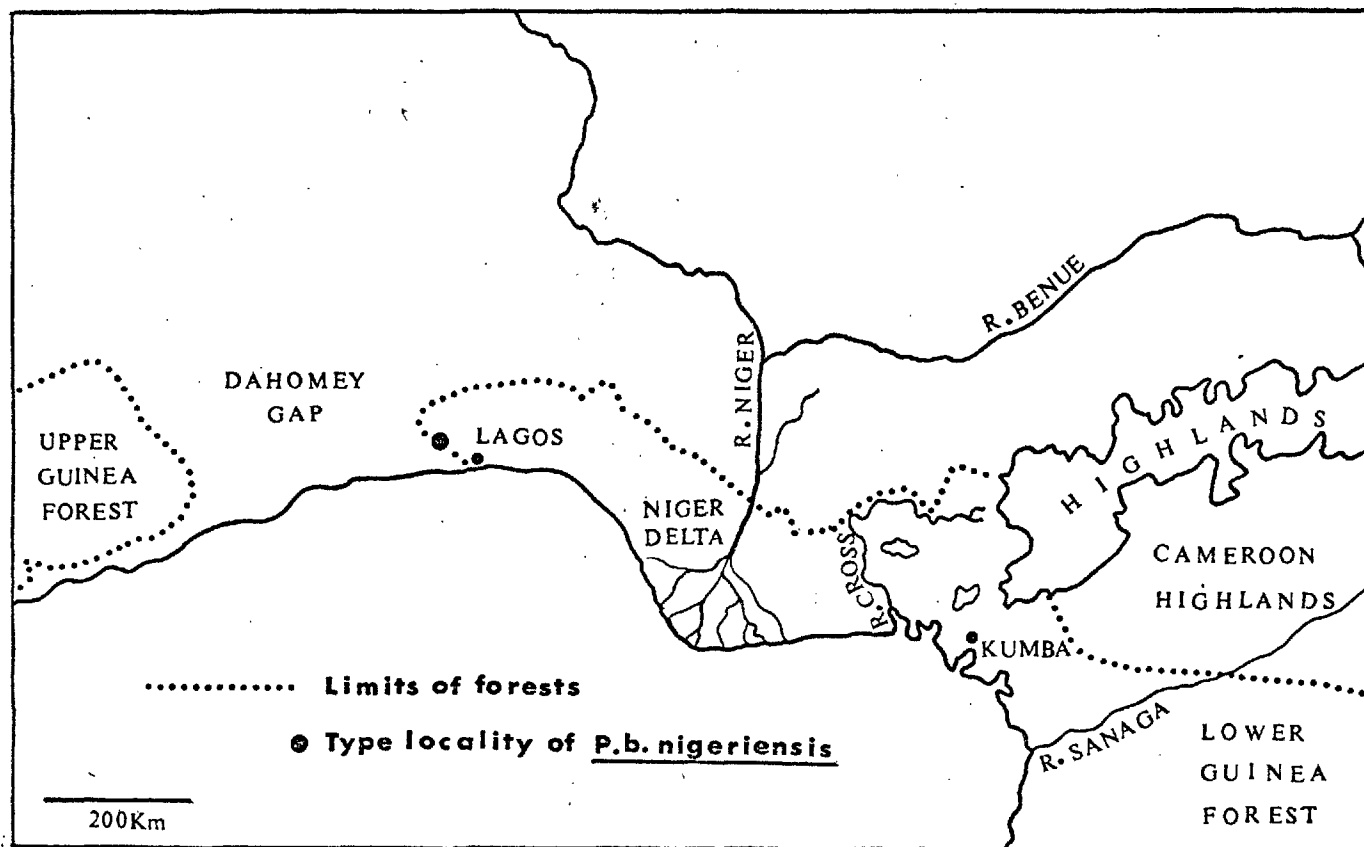


Figure 6. Map of West Africa showing faunal barriers to the east of the type locality of *P. berghei* (Nigeria) (black dot NW of Lagos) viz: the Niger Delta, R. Cross, Cameroon Highlands, and R. Sanaga.

Manwell (1957) thought it was not possible to apply Mayr's geographical definition of a subspecies to parasitic protozoa because any restriction or isolation applied to the host, rather than the parasite. But, for example, if a parasite is restricted to only one vertebrate host, its range must be the same as that of its host; host range of a parasite is broadly analogous to geographical range of a free-living animal.

By Mayr's definition, P. berghei (Nigeria) can clearly be considered as a subspecies of P. berghei separated from the other populations by past and present faunal barriers including the lower reaches of the River Niger, the River Cross, the Cameroon Highlands and the River Sanaga (fig.6). Although none of these barriers can be considered today to be insurmountable, all are believed greatly to have affected the present distribution of mammals, birds and treefrogs of the coastal forest of West Africa (Rand, 1951; Rosevear, 1953; Marchant, 1954; Booth, 1954, 1958; Schmitz, 1967; Jewell & Oates, 1969). In the recent past the montane forest of the Cameroon highlands was perhaps the most important, and it has been suggested that at the end of the Gamblian Pluvial in Africa, about 22,000 years ago, mountain forests (and their fauna) in the Cameroons may have been linked with those of the East African Highlands and thus have formed the division between the Upper and Lower Guinea Forests (rather than the more recent Dahomey Gap to the west of Nigeria) (see Moreau, 1933; Rand, 1951).

Marchant (1954), while acknowledging the importance of the

Cameroon Highlands as a faunal barrier, suggested that there was an even more influential one between East and West Nigeria i.e. an indentation of the coast before the formation of Coastal Plain Sands at a point which is now the Niger Delta.

The ecological barriers of West Africa, and an apparent absence of the rodent host of P. berghei (Nigeria) on the eastern side of the Cameroon and Rumpi Mountains are evidence of the geographical isolation of the new subspecies. By Mayr's definition, there should also ideally be some degree of morphological difference between P. berghei (Nigeria) and other populations of the species. In a search for such differences, special attention was paid to the length of the sporozoite because, like the gametocyte, this is a stage which has ceased growth until reaching a new host. Its mean length was found to be remarkably constant, and no statistically significant differences were detected when temperatures of sporogony were varied, or different methods of preparing stained smears were employed.

Since the ranges of lengths of sporozoites of the subspecies are wide and overlap with one another, the significance of the apparent differences in the means was investigated by conventional statistical methods. Published data were inadequate for this purpose, and new measurements were therefore made. Stained smears of sporozoites of P. b. yoelii were prepared from experimentally infected A. stephensi. Dr I. Landau kindly contributed smears of sporozoites of P. b. killicki. Two fresh isolates of P. b. berghei were provided by Dr J. Bafort; sporogony was obtained in A. stephensi in-

cubated at 19 - 21°C, and dried stained smears of sporozoites were prepared.

Representative results of the statistical comparison are given in figure 7 in the form of modified Dice-Leraas diagrams (Dice & Leraas, 1936; Davis, 1952, 1969; Hoare, 1956, 1959); the data on which they are based are given in Table 3 . The 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 rectangle represents 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 which Hoare (1959) considered of subspecific order in trypanosomes. 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 ($p = 0.05$).

P. b. berghei
R. L. L.

P. b. berghei
AN K A

P. b. yoelii
17 x

P. b. killicki
193 L

P. b. killicki
194 Z Z

P. berghei (Nigeria)
N67

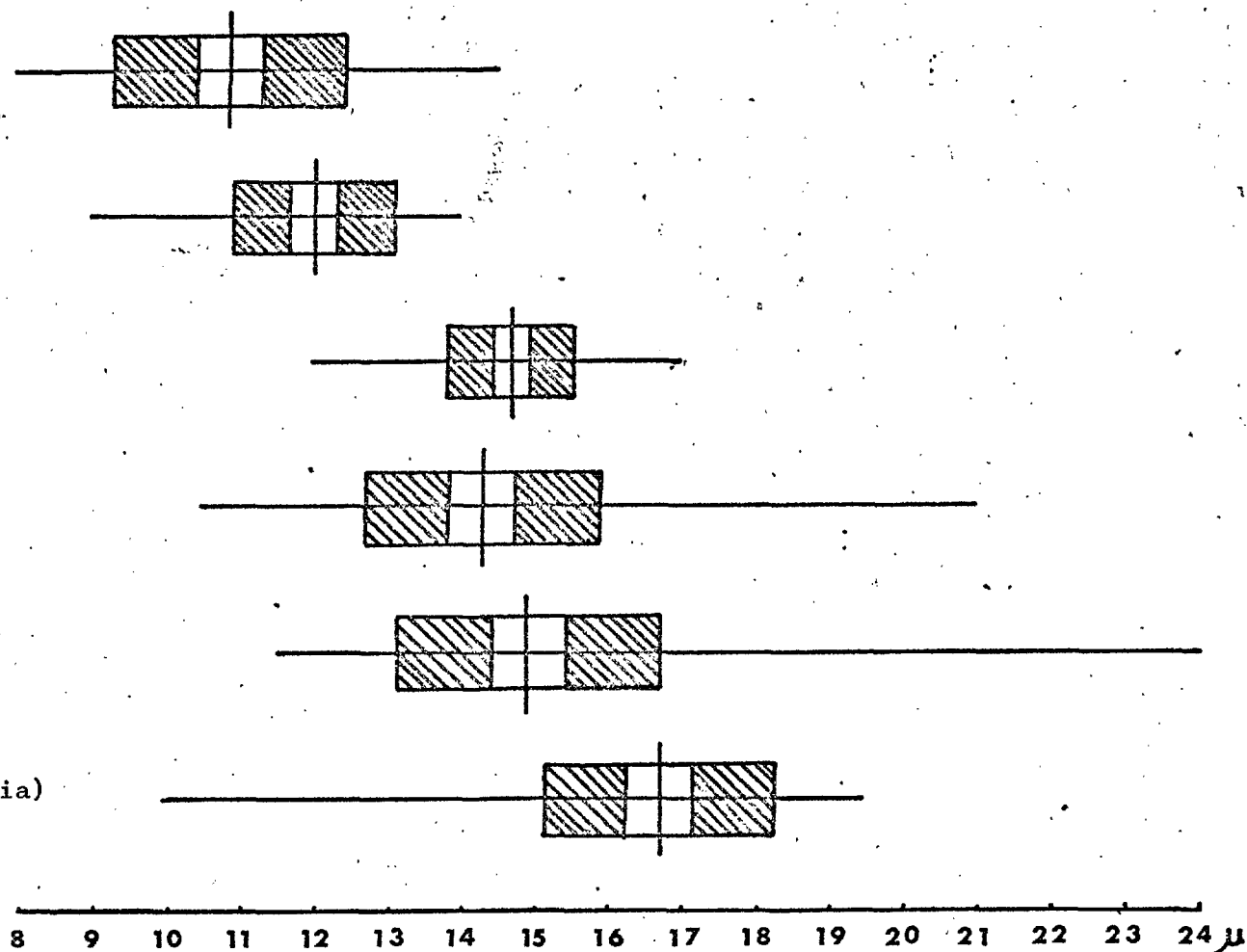


Figure 7. DICE-LERAAS DIAGRAMS OF LENGTHS OF SPOROZOITES OF P. BERGHEI SSPP.

TABLE 3

Mensural data of 50 sporozoites of isolates of

P. berghei from four localities

subspecies	strain	locality	mean	range	coeff of variation	Stnd dev.	Stnd 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>	193L	B'ville	14.32	10.5-21.0	11.1%	1.590	0.450
<u>killicki</u>	194ZZ	B'ville	14.94	11.5-24.0	12.0%	1.797	0.508
<u>(Nigeria)</u>	N67	Nigeria	16.72	10.0-19.5	9.3%	1.556	0.440

The populations examined fall into two groups: (i) P. b. berghei from Katanga, and (ii) P. berghei subspecies from Nigeria, the Central African Republic and Brazzaville. Within the first group, sporozoites of the two strains of P. b. berghei have significantly different means ($p = > 0.02$). Within the second group, sporozoites of P. berghei (Nigeria) have a mean length significantly different from that of P. b. yoelii or P. b. killicki ($p = > 0.02$), but those of the last two subspecies are indistinguishable.*

Except for the sporozoites of P. vinckei lentum (which measure $19.52 \pm 0.40 \mu\text{m}$; personal observation), those of P. berghei (Nigeria) are the longest of the genus. Apart from this striking morphological character, the new subspecies differs little phenotypically from the other two from lowland localities. The tissue stages grow at a slightly faster rate than that plotted for P. b. yoelii or P. b. killicki by Landau et al., (1970), and the size of the mature

* There is a discrepancy between my measurements of sporozoites of P. b. killicki and those of Landau et al. (1968). They gave the mean length of 20 sporozoites of strain 193L as $18 \mu\text{m}$ (range $15.5 - 21.0 \mu\text{m}$), whereas I found the mean length of 50 sporozoites of the same strain to be only $14.32 \mu\text{m}$ ($10.5 - 21.0 \mu\text{m}$) and of strain 194ZZ to be $14.94 \mu\text{m}$ ($11.5 - 24.0 \mu\text{m}$). The explanation is that in the present study sporozoites were measured strictly in the order in which they were seen, whereas the French workers selected sporozoites which were representative of the population (Landau, personal communication).

schizonts in the liver of the white rat is a little larger than that of the other two. The mean diameter of the mature oocyst of P. berghei (Nigeria) is the same as that of P. b. killicki (60 μ m at 24°C), but smaller than that of P. b. yoelii (75 μ m) (see Landau et al. 1970).

Studies on isolates of P. berghei from four separate localities in Africa have shown that this is a polymorphic species in which several populations can be recognized. The clearest distinction is between P. b. berghei from the Katangan Highlands and the three subspecies from the lowland localities (yoelii, killicki and "Nigeria") (see Table 4). The principal vertebrate host of P. b. berghei is Grammomys surdaster, while the only known rodent host of the lowland parasites is the related thicket-rat Thamnomys rutilans. The invertebrate host of P. b. berghei is Anopheles dureni millecampsi, a mosquito with a very limited distribution (Gillies & de Meillon, 1968) which cannot be the vector of the subspecies from other parts of Africa where the vectors are unknown. The optimum temperature for the sporogony of freshly isolated strains of P. b. berghei is normally lower than that of the lowland subspecies, its mature oocyst tends to be smaller and its sporozoite is the shortest of all.*

* One old strain of P. b. berghei (no K173) is said not to fit this description, and to have unusually large mature oocysts and much longer than normal sporozoites (Bafort, 1970). This strain had been maintained in the laboratory by syringe passage for over twenty years, and had long been agametocytic, when it was discovered that sub-curative doses of chloroquine resulted in the re-appearance of gametocytes and sporogony in the laboratory was obtained for the first time. Since it is impossible to tell to what degree the strain had been altered

Table 4

Differential characters of
P. berghei from highland and
 lowland localities

	Highland strains	Lowland strains
Geographical distribution:	Katanga only	C.A.R., Brazzaville, W. Nigeria.
Principal vertebrate hosts:	<u>Grammomys surdaster</u>	<u>Thamnomys rutilans</u>
Invertebrate hosts:	<u>An. durenii milled-</u> <u>campsi</u>	? <u>An. cinctus</u>
Optimum temperature of sporogony:	< 21°C	24°C
Mean diameter of mature oocysts:	< 45 µm	> 50 µm
Mean length of sporozoites:	< 13 µm	> 13 µm
Primary e.e. schizonts:	Growth rate slower than lowland strains	Growth rate faster than highland strains
Electrophoretic mobilities of enzymes.	Characteristic	Characteristic

The growth rate of the primary exoerythrocytic schizonts of the nominate subspecies in the liver of a standard laboratory animal, the white rat, is slower than that of the others, and the size of the mature schizonts is normally smaller (Landau & Killick-Kendrick, 1966b; Garnham et al., 1967; Landau et al., 1970).

Phenotypically, P. b. berghei is thus seen to differ markedly from all three populations from lowland localities. Not surprisingly, the biotope of this subspecies (the cool Katangan Highlands) and its hosts, both vertebrate and invertebrate, are also different. There is, moreover, evidence from the electrophoretic mobilities of four enzymes of P. berghei (Carter, 1970) that the nominate subspecies is very different genotypically from the lowland parasites, and that the highland and lowland populations no longer share the same gene pool. This may lead to the berghei-complex being divided into two separate species, one from the highlands of Katanga and the other from the lowland forests of West and Central Africa; in the latter there would appear to be more than one subspecies.

DIAGNOSIS: PLASMODIUM BERGHEI (NIGERIA)

BLOOD STAGES. Indistinguishable from other subspecies of Plasmodium berghei. In laboratory rodents there is usually, but not always,

* (contd.) by the long manipulation in the laboratory, it is perhaps wise not to consider its sporogonic stages to be typical of the subspecies. Moreover, Peters (personal communication), in whose laboratory the gametocytes were resurrected, now suspects that the line of the strain treated with chloroquine may have been mixed with P. b. yoelii or P. berghei (Nigeria).

a predilection for immature erythrocytes; polyparasitism is common; ring forms only occasionally have twin nuclear masses; trophozoites are not amoeboid; mature schizonts almost completely fill the host-cell, and produce 8 - 16 merozoites; pigment is golden or light brown; infected cells are neither enlarged nor stippled.

SPOROGENIC STAGES. Exflagellation of microgametes takes place in 6-14 mins at 16°C. The mean length of the microgametes is $16.8 \pm 0.29 \mu\text{m}$. Ookinetes are leaf-like and measure $11.4 \pm 0.27 \mu\text{m} \times 3 - 4 \mu\text{m}$; pigment is anterior to the nucleus. The optimum temperature of sporogony is 24°C. At this temperature, the mean diameter of mature oocysts is 60 μm ; sporozoites first invade the salivary glands on days 9 - 11; and the mean length of sporozoites in stained smears of crushed salivary glands is $16.72 \pm 0.22 \mu\text{m}$ (range 10.0 - 19.5 μm).

EXOERYTHROCYTIC STAGES. The minimum maturation time in the liver of white mice is 47 - 48 hrs. The mean diameter of mature forms at 49 - 50 hrs is 42 - 50 μm ; nuclei of infected parenchymal cells are commonly enlarged.

HOSTS. The type vertebrate host is Thamnomys rutilans (Peters, 1876) (Rodentia: Muridae); suitable experimental hosts include white mice, white rats and laboratory-bred Grammomys surdaster and Praomys natalensis. The natural invertebrate host is unknown; sporogony is obtainable in laboratory-bred Anopheles stephensi.

GEOGRAPHICAL RANGE. Believed to be restricted to Western Nigeria; the type locality is secondary forest and farmland on the edge of the Ilaro Forest Reserve near the village of Ilobi, Western State, Republic of Nigeria (longitude $3^{\circ} 03'E$, latitude $6^{\circ} 45'N$).

Type material will be deposited in the Wellcome Museum, London.

SUMMARY

The life-cycle of strain N67 of a new subspecies of Plasmodium berghei from Nigeria in laboratory-bred Anopheles stephensi and white mice and rats is described and compared with that of three other subspecies of P. berghei. From a statistical analysis it is shown that the sporozoites of the new subspecies are significantly longer than those of the others; less striking differences are (i) a tolerance of P. berghei (Nigeria) to higher than usual temperatures during sporogony, and (ii) the slightly larger size and faster rate of growth of the tissue stages of the new subspecies. The mature oocyst is the same size as that of P. b. killicki.

The type locality of the new subspecies is in Western Nigeria, and between this place and the other localities from which P. berghei is known there are four faunal barriers of varying importance. It is suggested that these have led to a geographical restriction to the range of P. berghei (Nigeria), and it is thought that the distribution of the new subspecies is not linked to that of P. b. berghei, P. b. killicki or P. b. yoelii. This is supported by the results of a survey for murine malaria parasites in the West Cameroons. No

parasites were found, and there was no evidence of the presence of thicket-rats (Thamnomys rutilans), the natural vertebrate host of P. berghei sspp. in the lowland localities. The new subspecies thus fits Mayr's concept of the taxon: a geographically defined population differing taxonomically from other similar divisions of the species.

The nominate subspecies stands apart from the others in a number of ways (biotope, natural vertebrate and invertebrate hosts, sizes of mature oocysts and of sporozoites, optimum temperature of sporogony, and rate of growth and size of the tissue schizonts), and it is suggested that current work on the electrophoretic mobilities of enzymes of P. berghei, which complements morphological studies, may lead to the division of the P. berghei group into two species, viz. one from the highlands of Katanga, and another from the lowland forests.

I wish to thank Prof. P.C.C. Garnham, F.R.S. for guidance and encouragement throughout the work; Dr George Murdie for advice on the statistical treatment of the lengths of sporozoites; Prof. L.J. Bruce-Chwatt for permission to quote the unpublished results of the surveys carried out with Mr F.D. Gibson in Nigeria; and Mr D.R. Rosevear of the British Museum (Natural History) for helpful discussions on the distribution of thicket-rats.

Support from a grant to Dr Elizabeth Canning from the Medical Research Council is gratefully acknowledged, and I thank Dr Canning

for arranging the facilities for the work to be done and for critically reading the manuscript. Studies in Africa were carried out under the auspices of the World Health Organisation.

Some of this work formed part of a thesis approved by the University of London for the degree of M.Phil.

REFERENCES

IN PART II

- BAFORT, J. (1970). The variability of Plasmodium berghei Vincke and Lips, 1948. Annales de la Société belge de médecine tropicale 50, 247-262.
- BAFORT, J., TIMPERMAN, G. & DELBAR, T. (1968). Observations on tissue schizogony and sporogony of rodent malaria. Annales de la Société belge de médecine tropicale 48, 535-540.
- BOOTH, A.H. (1954). The Dahomey Gap and the mammalian fauna of the West African forests. Revue de zoologie et de botanique africaines 50, 305-314.
- BOOTH, A.H. (1958). The zoogeography of West African primates: a review. Bulletin de l'Institut française d'Afrique Noire 20 series A, 577-622.
- BRUCE, D., HAMERTON, A.E. & BATEMAN, H.R. (1909). A trypanosome from Zanzibar. Proceedings of the Royal Society Series B 81, 14-30.
- BRUCE-CHWATT, L.J. & GIBSON, F.D. (1955). A Plasmodium from a Nigerian rodent. Transactions of the Royal Society of Tropical Medicine and Hygiene 49, 9-10.
- CARTER, R. (1970). Enzyme variation in Plasmodium berghei. Transactions of the Royal Society of Tropical Medicine and Hygiene 64, 401-406.
- DAVIS, B.S. (1952). Studies on the trypanosomes of some California mammals. University of California Publications in Zoology 57, 145-250.
- DAVIS, B.S. (1969). Trypanosoma (Megatrypanum) hoarei sp. nov. (Protozoa: Trypanosomatidae) from shrews of the genus Sorex in California. Parasitology 59, 469-491.
- DICE, L.R. & LERAAS, H.J. (1936). A graphic method for comparing several sets of measurements. Contributions from the Laboratory of Vertebrate Genetics of the University of Michigan 3, 1-3.

- GARNHAM, P.C.C., LANDAU, I., KILLICK-KENDRICK, R. & ADAM, J.P. (1967). Répartition et caractères différentiels des Plasmodiums de muridés. Bulletin de la Société de pathologie exotique 60, 118-127.
- GILLIES, M.T. & de MEILLON, B. (1968). The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region). 2nd. ed., Johannesburg: S. African Institute for Medical Research.
- HOARE, C.A. (1956). Morphological and taxonomic studies on mammalian trypanosomes. VIII: Revision of Trypanosoma evansi. Parasitology 46, 130-172.
- HOARE, C.A. (1959). Morphological and taxonomic studies on mammalian trypanosomes. IX: Revision of Trypanosoma dimorphon. Parasitology 49, 210-231.
- HOARE, C.A. & BROOM, J.C. (1938). Morphological and taxonomic studies on mammalian trypanosomes. IV: Biometrical study on the relationship between T. uniforme and T. vivax. Transactions of the Royal Society of Tropical Medicine and Hygiene 31, 517-534.
- JEWELL, P.A. & OATES, J.F. (1969). Ecological observations on the loroid primates of African Lowland Forest. Zoologica Africana 4, 231-248.
- KILLICK-KENDRICK, R. (1970a). The life-cycles of two murine malaria parasites from Nigeria (abstract). Journal of Parasitology, 56, 435-436.
- KILLICK-KENDRICK, R. (1970b). Studies on the life-cycles of murine malaria parasites from Nigeria. M. Phil. thesis, University of London.
- KILLICK-KENDRICK, R. (1971). The collection of strains of murine malaria parasites in the field, and their maintenance in the laboratory by cyclical passage. In Isolation and Maintenance of Parasites in vivo, Symposia of the British Society for Parasitology, eds A.E.R. Taylor & R. Muller, Oxford: Blackwell 9, 39-64.
- KILLICK-KENDRICK, R., SHUTE, G.T. & LAMBO, A.O. (1968). Malaria parasites of Thammomys rutilans (Rodentia, Muridae) in Nigeria. Bulletin of the World Health Organization 38, 822-824.

- LANDAU, I. & KILLICK-KENDRICK, R. (1966a). Note préliminaire sur le cycle évolutif des deux Plasmodium du rongeur Thamnomys rutilans de la République Centrafricaine. Comptes rendus hebdomadaire des séances de l'Académie des sciences 262, 1113-1116.
- LANDAU, I. & KILLICK-KENDRICK, R. (1966b). Rodent plasmodia of the République Centrafricaine: the sporogony and tissue stages of Plasmodium chabaudi and P. berghei yoelii. Transactions of the Royal Society of Tropical Medicine and Hygiene 60, 633-649.
- LANDAU, I., MICHEL, J.C. & ADAM, J.P. (1968). Cycle biologique au laboratoire de Plasmodium berghei killicki n. subsp. Annales de parasitologie humaine et comparée 42, 545-550
- LANDAU, I., MICHEL, J.C. ADAM, J.P. & BOULARD, Y. (1970) The life-cycle of Plasmodium vinckei lentum subsp. nov. in the laboratory: comments on the nomenclature of the murine malaria parasites. Annals of Tropical Medicine and Parasitology 64, 315-323
- MANWELL, R.D. (1957). Intraspecific variation in parasitic protozoa. Systematic Zoology 6, 2-6.
- MARCHANT, S. (1954). The relationship of the Southern Nigerian avifauna to those of the Upper and Lower Guinea. Ibis 96, 371-379.
- MAYR, E. (1954). Notes on nomenclature and classification. Systematic Zoology 3, 86-89.
- MAYR, E. (1963). Animal species and evolution. London: Oxford University Press.
- MAYR, E., LINSLEY, E.G. & USINGER, R.L. (1953). Methods and principles of systematic zoology. New York: McGraw-Hill.
- MOREAU, R.E. (1933). Pleistocene climatic changes and the distribution of life in East Africa. Journal of Ecology 21, 415-435.
- RAND, A.L. (1951) Birds from Liberia: with a discussion of barriers between Upper and Lower Guinea subspecies. Fieldiana Zoology (Chicago) 32, 561-653.
- ROSEVEAR, D.R. (1953). Checklist and atlas of Nigerian mammals, with a foreword on vegetation. London: Crown Agents for the Colonies.
- ROSEVEAR, D.R. (1969). Rodents of West Africa. London: British Museum (Natural History).

- SANDERSON, I.T. (1940). Mammals of the North Cameroons forest area being the results of the Percy Sladen Expedition to the Mamfe Division of the British Cameroons. Transactions of the Zoological Society of London 24, 623-725.
- SCHØITZ, A. (1967). The treefrogs (Rhacophoridae) of West Africa. A monograph. Spolia Zoologica Musei Hauniensis, Copenhagen 25, 346 pp.
- SHUTE, P.G. & MARYON, M. (1966). Laboratory technique for the study of malaria. 2nd ed. London: Churchill.
- WERY, M. (1968). Studies on the sporogony of rodent malaria parasites. Annales de la Société belge de médecine tropicale 48, 1-137.
- WILSON, E.O. & BROWN, W.L. (1953). The subspecies concept and its taxonomic application. Systematic Zoology 2, 97-111.
- YOELI, M. (1965). Studies on Plasmodium berghei in nature and under experimental conditions. Transactions of the Royal Society of Tropical Medicine and Hygiene 59, 255-276.

PART III
MALARIA PARASITES OF FLYING SQUIRRELS
IN THE IVORY COAST

Introduction

In 1967, Mr Pierre Hunkeler of the Centre Suisse in Abidjan, Ivory Coast, showed the writer fixed tissues of two flying squirrels which he had collected at Bolo, 50 km north of Sassandra. The spleen and the liver appeared to be pigmented, and when sections of the spleen were prepared, intracellular malaria pigment was found. Blood films from the animals had not been made. A visit was therefore made to the Ivory Coast in October, 1970, to search for malaria parasites of flying squirrels.

In the survey, made in collaboration with Mr Louis Bellier of O.R.S.T.O.M, Centre d'Adiopodoumé, malaria parasites, trypanosomes, haemogregarines and microfilariae were found. A note of the results has been published (Killick-Kendrick and Bellier, 1971); a description of the malaria parasites is given in the present section.

Materials and Methods

Locality.

The survey was carried out in farms and secondary forest

near two villages, Guéboua and Niakousoué (longitude $6^{\circ} 32''$ W, latitude $6^{\circ} 00''$ N) in the Lakota district of the Ivory Coast. The place was chosen because local hunters said flying squirrels were plentiful. They were hunted mainly in cocoa and coffee farms interspersed with tall trees left when the land was cleared.

A description of the topography and climate of the locality is given by Bellier and Killick-Kendrick (in press). Briefly, it lies at about 200 m. above sea-level in the southern densely forested part of the country. Mean monthly minimum temperatures at Gagnoa, 30 km. away, are $21-22^{\circ}\text{C}$, and mean monthly maximum temperatures range from $28-33^{\circ}\text{C}$. The rainy season begins in March (100-200 mm) and reaches a peak in June (200-300 mm); there is then a lull in July and August (0-50 mm) followed by an increase in September, October and November (50-100 mm). The survey was carried out from 16-23rd of October, towards the end of the rainy season.

Flying squirrels

African scaly-tailed flying squirrels are not true squirrels; they lie in the family Anomaluridae of the suborder Theridomyomorpha. Two genera, Anomalurus and Anomalurops form the subfamily Anomalurinae. Idiurus is the single genus of the subfamily Zenkerellinae (Wood, 1959; Simpson, 1945, 1959).

Descriptions of the forms in West Africa and notes on the little



(i)



(ii)

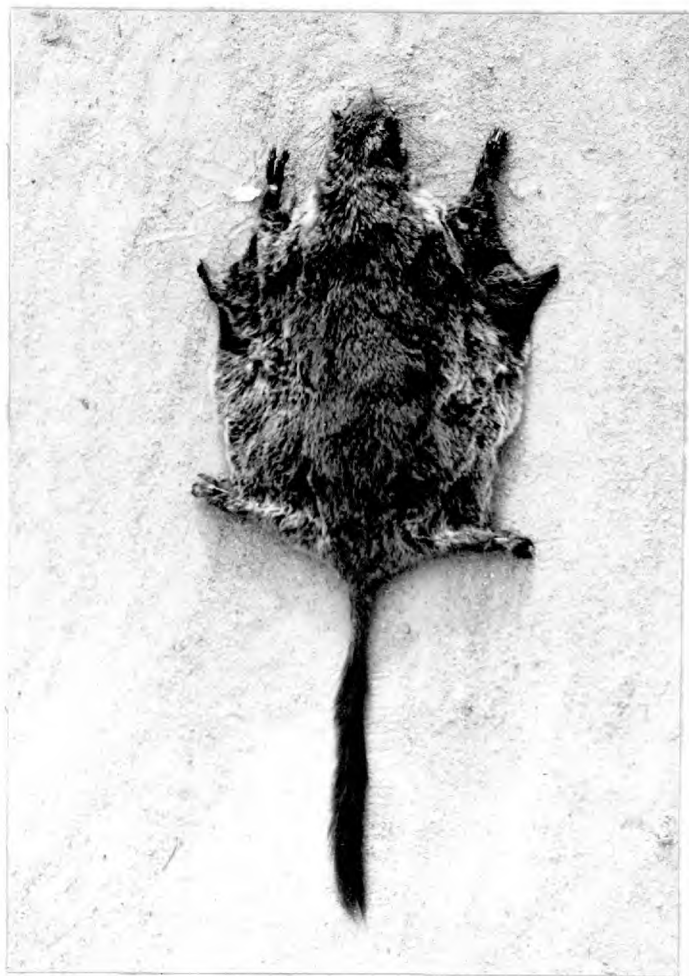
PLATE 5

Dorsal and ventral views of Anomalurus peli.

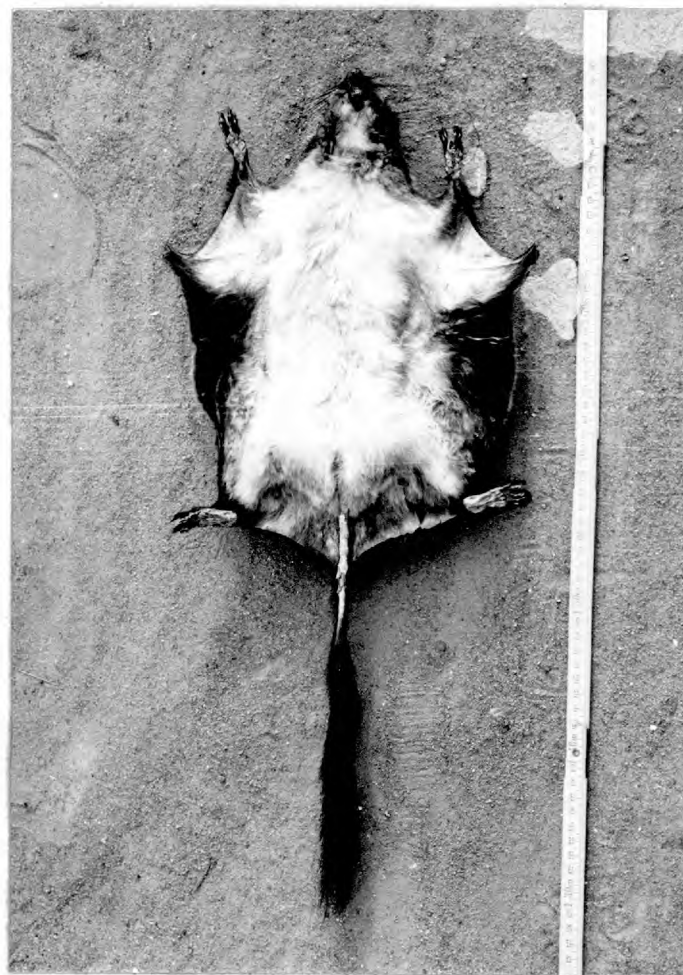
that is known of their habits are given by Rosevear (1969). They are strictly arboreal animals which never come to ground except by accident. It is thought they are wholly vegetarian, feeding on the fruits, flowers, leaves and bark of trees. They are nocturnal, except for Anomalurops beecrofti (Fraser, 1852) which is said to be most active in the late afternoon and early evening. Although this species is known from the Ivory Coast, no specimens were collected during the survey. Almost every female of the three species examined in October, 1970, was pregnant with a single foetus; the young would have been born at about the end of the rains.

Anomalurus peli (Schlegel and Miller, 1885). This animal is found only in the Upper Guinea Forest west of the Dahomey Gap. It is the largest of all anomalurids (Plate 5, figs i,ii); the weight of the 15 specimens collected in the survey ranged from 1.3-2.0 kg. All were adults, and were usually in pairs. Most specimens were shot at night as they fed in the forest canopy, but a few were flushed out of tree holes during the day by banging on the trees.

Anomalurus derbianus (Gray, 1842) (= A. fraseri Waterhouse, 1843; see Rosevear, 1969). The range of this animal is from the Upper Guinea Forest through Central Africa into parts of East Africa. It is a smaller animal than A. peli (Plate 6, figs. i,ii); the six specimens collected in the Ivory Coast weighed 660-830 gm. They were flushed out of tree holes (Plate 7,



(i)



(ii)

PLATE 6. Dorsal and ventral views of a skin of Anomalurus derbianus.

PLATE 7



Trees inhabited by Anomalurus derbianus; note holes.



(i)

PLATE 8

(ii)

(i) The pygmy flying squirrel. Idiurus macrotis. (Photo by Mr J.C. Jeanneret)

(ii) Hollow tree from which roosting I. macrotis were smoked.

fig i) during the day and shot. They were always solitary, and all were adult.

Idiurus macrotis Miller, 1898. This pygmy flying squirrel has a wide but patchy distribution throughout the Upper Guinea Forest, and eastwards across the Dahomey Gap into Central Africa. It is a tiny mouse-like animal weighing less than 30 gm (Plate 8, fig i). The 16 specimens collected in the survey were met during the day roosting in hollow trees (Plate 8 , fig ii), frequently in association with the bat Hipposideros cyclops. They were smoked out and shot. All were mature.

Techniques

Immediately the squirrels were shot, blood was taken from the heart with a syringe and needle. Thick and thin blood films were prepared and, because of the high humidity, were dried with gentle heat. Thin films were fixed momentarily in absolute methanol and were stained on the day of preparation in 10 per cent. Giemsa's stain diluted with phosphate buffered water at pH 7.2. Thick films were simulataneously lysed and stained in 3 per cent. Giemsa's stain at the same pH for 30 mins.

The Incidence of Malaria Parasites

The results of the survey are given in Table 5; prolonged

Table 5

Results of the survey of flying squirrels
of the Ivory Coast.

	<u>A.</u> <u>peii</u>	<u>A.</u> <u>derbianus</u>	<u>A.</u> <u>macrotis</u>
<u>Plasmodium</u>	2	0	0
<u>Trypanosoma</u>	6*	3	0
<u>Plasmodium</u> + <u>Trypanosoma</u>	3	2	0
<u>Hepatozoon</u>	0	0	4
Negative	2	1	12
Totals	15	6	16

* One was also infected with microfilariae morphologically similar to those of Onchocercella katangensis of Petrodromus tetradactylus (see Gedoelst, 1916; Yorke and Maplestone, 1931); the slide is deposited in the collection of Dr Ralph Muller, Dept. of Medical Helminthology, London School of Hygiene and Tropical Medicine.

searches of the blood films may yet reveal a few more scanty infections. Descriptions of the trypanosomes of Anomalurus spp. and the haemogregarines of Idiurus are given in Appendix 1.

No malaria parasites were found in 16 I. macrotis, the spleens of which were apparently normal with no sign of enlargement or pigmentation. Two typical spleens measured 16 X 2 mm and 20 X 1.5 mm.

Patent infections of malaria parasites were found in 5/15 A. peli and 2/6 A. derbianus. Every specimen, however, had an enlarged and pigmented spleen suggesting that all were or had been infected with malaria. Measurements of the spleens of 7 A. peli are given in Table 6. The spleen of one A. derbianus, which is a smaller animal than A. peli, measured 35 X 10 mm; this animal (No 70/34) had patent infections of malaria parasites and trypanosomes.

The stages of malaria parasites seen in thin films of the heart blood of the 5 A. peli and 2 A. derbianus with patent infections are listed in Table 7. Four out of 13 specimens of A. peli collected at Guéboua II had very light infections, whereas the only 2 collected at Niakousoué had moderately heavy infections and provided material for the descriptions given below. These animals were a breeding pair and were shot in the same tree during the day. They were only wounded, but the female, No 70/35, collapsed an hour later and died as blood was collected.

Table 6.

Spleen sizes of 7 Anomalurus peli
 with patent infections of Plasmodium (P),
Trypanosoma (T) or both.

Spleen size in mm. (length X maximum width)	infection		Survey number
75 X 28	P	T	70/25
70 X 23 *	P	T	70/37
65 X 22	P	T	70/35
65 X 22		T	70/24
60 X 20	P	T	70/22
60 X 18		T	70/52
55 X 25	P		70/23

* Thickness was 12 mm; weight was 6.7 gm.

TABLE 7

Stages of malaria parasites seen in the
heart blood of infected anomalurines, and
the places of collection

Species and survey number	Stages seen in thin smears of heart blood	Place collected
<u>A. peli</u> 70/22	1 microgametocyte	Guéboua II
<u>A. peli</u> 70/23	few rings	"
<u>A. peli</u> 70/25	very few rings	"
<u>A. peli</u> 70/35	rings, trophozoites, immature schizonts, rare macrogametocytes,	Niakousoué
<u>A. peli</u> 70/37	rings, trophozoites, rare immature schizonts	"
<u>A. peli</u> 70/42	one ring	Géboua II
<u>A. derbianus</u> 70/34	one trophozoite	Niakousoué
<u>A. derbianus</u> 70/65	one trophozoite one schizont	"

The male, No 70/37, was taken alive to Abidjan and maintained overnight in captivity. Although its wounds were slight, it was in a weak condition on the next day and was therefore sacrificed. Blood, taken from the heart with heparin as an anticoagulant, was inoculated intraperitoneally into 3 splenectomized and 4 intact mice. Smears were prepared from the lungs, liver, spleen, kidney, heart and skeletal muscle, and bone-marrow; they were fixed in methanol and stained in Giemsa's stain.

All six specimens of A. derbianus, two of which had patent infections of malaria parasites, were collected at Niakousoué. A single schizont was seen in a film from A. derbianus No 70/65; it had 16 nuclei and caused no reddening of the host-cell. The parasitaemias were, however, so low that the parasites cannot be fully described and given an identity.

Description of the Malaria Parasites of A. peli

Such striking differences were found in the morphology of the parasites of the specimens of A. peli collected at Niakousoué that they appear to be two different species; they are therefore described separately.

Parasite of A. peli No 70/35

All parasitized cells appeared to be normocytes, the normal diameter of which was 7-8 μ m. The youngest parasites were round

or oval ring forms lying eccentrically in the host cell and measuring 2 μ m in diameter. The nucleus was peripheral and lay within the smooth outline of the parasite; twin nuclear masses were never seen.

Trophozoites were round or oval and compact, with no sign of amoeboidicity (Plate 9, fig i). The cytoplasm stained blue, with a darker staining rim. On reaching a diameter of about 5 μ m, fine grains of golden pigment lay scattered around the edge of the parasite. Only one infected cell was seen with two parasites, both late trophozoites at the same point of growth (Plate 9, fig ii).

Although some infected cells were finely stippled (Plate 9, fig iii), this was probably not caused by the parasite; similarly stippled but uninfected cells were common in the material examined. The nuclei of asexual forms began to divide as the diameter of the parasite reached 6 - 7 μ m (Plate 9, fig iii). The infected cell then began to stain a dark red colour similar to but even more striking than that of freshly isolated strains of P. v. chabaudi (Landau, 1965). A few darkly red staining erythrocytes were found in all thin films from A. peli but since these were never infected with rings or trophozoites, the deep colour of the host cells of schizonts is presumed to be a change caused by the growing parasite and not simply a normal colour; all schizonts were in reddened cells (Plate 9, figs iii - vii). The maximum number of nuclei of the schizonts was 12 (Plate 9,

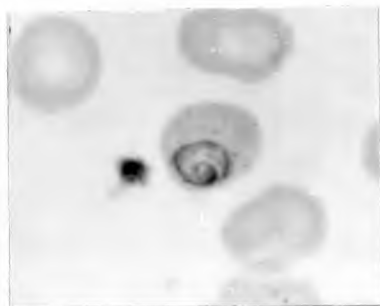
PLATE 9

Malaria parasite of Anomalurus peli No 70/35;

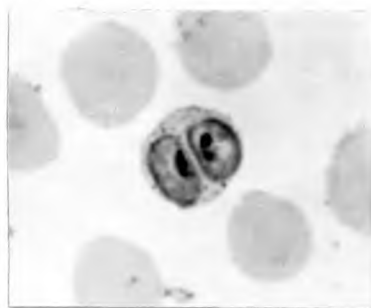
heart blood (X 1,800)



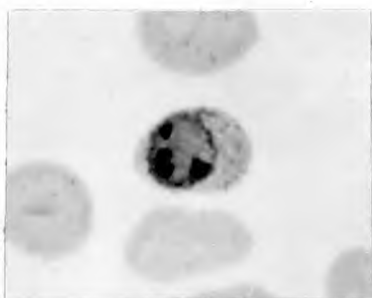
20 μ m



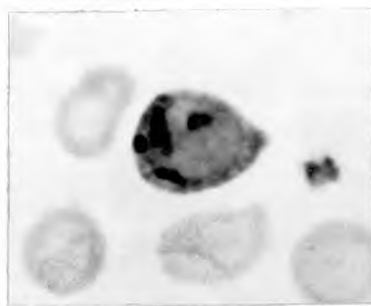
(i)



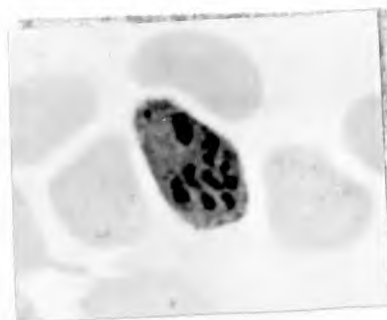
(ii)



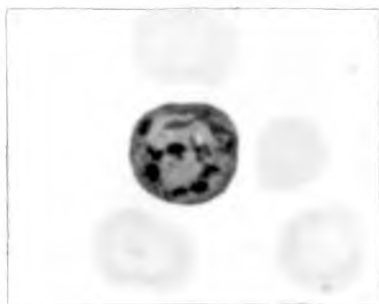
(iii)



(iv)



(v)



(vi)



(vii)



(viii)

figs vi and vii); one had nuclei budding off at the periphery of the parasite (Plate 9 , fig vii). Pigment in the late but still not quite mature schizonts was clumped into one or two golden masses which were strongly birefringent under polarized light. The host cells of these forms were slightly enlarged with a mean diameter of 9 μ m. Fully mature schizonts were not found and the number of merozoites produced is not known; it must, however, be 12 or more.

Two oval macrogametocytes in hypertrophied red cells measured 12 X 8.5 μ m. Along one edge of the parasites, a reddened rim of the host cell was visible (Plate 9 , fig viii). The morphology of the nuclei was unusual; they stained the usual red colour, but were composed of a cluster of darkly staining dots. The pigment of the gametocytes lay scattered throughout the blue cytoplasm in fine golden grains. No male gametocytes were found.

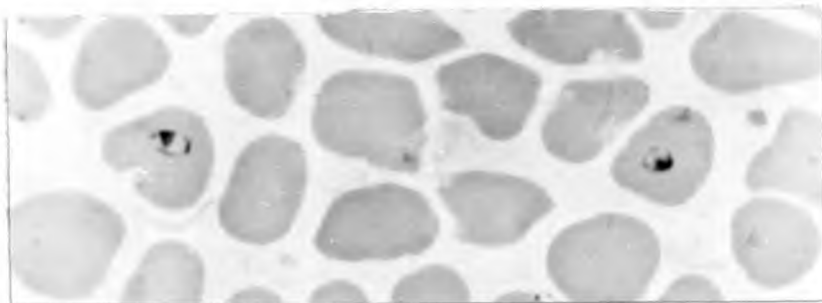
Parasite of A. peli No 70/37

With the exception of a single schizont and rare trophozoites, the only malaria parasites seen in a search of five films of heart blood from this animal were ring forms. Two of the films were made on the day of collection, and three on the next day. The rings were round with a thin wisp of blue cytoplasm

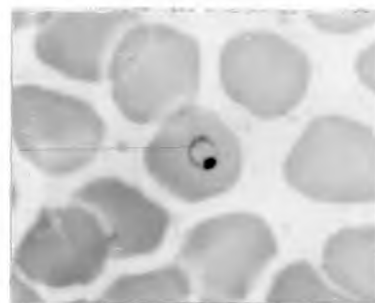
Malaria parasite of Anomalurus peli No 70/37; lung smear (X 1,500).



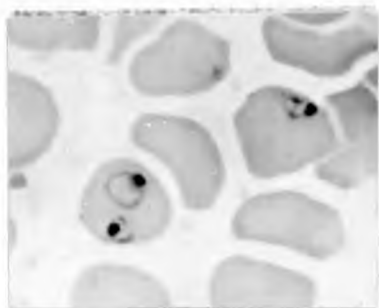
20 μ m



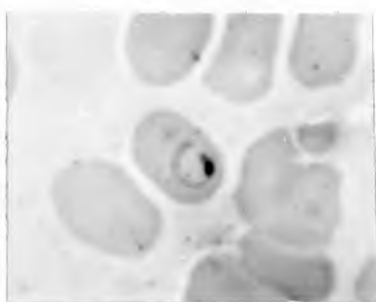
(i)



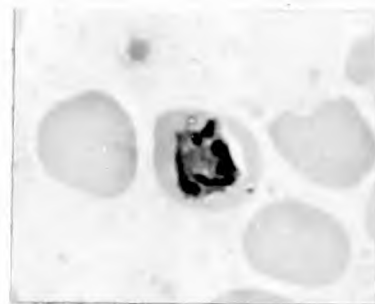
(ii)



(iii)



(iv)



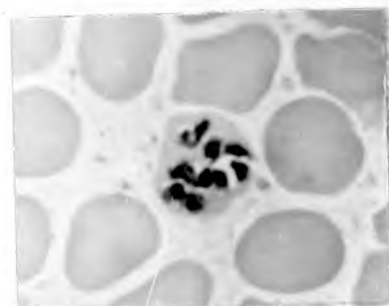
(v)



(vi)



(vii)



(viii)

and normally lay near the centre of the host cell. The nucleus stained deeply and appeared as a single round or oval dot lying at the periphery, sometimes protruding slightly (Plate 10, figs i - iii). Cells with double infections were not uncommon (Plate 10, fig iii).

Later stages were easily found in the smear of the lung (which was the main site of the erythrocytic schizogony) and to a lesser degree that of the spleen, but not in smears of heart and skeletal muscle, bone-marrow, liver or kidney. Trophozoites were either simply large rings (Plate 10, fig iv) or, as they grew larger, compact round bodies. Their nucleus was oval and fine, scanty, almost invisible pigment lay at the edge of the cytoplasm. The nucleus began to divide after the trophozoites had grown to a diameter greater than half that of the host cell. Immature schizonts tended to be angular in shape (Plate 10, fig v) and their nuclei stained very deeply ; lighter patches of red material were seen in the blue cytoplasm. Comparatively little pigment was visible at this stage, tending to lie in separate tiny grains at the periphery of the parasite. It was birefringent under polarized light, and formed into one or two golden clumps just before the schizont became fully mature. Mature schizonts produced 6-10 merozoites but the commonest number, seen in more than three-quarters of the mature

forms, was eight. The merozoites were angular or round bodies with a deeply staining nucleus against which lay a tiny wisp of blue cytoplasm. The parasites caused no enlargement, stippling or dark colouration of the host cell, but red cells containing mature schizonts tended to stain a little more palely than uninfected cells. No gametocytes of either sex were seen.

Intact and splenectomized mice inoculated with heparinized heart blood failed to become infected.

Discussion

The malaria parasites of Anomalurus in the Ivory Coast lie in the subgenus Vinckeia Garnham, 1964, in which are grouped 15 species of malaria parasites of mammals other than the supraleuroid primates (Garnham, 1966; Lien and Cross, 1968). In the definition of the subgenus, the erythrocytic schizonts are described, with some exceptions, as small with 8 merozoites or less. This character should perhaps be re-defined, since there are too many exceptions. Of the 15 named species of Vinckeia the schizonts of 7 are large and have more than 8 merozoites (P. cephalophi and P. brucei of duiker; P. voltaicum of a bat; P. anomaluri of Anomalurus; P. watteni of Petaurista; and P. berghei and P. vinckei of African murine rodents). The new parasites from the Ivory Coast are

also exceptions; both have large schizonts, and those of one produce 12 or more merozoites.

The parasites of A. peli may be differentiated, one from the other, by the erythrocytic schizonts. Those of the parasite of A. peli No 70/35 caused a deep reddening of the host cell and produced not less than 12 merozoites, whereas the schizonts of the parasite of A. peli No 70/37 changed the host cell very little, and produced 8 merozoites (range 6 - 10). No gametocytes were present in the blood films from A. peli No 70/37, and until they are seen, it is not known if the cluster of points which comprised the nucleus of the macrogametocytes in the blood of A. peli No 70/35 is a useful character to differentiate the parasites of the Ivory Coast.

The parasitaemias of the only two infected A. derbianus were so low that identifications were not possible. The parasites of these animals may have been P. anomaluri which Pringle (1960) described from the same species in Tanzania. It seems more likely, however, that they were one of the two parasites of A. peli, but the single schizont seen in the blood of A. derbianus 70/65 had 16 nuclei and caused no reddening of the host cell; this slender evidence suggests that the parasites of A. derbianus may have been neither of those of A. peli.

From a comparison of the Ivory Coast parasites with P. anomaluri (Table 8) it is concluded that the parasites of A.

Differences between three malaria
parasites of African flying squirrels

	<u>Plasmodium</u> <u>anomaluri</u> *	<u>Plasmodium</u> sp. No 70/35	<u>Plasmodium</u> sp. No 70/37
vertebrate host	<u>Anomalurus</u> <u>derbianus</u>	<u>Anomalurus</u> <u>pelii</u>	<u>A. pelii</u> and possibly <u>A.</u> <u>derbianus</u>
locality	Tanzania	Ivory Coast	Ivory Coast
ring forms	sometimes with 2 nuclear masses	never with 2 nuclear masses	never with 2 nuclear masses
trophozoites	a big ring, or compact, or amoeboid often with 2 or more vacuoles	compact or with 1 vacuole never amoeboid	compact or with 1 vacuole, never amoeboid.
number of mero- zoites	8 or more	12 or more	normally 8, range 6-10
gametocytes	nucleus with a "karyosome"	nucleus of ♀ a cluster of points	?
colour of pig- ment	brown	golden	golden
colour of host cell	unaffected	deep red as parasite grows.	slightly pale
multiple infections	absent	rare	common

* From the description of Pringle (1960)

peli are new. P. anomaluri differs from the parasite of A. peli No 70/35 by the presence of double nuclear masses in some rings, the morphology of the trophozoites, the lack of reddening of infected host cells, the colour of the pigment and the appearance of the nucleus of the macrogametocyte. It differs from the parasite of A. peli No 70/37 by the absence of double infections, the presence of double nuclear masses in some rings, the morphology of the trophozoites, the colour of the pigment and probably by a larger number of merozoites produced by mature schizonts. (which for P. anomaluri Garnham (1966) believed to be more than eight).

The apparent restriction of the erythrocytic schizogony of the parasite of A. peli No 70/37 mainly to the lungs may be an important distinguishing feature if it is found to be constant. Mr R.L. Jacobsen (personal communication) examined lung smears from one of three specimens of A. derbianus, all infected with P. anomaluri, which he collected in the type locality in Tanzania. No schizonts were present, and the preferred site of schizogony for this parasite remains unknown. Pringle (1960) found a few schizonts in heart blood, but none in the peripheral blood.

In the Ivory Coast, anomalurine malaria parasites also occur in another locality. Soon after the discovery of malaria pigment in the spleens of Anomalurus from Bolo in 1967, Hunkeler

returned there, collected two more specimens and prepared blood films; in 1970 the films were sent to the writer for examination. One was negative, but the other was heavily infected with a malaria parasite similar to that of A. peli No 70/37. Almost all the parasites were trophozoites, but a few very small rings were seen and, after many hours search, a few immature schizonts were found. Even after re-staining, they caused no reddening of the host cell. No gametocytes were seen.

The results of the survey in Lakota district, together with the observations of Hunkeler in another part of the Ivory Coast and Jacobsen in Tanzania, suggest that African flying squirrels are common hosts of malaria parasites. Rodhain et al. (1913) and Schwetz (1933) examined a few in Congo-Kinshasa but did not record malaria parasites. Since parasitaemias are commonly very low, this observation is not significant. Apart from those of the Belgian workers and workers in the Ivory Coast and Tanzania, there appear to be no other reports of the examinations of blood smears of African flying squirrels. No parasites of these animals are listed in Bray's (1964) checklist of parasitic protozoa of West Africa. This is not because the animals are scarce, but because they are overlooked; they are nocturnal; they are not hunted for food; and they are not exhibited in zoos because they are difficult to keep in captivity.

The transmission of the parasites of Anomalurus warrants

a special study. They are strictly arboreal, and the natural vector is presumably an anopheline which lives in the forest canopy or in tree holes. There are no known canopy-dwelling Anopheles in Africa (McCrae, personal communication), but further work may reveal an African mosquito with arboreal habits similar to Anopheles cruzi, the vector of P. brasilianum of monkeys in Brazil (Deane et al., 1971). Until the vectors of the anomalurine malaria parasites are discovered, the possibility that sporozoites of unknown identity in wild-caught sylvatic anophelines may be those of a parasite of flying squirrels must be borne in mind.

REFERENCES IN PART III

- BELLIER, L., & KILLICK-KENDRICK, R. (in press). Mammalia.
- 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, 26, 238-315.
- DEANE, L.M., DEANE, M.P., NETO, J.A.F., & ALMEIDA, F.B. (1971). On the transmission of simian malaria in Brazil. Rev. Inst. Med. trop. Sao Paulo, 13, 311-319.
- GARNHAM, P.C.C. (1966). Malaria parasites and other haemo-sporidia. Oxford: Blackwell.
- GEDOELST, L. (1916). Note sur la faune parasitaire du Congo Belge. Extrait de la Revue Zoologique africaine, 5, 1-90.
- KILLICK-KENDRICK, R. & BELLIER, L. (1971). Blood parasites of scaly-tailed flying squirrels in The Ivory Coast. Trans. R. Soc. trop. Med. Hyg., 65, 430-431.
- LANDAU, I. (1965). Description de Plasmodium chabaudi n.sp., parasite de rongeurs africains. C. r. hebd. Séanc. Acad. Sci., Paris, 260, 3758-3761.
- LIEN, J.C. & CROSS, J.H. (1968). Plasmodium (Vinckeia) watteni sp. n. from the Formosan flying squirrel Petaurista petaurista grandis. J. Parasit., 54, 1171-1174.
- PRINGLE, G. (1960). Two new malaria parasites from East African vertebrates. Trans. R. Soc. trop. Med. Hyg., 54, 411-414.
- RODHAIN, J., PONS, C., VANDENBRANDEN, F., & BEQUAERT, J. (1913). Rapport sur les travaux de la mission scientifique du Katanga Octobre 1910, à Septembre, 1912. Brussels.
- ROSEVEAR, D.R. (1969). Rodents of West Africa. London: British Museum(Natural History).

- SCHWETZ, J. (1933). Trypanosomes rares de la région de Stanleyville (Congo Belge). Annls Parasitol. hum. comp., 11, 287-296.
- SIMPSON, G.G. (1945). The principles of classification and a classification of mammals. Bull Am. Mus. nat. Hist., 85, 1-350.
- SIMPSON, G.G. (1959). The nature and origin of supraspecific taxa. Cold Spring Harbor Symposium on Quantitative Biology, 24, 255-271.
- WOOD, A.E. (1959). Eocene radiation and the phylogeny of the rodents. Evolution, 13, 354-361.
- YORKE, W., & MAPLESTON, P. (1931). Onchocercella nom. nov. Ann. trop. Med. Parasit., 25, 551.

PART IV

TAXONOMY OF MURINE
MALARIA PARASITES

INTRODUCTION

The concept of the protozoan species

The conventional view of an animal species has arisen from studies on metazoa, and doubt is sometimes expressed as to whether or not such a view is applicable to protozoa (e.g. Carter, 1951). But the general conclusions reached by authorities on metazoan taxonomy inevitably affect the thinking of protozoologists (Cotliss, 1967), and notions of species of protozoa have always been related to those of the metazoan species. Certainly, no other concept has gained acceptance. When difficulties have been met by protozoologists, new terms applicable to populations of protozoa have sometimes been coined (e.g. Sonneborn's syngens or Hoare's various demes).

Efforts to find an absolute definition of the species taxon, whether of metazoa or protozoa, are comparable to the search for the Philosopher's Stone and as likely to succeed. Huxley (1942) warned that too much should not be expected of the term species, and it should not be supposed that there is a hard and fast definition; there are bound to be border-line cases because evolution is usually gradual, and because species arise in a number of different ways. In his book The Origin of the Species, Darwin (1872) expressly declined to define a species, and commented

that "no one definition has satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species".

Echoes of Darwin can be heard in Tate-Regan's (1926) oft misquoted definition: "a species is a community, or a number of related communities, whose distinctive morphological characters are in the opinion of a competent systematist, sufficiently definite to entitle it, or them, to a specific name. Cain (1963) put it more succinctly: "a species is a species if a competent taxonomist says it is." This definition shifts the need to define "species" to the need to define "competent" (Huxley, 1942) and, while undoubtedly reflecting what often happens, it ignores the reasoning behind the decision; it suggests, perhaps too facilely, that there is no point in trying to reach a definition. Tate-Regan's definition could well be attributed to Noah, since it was presumably by such arbitrary means that the animals were selected for the ark.

The early species to be described and named were, like palaeontological species, wholly phenotypic in that they were based on morphology alone; if an animal looked different from another it was given a different name. From the time of Wallace and Darwin, the idea slowly grew of the "biological species" which, in addition to morphology and general biological characters, greatly depended upon the ability or lack of it for populations

to interbreed freely in natural conditions; the important point became whether a population shared, or could potentially share, the gene pool of another. Mayr (1963) defined species as "groups of actually or potentially interbreeding populations which are reproductively isolated from other such populations." Similarly, Cain (1963) briefly described a species as "the lowest rank of groups that cannot interbreed in nature". Sheppard (1967), who doubted that a species could ever be defined adequately, also suggested a definition based solely on interbreeding. He said: "in general (the species) can be described as a population or group of populations which are capable of exchanging genes, one with another in nature, if they come into contact." Emerson (in Riley, 1948, p. 516) defined a species as "a genetically distinctive, reproductively isolated, natural population." He added the important qualification that "the genetic distinction may be morphological, physiological or behaviouristic."

Earlier, Dobzhansky (1937) offered a definition which, unlike those quoted above, included as a cornerstone the dynamic nature of evolution of which species are a product. He viewed the species as "that stage of the evolutionary process at which the once actually or potentially interbreeding array of forms became segregated into two or more separate arrays which are physiologically incapable of interbreeding." Yet another emphasis is seen in the definition of Carter (1951) who incorporated the idea of phenotypic

variation within the species. He thought an "animal species is a group of animals formed into communal populations or demes, which are often differentiated into groups within the species differing both morphologically and in habit, but which will live together a completely communal life and will naturally breed together. They will not readily do so with animals outside the species group." This definition was modified from that of Mayr (1942) and, in Carter's opinion, was applicable only to sexual metazoan animals.

Only in Huxley's (1942) definition is account taken of degrees of interbreeding. He believed a species could be "regarded as a geographically definable group whose members actually interbreed or are potentially capable of interbreeding in nature, which normally in nature does not interbreed freely or with full fertility with related groups, and is distinguished from them by constant morphological differences.

In experiments investigating interbreeding, the fertility of hybrids as well as the number of hybrid offspring should be determined before it is assumed that two populations are conspecific.*

* Manwell (1957) discusses the likelihood of hybrids of malaria parasites occurring in nature. The recent demonstration of genetic recombination in strains of P. berghei (Walliker et al., 1971) confirms this possibility. However, the chance of isolating a hybrid and mistaking it for a new species seems remote.

A recurrent theme in the definitions is the presence or absence of interbreeding in nature (Huxley, Carter, Emerson, Sheppard) and observations in the laboratory, when interbreeding is observed, must be interpreted cautiously. Although interbreeding may take place in experimental conditions, it does not necessarily follow that it takes place in nature.

It is, however, seldom possible to check if an animal will interbreed with related groups and, in practice, a species is generally recognised by morphological and other biological characters which are sufficiently marked to make it reasonable to assume that the animal does not share the gene pool of related species. In difficult groups precise methods of investigating genotypes, such as observing the electrophoretic mobility of enzymes or mapping chromosomes, are of great value.

Huxley (1942) listed three points which he believed should be jointly considered when recognizing a species. There should be:

- (i) "a geographical area consonant with a single origin",
- (ii) " a certain degree of constant morphological and presumable genetic difference from related groups", and
- (iii) "an absence of intergradation with related groups."

Several difficulties arise when attempting to apply modern definitions of a species to the protozoa. The greatest of these is the position of agamospecies - species which are thought to

lack any sexual forms. Among parasitic protozoa, trypanosomatids are perhaps the most important and best studied. There is no convincing evidence that conjugation occurs in this group, and they are considered to be asexual. But so, for a long time, was Escherichia coli. Many trypanosomatids are parasites of invertebrates only, and if conjugation does occur in this group, it would seem most likely to take place in invertebrate rather than vertebrate hosts. Conjugation of Escherichia coli was unsuspected until recombination was demonstrated by Lederberg and Tatum (1946) using nutritional dependence as a marker. Similar experiments with the invertebrate stages of trypanosomatids in culture have not been done and until they are, it cannot be said that conjugation does not occur, although it may well be a rare happening and most unlikely to be seen.

Trypanosomatids are an example of apparent agamospecies in which conjugation has yet to be investigated fully. But agamospecies must not be assumed simply to be sexual organisms in which conjugation has not been demonstrated. Some no doubt are, but others will be found to be truly agametic. Definitions of species based on the inability to share the other gene pools obviously do not apply to such organisms.

A second difficulty, even with protozoa such as malaria parasites in which genetic recombination occurs, is the question of distribution. With parasitic protozoa Huxley's "geographical area consonant with a single origin" must be interpreted in the

light of the distribution, habits and phylogenetic relationships of the hosts. The zoogeography of the host may be the key to the origin of a parasite rather than simply the geographical area in which the parasite is encountered. A striking instance of this is T. evansi, the origins of which were not understood until the migrations of the principal host, the dromedary, were related to the present day distribution of the parasite (Hoare, 1957). Similarly, the status of Polychromophilus deanei, a haemoproteid of the S. American bat, Myotis nigricans nigricans, and the nycteribiid fly, Basilia, cannot be understood without considering the origins of the hosts. Except for Po. deanei mammalian haemoproteids are absent from the New World. Myotis is one of the few Old World genera of bats to have found its way to the Americas, and it is thought that an ancestor of M. n. nigricans must have been accompanied in its migrations by a haemoproteid and a nycteribiid fly, all of which became established in S. America (Garnham et al., 1971).

To sum up, it seems as though there is little difficulty in applying the concept of the genospecies to the parasitic protozoa which mate; as Manwell (1937) said of malaria parasites "we should conceive of a species of malaria in genetic terms as we do most other types of living things. It should be a strain of malaria plasmodium which will not hybridize with others in the

invertebrate host, and it should, of course, have certain more-or-less unique morphological characters". Protozoa which do not mate must be considered differently. At present it is seldom practicable to test the ability to interbreed, and relevance must still be placed upon the phenotype and biological characters other than interbreeding. New methods of recognition of enzymes (Carter, 1970; Tait, 1970), which are a more precisely measurable expression of genes than are many characters of the phenotype, will doubtless become more widely used. Finally, when considering the origins of parasitic protozoa, the distribution, habits and phylogeny of the hosts are as important as the present geographical distribution of the parasites.

The Subspecies

Trinomials were first used by the ornithologist Hermann Schlegel in 1844 to designate geographical subdivisions of species of birds (Sibley, 1954). Since that time subspecies have been a constant cause of polemic (see Wilson and Brown (1953), and subsequent contributions on subspecies of different groups of animals by various authors in Systematic Zoology, 1953-1958.)

The subspecies problem is partly one of temperament : splitters like them, lumpers do not. Prolonged study of a group of animals may lead a specialist to the point where he confidently gives subspecific status to certain populations, but less experienced

workers find it difficult to accept his reasoning. The validity of a trinomial depends to some degree upon the reputation of the taxonomist who gives the name (Tate Regan, 1926), and since the recognition of an intraspecific population is often a nice question of judgement perhaps that is as it should be.

The subspecies is the lowest taxon given formal recognition in the International Rules of Zoological Nomenclature. It has been defined by many workers, and, although it is generally thought to be an arbitrary subdivision of a continuum (Simpson, 1945; see also Huxley, 1942), the consensus of opinion is that the subspecies is a population or group of populations inhabiting a geographical subdivision of the range of a species and differing from other populations by diagnostic morphological characters (Mayr, 1963). It follows that subspecies cannot be sympatric, since their identities would be lost by interbreeding.

With vertebrates, Huxley (1942) pointed out that the distribution of a subspecies may be either geographical or ecological. This is an important point when considering subspeciation of parasitic protozoa. There are many instances in which the first step in speciation may be considered to have taken place when a parasite has acquired a strong host restriction (e.g. T. lewisi of rats and T. musculi of mice). Such a parasite may be found in a host in the same place as another which harbours a related parasite. A typical factor giving rise to this is a marked feeding preference of a haematophagous vector which

could well result in parallel evolution in the same locality. Subspecies of parasites may therefore be sympatric in locality, but they will then be parts of different biocenoses, and will not have hosts in common. In this respect the concept of subspecies of parasites differs from that of Mayr's. The mobility of hosts led Manwell (1957) to reject Mayr's largely geographical definition of the subspecies, but if the host is considered to be part of the "geography" of a parasite, Mayr's definition would seem to be acceptable to the parasitologist.

There are a few observations which give an idea of the time taken for subspecies of mammals to appear in populations which become isolated. A population of the normally marine seal Phoca vitulina isolated in a fresh-water lake in Canada 3-8,000 years ago is now recognized by its morphology as a subspecies. This period represents 300-1600 generations. Scattered data for rodents isolated on islands suggest that subspecific differentiation may occur in even less than 300 generations - the lowest figure for the seal (Simpson, 1944). There are no similar observations for parasitic protozoa, but if it took as little as 300 generations for a morphological subspecies of a malaria parasite to arise, and if there were 5 generations from zygote to zygote each year, the time taken would be only 60 years; with continual cyclical passage in the laboratory it would take 17 years.

The vagueness of the subspecies is a reflection of evolution. Darwin (1872) thought in terms of a hierarchy of intraspecific populations, all leading towards, but not necessarily attaining, the rank of species. It will always be a vague taxon, but while it is a useful means of naming a population, it will, no doubt, continue to be used.

Demes

A deme is a term first used by Gilmour and Gregor (1939) to denote any specified assemblage of taxonomically closely related populations. Its meaning has become slightly altered and it now means any interbreeding community or population (Huxley, 1942; Mayr, 1963), which is not considered to have attained a formal taxonomic rank. A series of demes with character gradients is termed a cline (Huxley, 1942; Carter, 1951). Although neighbouring populations differ little, there may be marked differences in the extremes of a cline.

Among parasitic protozoa, the deme has been widely used by Hoare (1955, 1967) for describing populations of trypanosomes. He recognized five kinds of demes, viz. the plastodeme for antigenic variants; the serodeme for immunologically distinct strains; the xemodeme for populations largely restricted to different hosts; the clinodeme for neighbouring populations differing in mean length and virulence; and the nosodeme for a

population which, like the clinodeme, differs from others in its virulence and size, but which is not part of a geographically continuous series.

The deme has been used to describe populations of P. falciparum (Garnham, 1966a), and may perhaps be a useful way of naming populations of other malaria parasite (e.g. to denote populations of P. vivax with differences in patterns of relapse - there may, in fact, be a cline of P. vivax).

Syngen

This is a term coined by Sonneborn (1957) to describe geographically separate populations of the ciliate Paramecium aurelia which he had earlier subdivided into physiological subspecies or varieties.

Some syngens differ from others by differences in size, surface structures, growth rates, tolerance to high temperatures etc. These differences are, however, slight and difficult to use as a means of recognition. More usually, identification of the 14 known syngens is by mating, in culture, with previously identified stocks. Different syngens may conjugate, but there is then seldom an exchange of nuclei; if there is, the organisms which develop are not viable.

Tait(1970), while investigating the electrophoretic mobilities of enzymes of the mitochondria of P. aurelia (succinic dehydrogenase,

isocitrate dehydrogenase, glutamate dehydrogenase, beta-hydroxybutyrate dehydrogenase and fumarase), found a new way of recognising 12 out of the described 14 syngens. As long as all four enzymes were examined, their characteristic mobilities were a means of identification.

The term syngen has not been generally adopted by protozoologists for organisms other than ciliates. In the light of modern definitions of species, a syngen would seem to be a species.

THE TAXONOMY OF P. BERGHEI AND SUBSPECIES

P. berghei Vincke and Lips, 1948 lies in the subgenus Vinckeia Garnham, 1964, in which are grouped all species of Plasmodium parastizing lemurs and lower mammals. The characters of the subgenus are small erythrocytic schizonts usually producing eight or fewer merozoites, an absence of stippling of infected erythrocytes, a spherical gametocyte with widely distributed pigment, an exoerythrocytic schizogony of three days or less, and the presence of so-called secondary exoerythrocytic schizonts (Garnham, 1966a).

Some of the characters are now not very satisfactory. Anomalies in the morphology of schizonts of seven of the fifteen named species have been discussed in Part III. Primary exoerythrocytic schizonts have been seen in only two species, P. berghei and P. vinckei, and this is slender evidence for a short tissue schizogony in the whole subgenus. Tissue schizonts which may be secondary forms have been found in the livers of the hosts of only two species (P. berghei and P. traguli) and a plausible alternative to the Shortt-Garnham theory of the formation of secondary forms has been put forward (Garnham, 1966b) in which it is suggested that so-called secondary forms may be derived directly from sporozoites and not from the product of quickly maturing primary forms. The value of the presence of

"secondary forms" as a character of the subgenus Vinckeia will have to be re-assessed as new facts emerge.

In spite of the unsatisfactory definition of Vinckeia its erection was justified. The characters of the other two subgenera of mammalian malaria parasites are clear, and the grouping of the species parasitizing supralemuroid primates into the subgenera Plasmodium and Laverania obviously reflects their evolution (see Garnham, 1966a). This step could not be taken without giving taxonomic recognition to the malaria parasites of lemurs and lower primates. The best course at the moment might be thought to be to define Vinckeia by the characters of the best known species, P. berghei and P. vinkei, the whole life-cycles of which have been seen since Vinckeia was created, and to classify the others as Vinckeia incertae sedis. Unfortunately the type species of Vinckeia is the little studied P. bubalis Sheather, 1919 of the domestic buffalo, and a redefinition of the subgenus should await the discovery of the full life-cycle of this parasite.

Populations of P. berghei from four localities in Africa (see map, fig 5 in Part II of this thesis) have been designated as subspecies. They are: P. berghei berghei Vincke and Lips, 1948 from Katanga; P. b. yoelii Landau and Killick-Kendrick, 1966 from the C.A.R.; P. b. killicki Landau et al., 1968 from Brazzaville; and P. berghei (Nigeria) (present work). Bafort

(1968, 1970, 1971) has opposed the division into subspecies, although at no time has he suggested synonymy. He rightly drew attention to the pleomorphism of some of the stages in the life-cycle of P. berghei, but he appears not fully to have understood the need to make comparisons in identical conditions.

It is true that the size of primary tissue schizonts, and maturation rates, vary in different hosts, but the measurements distinguishing the tissue stages of P. berghei and P. b. yoelii were made on forms of the same age, in the liver of the same species of host. It was noted that the measurements of P. b. berghei differed from those made by Yoeli (1965) but in Yoeli's early work more than one inoculation of sporozoites was given, and the age of the schizonts was, therefore, uncertain. (Landau and Killick-Kendrick, 1966).

In an attack on the concept of subspecies of P. berghei Bafort (1970) points out that "Hoare (1966) drew attention to the fact that in trypanosomes the application of biometry to the differentiation of infraspecific strains or races may be misleading on account of the wide variation sometimes observed even within populations of the same strain." But the Anglo-French workers have not used biometrical methods to recognize strains or races: they have used them to recognize populations they believe to warrant the status of a formal taxon, the subspecies.

Bafort's selected quotation of Hoare's paper may give the

erroneous impression that Hoare rejects the use of biometry.

On the contrary, Hoare's application of biometrical method in a lifetime study of mammalian trypanosomes has been the means of solving many problems. In the same paper quoted by Bafort, Hoare (1966) says "In addition to measurements characterizing different species of trypanosomes, statistical methods also serve as a valuable aid for the separation of related groups and for the study of variation."

It is in this way that the biometry has been used by those who named the subspecies (Landau and Killick-Kendrick, 1966; Landau et al., 1968, 1970).

Bafort (1970) goes on to examine "several other features that have been considered as differences." Firstly, he discusses virulence: this was not used as a criterion to recognize the subspecies. Secondly, he considers epizootiology, in particular the generally low prevalence of infections in rodents in Katanga and the very high rate of infection in thicket-rats in the C.A.R. He suggests that the picture in Katanga is incomplete and in support of this quotes Vincke (1954) who, to his (Vincke's) surprise, found it easy to isolate strains from rats in one particular locality. While it may be true that the complete picture in Katanga is unknown, it is equally true that no figures have been published of rates of infection of rats in Katanga as high as those reported in the C.A.R. where every adult thicket-rat

was found infected with P. v. chabaudi and more than half were additionally infected with P. b. yoelii (Landau and Chabaud, 1965).

Bafort's third point is type localities and the question of geographical barriers between the populations. He puts the reasonable view that there are no barriers within the Congo Basin. But the present apparent restriction of murine malaria to the periphery of the Basin may well be a consequence of past barriers, rather than present ones. It is plausible to suppose that the present populations are relicts following the time when the Congo Basin was a lake (Chapin, 1932). The faunal barriers between the type locality of P. berghei (Nigeria) and those of other subspecies are fully discussed in Part II of this thesis.

Fourthly, the question of the vertebrate hosts is discussed, and it is implied that, because 55 different vertebrates have been experimentally infected with P. berghei great significance should not be placed on the differences in natural hosts.

(Thamnomys rutilans in the C.A.R. and Brazzaville and Grammomys (or Thamnomys) surdaster and, of lesser importance, Praomys jacksoni and Leggada bella in Katanga). But the experimental infections were induced by the inoculation of infected blood usually with highly virulent strains, and therefore mean little or nothing in relation to natural, sporozoite-induced infections.

The fact that constant differences in hosts represent different selective forces is ignored.

In his final point, that of the different invertebrate hosts, Bafort acknowledges that the parasites in the C.A.R. and the Congo-Brazzaville (where the vectors are not known) cannot have the same invertebrate host as P. berghei of Katanga (where the vector is A. d. millecampsi, a mosquito with a very limited distribution). He quotes Vincke (1967) as saying that the most probable vector in Brazzaville is A. cinctus, a mosquito close to A. d. millecampsi. Vincke made no special claim for A. cinctus, but simply included it as one of a list of exophilic, zoophilic species to which he wished to draw attention. Possibly, A. cinctus was singled out because of the then unpublished fact, widely known to workers in the field, that Adam had found sporozoites in the salivary glands of a specimen of A. cinctus caught in the type locality of P. b. killicki and P. v. lentum.*

* Too much should not be made of this observation. Adam found and photographed four long sporozoites. I have seen the photographs and confirm that the bodies look like sporozoites of P. b. killicki or P. v. lentum. However, blood meals from wild-caught A. cinctus in the type-locality have now been tested and shown to be of a primate (Boreham, personal communication), and since it is suspected that A. cinctus is the vector of a trypanosome of galagoes (Frezil, personal communication), this was probably the primate. No meals of rodent blood were identified.

Bafort belittles the difference in invertebrate hosts by comparing
it with the very large number of anopheline hosts of the malaria parasites of
man.

The comparison is hardly valid. Malaria parasites of man are transmitted mainly by domestic mosquitoes, whereas those of forest animals are typically in delicately balanced situations where transmission is solely by one sylvatic or cavernicolous mosquito (e.g. P. atheruri, P. voltaicum, P. traguli). As with the vertebrate host, restriction to a single mosquito for long periods would be a selective force. Evidence that murine malaria parasites have been affected by such a restriction is provided by observations on the poor development of all subspecies in laboratory bred mosquitoes known not to be natural hosts (reviewed by Killick-Kendrick, 1971).

Bafort et al. (1968) believe that valid comparisons of the morphology of different populations of P. berghei can be made only in natural hosts. This counsel is impracticable since (i) invertebrate hosts in lowland localities are unknown, (ii) the invertebrate host of highland parasites cannot be bred in the laboratory, (iii) P. b. berghei is believed to have at least three vertebrate hosts, only one of which is available in the laboratory and (iv) the vertebrate host of lowland subspecies has not been bred in the laboratory. Landau et al. (1970) point out that a better comparison is possible in standard laboratory bred rodents and mosquitoes. In the extensive studies on the tissue schizonts and blood stages of malaria parasites of Asian monkeys, the rhesus monkey has become the standard host in which comparisons are made.

If it is accepted that Mayr's geographical definition (with the modification that restriction of parasites in Nature to particular hosts is analagous to geographical restriction of free-living animals) is applicable to malaria parasites, it seems clear that P. berghei is correctly sub-divided. Mayr's corollary that there should be some morphological differences is satisfied. Landau et al. (1970) review the morphological criteria and suggest that the three most important are (i) the size of the mature oocyst in A. stephensi at known temperatures, (ii) the length of the sporozoite in the glands of A. stephensi and (ii) the rate of growth of primary exoerythrocytic schizonts in the livers of white rats and mice. Much emphasis has been placed on extremes of ranges, particularly odd giant forms (Bafort, 1968, 1970, 1971), while such stages must be included in randomly measured samples, to give them undue prominence is misleading.

Sizes of sporozoites overlap, and the significance of differences in mean lengths must be judged by statistical methods (Killick-Kendrick, 1970; Part II of this thesis). Conclusions based on means with little difference and overlapping ranges alone, giving such inadequate data that standard errors of the means and standard deviations of the series cannot be calculated. (Bafort, 1971, p. 142), can be described as mathematically meaningless. Methods of sampling populations must be standardized, and it is best if comparisons are made by a single observer.

The differences in the sporogonic and exoerythrocytic stages of P. b. berghei, P. b. yoelii, P. b. killicki and P. berghei (Nigeria) have been discussed in Part II of this thesis, and the point has been made that there is a sharp difference between P. b. berghei from the highlands of Katanga and the three other populations from the lowland forests (summarized in Table 4). In the writer's view, the point at issue is not whether or not P. berghei is correctly split into subspecies; it is whether or not the P. berghei complex is composed of two species which do not share the same gene pool, and which are so divergent that they have lost the ability to cross-breed.

Support for the view that there are two species is provided by Carter's (1970) study on the electrophoretic mobilities of enzymes of populations of P. berghei in different parts of Africa. Carter's (1971) most recent work, as yet unpublished, confirms his earlier opinion that P. b. berghei stands apart from the lowland subspecies. The electrophoretic patterns of enzymes of highland and lowland are characteristic and constant, and differ so markedly that, on analogy with Tait's (1970) enzymatic differences between syngens of Paramecium, they would seem unlikely to interbreed.

Carter's approach is a great advance in methods of studying populations of malaria parasites. Electrophoretic mobilities

of enzymes are accurately measurable expressions of genes, and for the first time a method of investigating the genetic make-up of a population of malaria parasites, other than by examining its phenotype, is available. Advances in the recognition of parasites following Carter's pioneer work may well be comparable to the progress which followed Romanowsky's discovery of new ways of staining blood films.

TAXONOMY OF P. VINCKEI AND SUBSPECIES

The characters of the subgenus Vinckeia of which P. vinckei Rodhain, 1952 is a member, have been discussed in the section above.

Like P. berghei, populations of P. vinckei from different parts of Africa have been designated as subspecies viz. P. v. vinckei Rodhain, 1952 from Katanga; P. v. chabaudi Landau 1965 emend. Bafort, 1968 from the C.A.R.; and P. v. lentum Landau, Michel, Adam and Boulard, 1970 from Brazzaville. A fourth population from Nigeria, studied by Killick-Kendrick (1970a,b.) and Bafort (in press) has not yet been named.

The arguments put forward for speciation in P. berghei (selective pressures of hosts and differences in biocenoses) apply equally to P. vinckei. The morphological differences of

the named subspecies of P. vinckei have been discussed in detail by Landau et al. (1970). Their conclusion that P. vinckei, P. v. chabaudi and P. v. lentum are separable on the basis of the size of the mature oocyst, the length of the sporozoite and the rate of growth of the tissue schizonts is again challenged by Bafort (1971), whose comprehensive studies on P. vinckei have been restricted almost completely to a single strain of one subspecies, P. v. vinckei.

It is difficult at the moment to speculate on the status of the geographical populations of P. vinckei, which have been less studied than those of P. berghei. The vertebrate host of the nominate subspecies in Katanga has not been found and, as with the lowland populations of P. berghei, the invertebrate hosts of P. v. chabaudi, P. v. lentum and P. vinckei (Nigeria) are unknown. Infective sporozoites of P. vinckei (Nigeria) have proved difficult to obtain (Killick-Kendrick, 1970 a,b.) and it was not until recently that the exoerythrocytic schizogony of this parasite was demonstrated. (Bafort, in press).

Populations of P. vinckei do not seem to have quite the same relationships with each other as do those of P. berghei. Carter (1971, but unpublished) has studied the electrophoretic mobilities of (i) one of the two known isolates of P. v. vinckei (ii) 22 isolates of P. v. chabaudi, (iii) the only two isolates of P. v. lentum, and (iv) two of the three known isolates of P. vinckei (Nigeria). This work is continuing and it is

hoped to obtain even larger numbers of isolates. Carter tentatively concludes that the patterns so far, revealed suggest a linear relationship in the following order:

P. v. vinckei - P. v. chabaudi - P. v. lentum - P. vinckei (Nigeria), and postulates that this reflects a phylogenetic relationship following the geographical distribution of the parasites. Geographically, however, a more acceptable series from east to west would perhaps be P. v. vinckei - P. v. lentum - P. v. chabaudi - P. vinckei (Nigeria). Possibly populations of P. vinckei will be found to represent a cline, although the morphological and enzymatic differences (Landau et al., 1970; Killick-Kendrick, 1970a; Carter, 1971) seem to be of an order greater than those of demes, and the point of evolution of the separate populations would then be more suitably expressed by sub-specific designations.

ORIGINS OF MALARIA PARASITES OF AFRICAN RODENTS

Speculations on the evolution of the suborder Haemosporina, in which lie the three families Plasmodiidae, Haemoproteidae and Leucocytozoidae, have been made by many workers. Recent notable and contributions are those of Bray (1957), Baker (1965) who subscribe

to the view that malaria parasites sensu lato evolved from adeleid haemogregarines of vertebrates, although Garnham (1966a) points out that this view ignores fundamental differences in the microgametes, oocysts and tissue schizogony separating malaria parasites and adeleid haemogregarines.

A Coccidian ancestry of Haemosporina is generally accepted (Baker, 1965), but whether the ancestors were parasites of vertebrates or invertebrates is still debated. Bray(1957) and Baker (1965) argue that the host was a vertebrate whereas Huff (1945) put the opposite view. Perhaps the strongest argument in support of Huff is the restriction of the invertebrate hosts of Haemosporina to a single order of insects, the Diptera, suggesting an origin from parasites of the ancestors of these insects. Baker (1965), while recognizing the weight of this point, rejects it in the face of evidence for the opposite view without discussing it further. But, if the evolution of the Classes of the vertebrate hosts of the true malaria parasites (Reptilia, Aves and Mammalia) are compared with the evolution of the dipteran hosts (Table 9), the pattern which emerges adds support to the notion that malaria parasites arose from parasites of insects.

Insects arose in the Silurian period, before the first vertebrates. During the Mesozoic era (the Age of Reptiles), the first Diptera, first blood-sucking arthropods (Hocking, 1971)

Table 9. Time-scale of the evolution of some vertebrate and invertebrate hosts of malaria parasites (time in millions of years in parentheses). (from: Hocking, 1971; Manwell, 1955; Mattingly, 1965; Romer, 1941; Simpson, 1953; Tarling and Tarling, 1971; Wood, 1959).

PRECAMBRIAN

PALEOZOIC

Cambrian (480)

Ordovician (430)

Silurian (390)

Devonian (350)

Carboniferous (300) First vertebrates, first amphibians;
first reptiles

Permian (215)

MESOZOIC (Age of Reptiles)

Triassic (190)

Jurassic (155) First Diptera; first blood-sucking arthropods
first true mammals; first birds.

Cretaceous (120)

CENOZOIC (Age of Mammals)

Tertiary

Paleocene (55)

Eocene (45) Mosquitoes present; first bats, first "squirrels"
first porcupines.

Oligocene (30) Anopheles not yet arisen?

Miocene (19) First murids.

Pliocene (7)

Quaternary

Pleistocene (1)

Recent (0.5)

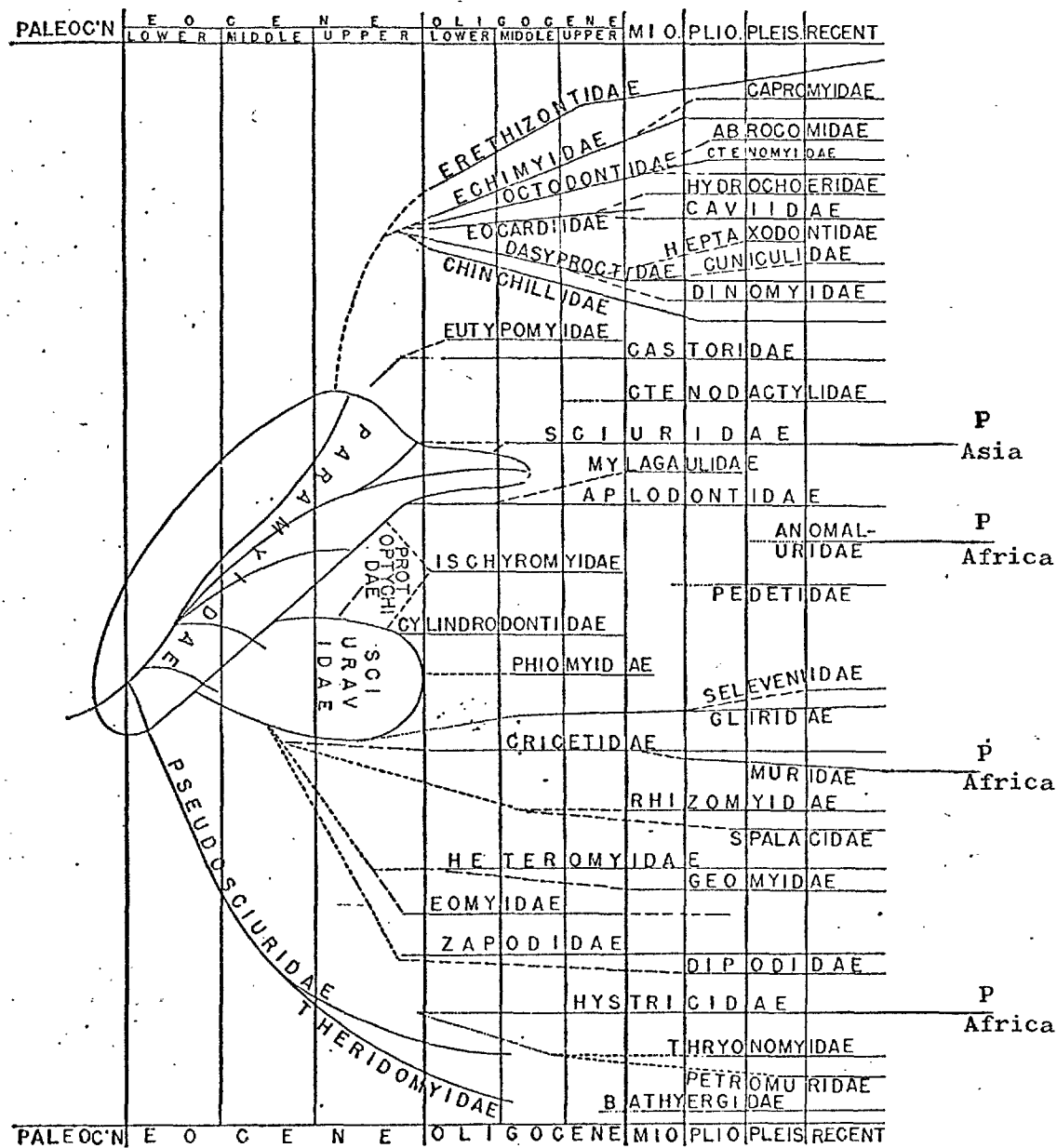
first birds and first true mammals arose. Perhaps it was during this era that the malaria parasites of lizards arose, the vectors of which are now believed to be phlebotomine sandflies (Ayala and Lee, 1970). Saurian malaria is widely distributed throughout the Tropics, and it is logical to assume the parasites of lizards arose before the Continental Drift led to a final separation between the Old and New Worlds (about 1,000,000 years ago; Tarling and Tarling, 1971). Mosquitoes (though not Anopheles) were present in the Oligocene (Manwell, 1955).

Malaria parasites of birds presumably evolved, probably from those of a saurian ancestor, sometime from the mid-Mesozoic to mid-Cenozoic.

The Eocene is probably the time of the evolution of mammalian malaria parasites. This comparatively late arrival is perhaps roughly about the time of the appearance of Anopheles.

The phylogeny of the groups of African rodents which are hosts of malaria parasites is shown in fig 8.

Murid rodents arose in the Miocene and it is curious that modern descendants of the ancestors of this Old World groups, the cricetid stock, have no malaria parasites. This suggests that murine malaria parasites probably arose very recently, and originated from parasites of hosts other than the stock from which murids evolved. A likely source would seem to be precursors of the modern anomalurine malaria parasites, and it is suggested that as thicket-



Wood's (1959) suggested phylogeny of the families of rodents showing within which families lie hosts of malaria parasites.

Figure 8.

rats began to acquire arboreal habits they came into contact with anopheline vectors transmitting malaria among anomalurids above the ground; in this way the ancestor of P. berghei and P. vinckei may well have arisen. If this were so, one could predict that the characters of anomalurine malaria parasites, particularly those of the tissue schizogony, will be found to echo those which are known for the murine parasites. In the size and, to a lesser degree, the number of merozoites, the characters of the erythrocytic schizonts of anomalurine parasites already show some similarities with schizonts of the murine forms.

A similar relationship may be postulated for P. atheruri and P. voltaicum. These are parasites of cavernicolous mammals, porcupines and bats, and are transmitted by highly specialized species of Anopheles in caves. The small size of the schizont is a remarkably fixed character in P. atheruri.* When established

* Another fixed character of P. atheruri would appear to be its ability to undergo successful sporogony. I have attempted to obtain infective sporozoites of the mouse-adopted strain in 8 species of Anopheles with the following results (temperatures in parentheses); ookinetes seen, but no oocysts: A. albimanus (21°C), A. melas (24°C), A. sundaicus (24°C), A. farauti (28°C), and A. freeborni (28°C); oocysts grew and matured by glands not invaded: A. stephensi (24°C, 28°C), and A. gambiae (20°C); complete sporogony but sporozoites (from glands) not infective to mice (splenectomized): A. l. atroparvus (22°C) and A. freeborni (22°C).

in mice, the strain isolated in Brazzaville by Landau et al.

(1969) has a general resemblance to P. berghei except that the schizonts retain their small size; it is noteworthy that the schizonts of P. voltaicum are also small.

Bats and porcupines are both much older animals than murine rodents, and in this instance it is impossible to suggest in the ancestor of which animal malaria parasites may have first arisen.

REFERENCES IN PART IV

- AYALA, S.C., & LEE, D. (1970). Saurian malaria: development of sporozoites in two species of phlebotomine sandflies. Science, N.Y., 167, 891-892.
- BAFORT, J. (1970). The variability of Plasmodium berghei Vincke & Lips, 1948. Annls Soc. belge Méd. trop., 50, 247-262.
- BAFORT, J.M. (1971). The biology of rodent malaria with particular reference to Plasmodium vinckei Rodhain, 1952. Annls Soc. belge Méd. trop., 51, 1-204.
- BAFORT, J.M. (in press). Le cycle biologique de Plasmodium vinckei de Nigeria. Proc. 1st European Multicolloquium in Parasitology, Rennes, 1971.
- BAKER, J.R. (1965). The evolution of parasitic protozoa. In Evolution of parasites, A.E.R. Taylor, (ed), 3rd Symp. Brit. Soc. Parasit., Oxford: Blackwell, pp 1-27.
- BRAY, R.S. (1957). Studies on the exo-erythrocytic cycle in the genus Plasmodium. London: Lewis.
- CAIN, A.J. (1963). Animal species and their evolution. Revised 1954 edition London: Hutchinson.
- CARTER, G.S. (1951). Animal evolution. London: Sidgwick & Jackson.
- CARTER, R. (1970). Enzyme variation in Plasmodium berghei. Trans. R. Soc. trop. Med. Hyg., 64, 401-406.
- CARTER, R. (1971). The study of enzyme variation as a means of classification in rodent malaria parasites. Unpublished document presented at a Round-Table Discussion on Taxonomic Problems Relating to Malaria Parasites Fogarty International Centre, Bethesda, U.S.A., November 22-24, 1971.
- BAFORT, J., TIMPERMAN, G., & DELBAR, T. (1968). Observations on tissue schizogony and sporogony of rodent malaria. Annls Soc. belge Méd. trop., 48, 535-540.

- CHAPIN, J.P. (1932). The birds of the Belgian Congo.
Part 1. New York: American Museum of Natural History.
- CORLISS, J.O. (1967). Newer trends in the systematics of the protozoa. Symp. on Newer Trends in Taxonomy, No 24, 26-33.
- DARWIN, C. (1872). The origin of species by means of natural selection or the preservation of favoured races in the struggle for life. 6th ed., London: Murray.
- DOBZHANSKY, T. (1937). Genetics and the origin of the species. New York: Columbian University Press.
- GARNHAM, P.C.C. (1966a). Malaria parasites and other haemosporidia. Oxford: Blackwell.
- GARNHAM, P.C.C. (1966b). Immunity against the different stages of malaria parasites. Bull. Soc. Path. exot., 59, 549-557.
- GARNHAM, P.C.C., LAINSON, R., & SHAW, J.J. (1971). A contribution to the study of haematozoon parasites of bats. A new mammalian haemoproteid, Polychromophilus deanei n. sp. Mem. Inst. Osw. Cruz., 69, 119-125.
- GILMOUR, J.S.L., & GREGOR, J.W. (1939). Demes: a suggested new terminology. Nature, London, 144, 333.
- HOARE, C.A. (1955). Intraspecific biological groups in pathogenic protozoa. Refuah vet., 12, 263-258.
- HOARE, C.A. (1957). The spread of African trypanosomes beyond their natural range. Z. Tropenmed. Parasit. 8, 157-161.
- HOARE, C.A. (1966). The classification of mammalian trypanosomes. Ergebn. Mikrobiol., 39, 43-67.
- HOARE, C.A. (1967). Evolutionary trends in mammalian trypanosomes. Advances in Parasitology, 5, 47-91.

- HOCKING, B. (1971). Blood-sucking behaviour of terrestrial arthropods. Ann. Rev. Ent., 16, 1-26.
- HUFF, C.G. (1945). A consideration of the problem of evolution of malarial parasites. Rev. Inst. Salubr. Enferm. trop. Mex., 6, 253-257.
- HUXLEY, J. (1942). Evolution, the modern synthesis. London: Allen & Unwin.
- KILLICK-KENDRICK, R. (1970a). Studies on the life-cycles of murine malaria parasites from Nigeria. M. Phil. thesis, University of London.
- KILLICK-KENDRICK, R. (1970b). The life-cycles of two murine malaria parasites from Nigeria. J. Parasit., Suppl., 56, No 4, Sect II, Part 2, 435-436.
- KILLICK-KENDRICK, R. (1971). The collection of strains of murine malaria parasites in the field, and their maintenance in the laboratory by cyclical passage. In Isolation and Maintenance of Parasites in vivo, Eds A.E.R. Taylor & R. Muller, Symposia of the British Society for Parasitology, vol. 9, Oxford: Blackwell, pp. 39-64.
- LANDAU, I., 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, 8-9.
- LANDAU, I., & CHABAUD, A.G. (1965). Infection naturelle par deux Plasmodium du rongeur Thamnomys rutilans en République Centre Africaine. C. r. hebd. Séanc. Acad. Sci., Paris, 261, 230-232.
- LANDAU, I., & 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, 633-649.

- LANDAU, I., MICHEL, J.C., & ADAM, J.P. (1968).
Cycle biologique au laboratoire de Plasmodium
berghei killicki n. subsp.
Annls Parasit. hum. comp., 43, 545-550.
- LANDAU, I., MICHEL, J.C., ADAM, J.P., & BOULARD, Y.
(1970). The life-cycle of Plasmodium vinckei
lentum n. subsp. in the laboratory; comments
on the nomenclature of the murine malaria para-
sites. Ann. trop. Med. Parasit., 64, 315-
323.
- LEDERBERG, J., & TATUM, E.L. (1946). Gene recom-
bination in Escherichia coli. Nature, Lond.,
158, 558.
- MANWELL, R.D. (1936). The problem of species, with
special reference to the malaria parasite.
Ann. trop. Med. Parasit., 30, 435-439.
- MANWELL, R.D. (1955). Some evolutionary possibilities
in the history of malaria parasites. Indian
J. Malar., 9, 247-253.
- MANWELL, R.D. (1957). Intraspecific variation in para-
sitic protozoa. Syst. Zool., 6, 1-6.
- MAYR, E. (1942). Systematics and the origin of
species.
New York: Columbia University Press.
- MAYR, E. (1963). Animal species and evolution.
London: Oxford University Press.
- RILEY, H. P. (1948).
Introduction to genetics and cytogenetics.
New York: Hafner.

- SHEPPARD, P.M. (1967).
Natural selection and heredity.
3rd edition. London: Hutchinson.
- SIBLEY, C.G. (1954). The contribution of avian taxonomy.
Syst. Zool., 3, 105-110.
- SIMPSON, G.G. (1944). Tempo and mode in evolution.
New York: Columbia University Press, p. 19.
- SIMPSON, G.G. (1945). The principles and classification and a classification of mammals.
Bull. Am. Mus. nat. Hist., 85, 1-350.
- SONNEBORN, T.M. (1957). Breeding systems, reproductive methods, and species problems in Protozoa.
In The Species Problem. Ed. E. Mayr,
Washington: American Association for the Advancement of Science.
- TAIT, A. (1971). Enzyme variation between syngens in Paramecium aurelia.
Biochemical Genetics, 4, 461-470.
- TARLING, D.H., & TARLING, M.P. (1971).
Continental drift.
London: Bell.
- TATE-REGAN, C. (1926). Organic evolution.
Report of the 93rd Meeting of the British Association for the Advancement of Science.
London: British Association.
- VINCKE, I. (1954). The natural history of Plasmodium berghei.
Indian J. Malar., 8, 245-256.
- VINCKE, I.H. (1967). The potential vectors of P. chabaudi and P. b. yoelii.
Trans. R. Soc. trop. Med. Hyg., 61, 154-155.

- WALLIKER, D., CARTER, R., & MORGAN, S. (1971).
Genetic recombination in malaria parasites.
Nature, London, 232, 561-562.
- WILSON, E.O., & BROWN, W.L. (1953). The subspecies
concept and its taxonomic application.
Syst. Zool., 2, 97-111.
- YOELI, M. (1965). Studies on Plasmodium berghei in
nature and under experimental conditions.
Trans. R. Soc. trop. Med., 59, 255-276.

PART V
PROTOZOA OF THE BLOOD CELLS
OF RODENTS: AN ANNOTATED
CHECKLIST AND HOST-INDEX

INTRODUCTION

The protozoa of the erythrocytes or leucocytes of rodents lie in five of the families in the subphylum Sporozoa, namely Hepatozoidae, Plasmodiidae and Haemoproteidae (Telosporea; Eucoccida), and Babesiidae and Dactylosomidae (Piroplasmea; Piroplasmida). Trypanosomes, which are not dealt with in the present work, are common extracellular parasites of the blood of rodents. A check-list which includes the species infecting rodents is given by Hoare (1972) in his comprehensive treatise on the mammalian trypanosomes.

In addition to the true protozoa parasitizing the blood-cells of rodents, there are a number of other organisms which share the same habitat. Bartonella Strong et al., 1915, Anaplasma Theiler, 1910, Grahamella Brumpt, 1911, Haemobartonella Tyzzer and Weinman, 1939, and Eperythrozoon Schilling, 1925 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 (Philip, 1957; Weinman, 1957). Records of these organisms are not included in this check-list. They are common in rodents and are easily revealed in Giemsa-stained blood films, although less easily assigned to a genus (see Tyzzer, 1942). They are seen singly or in groups in or on erythrocytes, and appear as small dots or rod-shaped bodies.

An apparently similar parasite of the leucocytes of voles

was described by Coles (1914), who found it in an English Microtus agrestis. It was later seen in the neutrophils and eosinophils of M. pennsylvanicus in the U.S.A. and named Cytoecetes microti Tyzzer, 1938. Possibly Cytoecetes should be grouped with Ehrlichia, a tick-borne virus of the lymphocytes of cattle (Foggie, 1962).

In the preparation of the check-list the exact identity of some of the rodents has given difficulty, but an attempt has been made to correct 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, and as far as possible generally accepted modern names have been substituted. Trinomials have been ignored in some groups when the authorities seem not to be in agreement.

To speculate on possible relationships between parasites, it is helpful if the relationships of the hosts are understood. The abundance and diversity of rodents makes this difficult, but Wood's (1955) revision of the higher taxa (followed in the host-index) is valuable to workers interested in the hostal distribution of the parasites of rodents. He rejected the three pragmatic but non-phylogenetic suborders commonly recognised (Sciuromorpha, Myomorpha and Hystricomorpha) and replaced them, with reservations, with seven less arbitrary groups. Wood's revision met with strong support from Simpson (1959) who adopted new classification and freely acknowledged that his earlier views

on the suborders (Simpson, 1945) had become indefensible; they are, nevertheless, still accepted by some (e.g. Rosevear, 1969).

Great significance cannot be placed on the geographical distribution suggested by some of the records which in many instances appear more clearly to reveal the distribution of protozoologists.

CHECKLIST OF THE PROTOZOA IN THE BLOOD-CELLS 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. The records for each genus of parasite are arranged in chronological order according to the date of naming or, for unnamed species, the date of the first report.

HEPATOZOIDAE

Hepatozoidae includes the genus Hepatozoon Miller, 1908, a widespread parasite of the leucocytes, or in a few instances (indicated by an asterisk (*) before the name in the check-list) the erythrocytes, of rodents and many other vertebrates. The only stage in the blood is the characteristically banana-shaped

gametocyte. The haemogregarines of rodents have been given several different generic names, but in the check-list they are all referred to the genus Hepatozoon. Except for differences in size and host-cell, the gametocytes look very much alike, and the few life-cycles which have been seen (e.g. H. muris, H. pitymysi, H. balfouri, H. griseisciuri, H. erhardovae) are typical of the genus Hepatozoon. At the moment there is no reason to suppose the haemogregarines of rodents are represented by more than this one genus.

There is some confusion in the naming of the species of Hepatozoon of rodents, caused by the old practice of calling all haemogregarines of murids, muris. In the check-list I have used the names given usually because of differences of host rather than of morphology. This gives identities to haemogregarines which may later be shown not to deserve them.

1. *Hepatozoon balfouri (Laveran, 1905); Jaculus gordonii (109)
 Sudan (Balfour, 1905, 1906); J. orientalis (110)
 Tunisia (Laveran, 1905a), Egypt (Hoogstraal, 1961);
 J. jaculus (111) Sudan (Balfour, 1905, 1906), Egypt
 (Hoogstraal, 1961; Furman, 1966).

2. Hepatozoon muris (Balfour, 1905): R. norvegicus (89)
 R. rattus (90) cosmopolitan. Rattus assimilis (87)
 R. conatus (88) Queensland, Australia (Mackerras, 1959);

As rats have moved around the world in the company of man, their haemogregarine has moved with them. Early records of H. muris 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 species of Rattus has not been confirmed by transmission experiments in the laboratory.

H. perniciosum Miller, 1908, a parasite of white laboratory rats (R. norvegicus) and the type species of Hepatozoon Miller, 1908 is a synonym of H. muris (see Brumpt, 1946).

3. *Hepatozoon gerbilli (Christophers, 1905); Tatera indica (65) Madras, India, (Christophers, 1905); Rhombomys opimus (72) S. Vostok, U.S.S.R. (Zasuchin et al., 1934); Tatera indica ceylonica (66) Ceylon (Manawadu, unpublished).
4. Hepatozoon funambuli (Patton, 1906); Funambulus pennanti (3) India.
5. Hepatozoon musculi (Porter, 1908); Mus musculus (100) England (Porter, 1908), Italy (Sangiorgi, 1912), U.S.S.R. (Yakimoff and Schokhor, 1917; Zasuchin, 1936), Czechoslovakia (Erhardova, 1955).

6. Hepatozoon citellicolum (Wellman and Wherry, 1910);
Citellus beecheyi (26) U.S.A.
7. Hepatozoon sp. Kleine, 1910; Myiomys dybowskii (80)
Tanzania.
8. Hepatozoon criceti (Nöller, 1912), Cricetus cricetus (52)
Austria.
9. Hepatozoon mereschowskii Tartakowsky, 1913; Citellus
guttatus (28) C. musicus (29) U.S.S.R.
10. Hepatozoon arvalis (Martoglio, 1913); rodent, Ethiopian
highlands.

Martoglio identified the host as Arvicola (= Microtus) arvalis but, except for M. guentheri which is known from Libya, voles are absent from Africa (Ellerman and Morrison-Scott, 1966). Brumpt (1946) suggested Martoglio's rodent was perhaps Arvicanthis abyssinicus (= A. niloticus), and his parasite the same as H. arvicanthis Schwetz and Collart, 1930).

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

11. Hepatozoon plicatum (Martoglio, 1913);? Pectinator

spekei (40) Somali Republic.

12. Hepatozoon sp. Rodhain, Pons, Vandenbranden and Bequaert, 1913; rodent (see note in the host-index under Fam. Dipodidae), Sankisia, Democratic Republic of the Congo.

13. Hepatozoon microti (Coles, 1914); Microtus agrestis (58) England (Coles, 1914; Jacobs, 1953), Germany (Krampitz, 1964), Czechoslovakia (Černa, 1957); voles (64) Volga Delta, U.S.S.R. (Dubinin, 1953).

14. Hepatozoon sciuri (Coles, 1914); Sciurus vulgaris (2) England (Coles, 1914, Vizoso, quoted by Dasgupta and Meedeniya, 1958), China (Nauck, 1927), Italy (Francini, 1932), central and southern Europe (Krampitz, 1964).

15. Hepatozoon sylvatici (Coles, 1914); Apodemus sylvaticus (76) England (Coles, 1914), W. Germany (Krampitz, 1964), France (Rioux and Golvan, 1969); Apodemus flavicollis (75) Czechoslovakia (Erhardova, 1955), central and southern Europe (Krampitz, 1964).

16. Hepatozoon akodoni (Carini and Maciel, 1915); Akodon fuliginosus (48) São Paulo, Brazil.

17. Hepatozoon pitymysi Splendore, 1918; Pitymys savii (57) Italy (Splendore, 1918, 1920), Sicily (Krampitz, 1957).

Lavier's (1921) suggestion that the haemogregarines of M. arvalis and P. savii were the same is not supported by the work of Krampitz (1964), who demonstrated a marked host restriction of one of the haemogregarines of voles. Possibly these parasites are similar to the large group of lewisi-like trypanosomes of rodents which are morphologically indistinguishable but which exhibit strong host restrictions (e.g. Molyneux, 1969).

18. Hepatozoon getulum Sargent, 1921; Atlantoxerus getulus (20) Tunisia.

Landau (personal communication) found gametocytes of two unidentified haemogregarines, one large and one small, in the leucocytes of A. getulus from Algeria.

19. Hepatozoon sp. (Léger, 1922); Euxerus erythropus (23) Republic of Senegal..

20. Hepatozoon sp. Donovan (Wenyon, 1926); Petaurista petaurista (36) India.

This record is in Wenyon's check-list under the host's old name Pteromys petaurista: Donovan's report was apparently unpublished.

21. Hepatozoon arvicanthis Schwetz and Collart, 1930;
 Arvicanthis niloticus (81) Democratic Republic of the Congo.

22. Hepatozoon lusitanicum Najera Angulo, 1937; Eliomys
 quercinus (116) Spain.

23. Hepatozoon sp. Tyzzer, 1939; Microtus pennsylvanicus (63)
 Island of Martha's Vineyard, Mass. U.S.A.

24. *Hepatozoon alactaguli Brumpt, 1946; Allactaga major (112)
 Saratov, U.S.S.R. (Shingareva, 1910), Urdu, Kazakstan,
 U.S.S.R. (Zasuchin, 1936); Allactaga sibirica (113)
 Mongolia and Manchuria (Jettmar, 1932); Alactagulus
 pumilio (114) S. Vostok, U.S.S.R. (Zasuchin, 1931);
 Dipus sagitta (108) Mongolia and Manchuria (Jettmar, 1932),
 Kazakstan, U.S.S.R. (Zasuchin, 1936).

- Complete life-cycles must be seen and cross infectivity tests
performed before it can be decided if more than one species of
Hepatozoon is present in these jerboas.

25. Hepatozoon arvicolae Brumpt, 1946; Microtus montebelli
 (61) Japan (Miyairi, 1932).

26. *Hepatozoon cricetomysi Brumpt, 1946; Cricetomys gambianus
 (73) Democratic Republic of the Congo (Rodhain, 1915),

Central African Republic (Landau, unpublished).

27. *Hepatozoon dendromus Brumpt, 1946; Dendromus
? insignis (106) Tanzania (Kleine, 1910).

In spite of confusion, the correct spelling of the name of this rodent is apparently Dendromus not Dendromys (Simpson, 1945, Rosevear, 1969). Brumpt incorrectly called the host Dendromys (following Kleine, 1910), and the parasite H. dendromysi. This was an inadvertent error caused by the current misspelling of the rodent's name, and the spelling of the name of the parasite is here corrected (Article 32 a(ii) of the International Code of Zoological Nomenclature, 1964).

28. Hepatozoon graomysi Brumpt, 1946; Graomys medius (49)
Argentina (Romana, 1945).

Brumpt misspelt the name of the rodent Groamys, and named the parasite H. groamysi. In his publication there is clear evidence of this inadvertent error, and the spelling is here corrected (Article 32a(ii) of the International Code of Zoological Nomenclature, 1964).

29. Hepatozoon lavieri Brumpt, 1946; Microtus arvalis (59)
France (Lavie, 1921), Czechoslovakia (Erhardova, 1955;
Černa, 1957), Austria (Krampitz, 1964).

30. Hepatozoon sp. Latyshev, 1949; Meriones erythrourus (69)
Turkmenia, U.S.S.R.

31. Hepatozoon sp. Latyshev, 1949; Spermophilopsis leptodactylus (25) Turkmenia, U.S.S.R.

32. Hepatozoon sp. (Dubinin, 1953); Arvicola terrestris
(56) Volga Delta, U.S.S.R.

Dubinin (1953) thought this haemogregarine was perhaps H. arvalis, the parasite which Martoglio (1913) recorded from a rodent misidentified as Microtus arvalis. Krampitz's (1964) work suggests a host restriction of the haemogregarines of voles, and it is likely that Dubinin's parasite is new.

33. Hepatozoon sp. Erhardova, 1955; Microtus oeconomus (62)
Czechoslovakia (Erhardova, 1955), central and southern Europe (Krampitz, 1964).

34. Hepatozoon griseisciuri Clark, 1958; Sciurus carolinensis
(1) Maryland, U.S.A. (Herman and Price, 1954; Clark, 1958),
Virginia, U.S.A. (Parker, 1968), England (Vizoso, quoted
by Dasgupta and Meedeniya, 1958).

Clark (1958) found that the sporocysts of H. griseisciuri in the mites Euhaemogamasus ambulans and Echinolaelaps echidninus contained only 4 large sporozoites, and in this character the

haemogregarine of the grey squirrel differs from other known species. Dasgupta and Meedeniya (1958), however, described sporocysts of an Hepatozoon in the flea Orchopeas wickhami, from English squirrels, which contained 12 sporozoites. The species of squirrel from which the infected fleas were collected is not clearly stated, but since these workers examined fleas from both S. carolinensis and S. vulgaris, it is possible that their description is of the sporogonic stages of H. sciuri a parasite of the latter squirrel.

35. Hepatozoon leptosoma Wood, 1962; Peromyscus maniculatus (46) California, U.S.A.

The gametocytes of this parasite differed from those of other Californian rodents by their size ($10.5\mu \times 2.1\mu$) and slender shape.

36. Hepatozoon sp. Wood, 1962; Microtus californicus (60), California, U.S.A.

Wood (1962) tentatively identified this haemogregarine as H. citellicolum, a parasite of the gopher, Citellus beecheyi. These hosts are not closely related, and until the life-cycles of the parasites are compared the Hepatozoon of M. californicus is better left with a separate identity.

37. Hepatozoon sp. Wood, 1962; Peromyscus boylii (44) P. truei (47) California, U.S.A.

Wood (1962) thought this haemogregarine was probably H. muris, a parasite of Rattus spp. From the host restriction of the well studied haemogregarines of European rodents (Krampitz, 1964), it seems probable that Wood's parasite is new. Morphologically its gametocytes were different from those of the haemogregarines found in Peromyscus maniculatus and Microtus californicus in the same locality.

38. Hepatozoon sp. Keymer, 1964; Tatera leucogaster (67)

Luangwa valley, Zambia (see also Keymer, 1966).

Keymer suggests this haemogregarine may be H. gerbilli described from T. indica in India.

39. Hepatozoon erhardovae Krampitz, 1964; Clethrionomys glareolus (54) England (Jacobs, 1953; Baker et al., 1963), Sweden (Tadros, unpublished), Czechoslovakia (Erhardova, 1955; Černa, 1957), W. Germany (Krampitz, 1964); Clethrionomys rutilus (55) W. Germany (Krampitz, 1964).

40. Hepatozoon sp. Dasgupta, 1965; Petaurista magnificus (35) Darjeeling, India.

41. Hepatozoon sp. Keymer, 1966; Aethomys ?kaiseri (86) Chitala area, Malawi (Keymer, 1966), Uganda (Minter, unpublished).

42. *Hepatozoon sp. Keymer, 1966; Lophuromys flavopunctatus
 (102) Otomys kempi (107) Myika Plateau, Zambia.

Keymer (1966) found a single intra-erythrocytic haemogregarine in the blood of one Otomys, and a profuse schizogony in the myocardium, connective tissue, diaphragm, spleen and lungs of a second in the blood of which no gametocytes were found. Schizonts were also seen in the lungs and heart of a Lophuromys which once again had no parasites in the blood. The tissue forms may have been stages in the life-cycle of a parasite other than Hepatozoon.

43. Hepatozoon sp. Krampitz, Sachs, Schaller and Schindler,
 1968; Mastomys natalensis (95) Uganda.

44. Hepatozoon epsteini Kakabadze and Zasuchin, 1969;
 "grey rats" (94) Sukhumi, e. shore of Black Sea, U.S.S.R.

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

45. Hepatozoon sp. Killick-Kendrick and Bellier, 1971;
 Idiurus macrotis (42) Ivory Coast, West Cameroons.

46. Hepatozoon sp. Killick-Kendrick and Bellier, 1971;
 Idiurus macrotis (42) Ivory Coast.

This second, less common, haemogregarine of the pygmy flying squirrel has the longest gametocytes (27.5-38.5µm) of any

Hepatozoon of mammals.

47. Hepatozoon sp. Bruce-Chwatt and Gibson (unpublished);
 Hybomys trivirgatus (85) W. Nigeria.
48. Hepatozoon sp. Bruce-Chwatt and Gibson (unpublished);
 Praomys tullbergi (97) W. Nigeria.
49. Hepatozoon sp. Lainson (unpublished); Holochilus brasil-
 iensis (50) Brazil.
50. Hepatozoon sp. Lainson (unpublished); Oryzomys capito
 (43) Brazil.
51. Hepatozoon sp. Landau (unpublished); Hybomys univittatus
 (84) Central African Republic.
52. Hepatozoon sp. Landau (unpublished); Xerus rutilus (21)
 Ethiopia.
53. Hepatozoon sp. Markus (unpublished); Xerus inauris (22)
 S. Africa.

PLASMODIIDAE

Plasmodiidae contains the single genus of true malaria parasites, Plasmodium Marchiafava and Celli, 1885. The species infecting rodents form part of the subgenus Vinckeia Garnham, 1964. The two found in murine rodents of Africa are sub-divided into a number of subspecies based on differences in distribution and the morphology of the sporogonic and exoerythrocytic stages (Landau and Killick-Kendrick, 1966; Garnham et al., 1967; Bafort, 1968; Landau et al., 1968, 1970; Killick-Kendrick, in press).

54. Plasmodium berghei berghei Vincke and Lips, 1948;
Grammomys surdaster (78) (Vincke and Lips, 1948;
Vincke, 1954). Praomys jacksoni (96). Leggada bella
(101) (Vincke, 1954) Democratic Republic of the Congo.
55. Plasmodium berghei yoelii Landau and Killick-Kendrick, 1966;
Thamnomys rutilans (77) Central African Republic
(Landau and Chabaud, 1965).
56. Plasmodium berghei killicki Landau, Michel and Adam,
1968; Thamnomys rutilans (77) Brazzaville, People's
Republic of the Congo (Adam et al., 1966).

57. Plasmodium berghei (Nigeria) Killick-Kendrick (in preparation); Thamnomys rutilans (77) W. Nigeria.

58. Plasmodium vinckei vinckei Rodhain, 1952; ?Grammomys surdaster (78) 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 millecampsi (Rodhain, 1952; Bafort, 1967). Since A. d. millecampsi appears to feed exclusively on rodents (see Gillies 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 G. surdaster.

59. Plasmodium vinckei ssp. Bruce-Chwatt and Gibson, 1955; Praomys tullbergi (97) (Bruce-Chwatt and Gibson, 1955); Thamnomys rutilans (77) (Killick-Kendrick et al., 1968), 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 more than 1,000 Praomys was infected (Bruce-Chwatt, personal communication). This suggests either that the single infected Praomys was a misidentified Thamnomys, or that the Praomys had a very rare accidental infection of a parasite of Thamnomys.

60. Plasmodium vinckei chabaudi Landau, 1965; Thamnomys

rutilans (77) Central African 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).

61. Plasmodium vinckei lentum Landau, Michel, Adam and
Boulard, 1970; Thamnomys rutilans (77) Brazzaville,
People's Republic of the Congo (Adam et al., 1966).
62. Plasmodium atheruri van den Berghe, Peel, Chardome and
Lambrecht, 1958; Atherurus africanus (118) Republic
of Cameroon (Languillon, 1957), Brazzaville, People's
Republic of Congo (Landau et al., 1969a), Irangi Forest,
Democratic Republic of the Congo (van den Berghe et al.,
1958).
63. Plasmodium anomaluri Pringle, 1960; Anomalurus derbianus
(41) Chemka, Tanzania.
64. Plasmodium booliati Sandosham, Yap and Omar, 1965;
Petaurista petaurista (36) Malaya.
65. Plasmodium watteni Lien and Cross, 1968; Petaurista
petaurista grandis (37) Taiwan.

66. Plasmodium spp. Killick-Kendrick and Bellier, 1971;

Anomalurus peli (41A) A. derbianus (41) Ivory Coast

Two unnamed malaria parasites, both thought to be different from P. anomaluri, were found in A. peli.

HAEMOPROTEIDAE

Haemoproteidae includes the genera Hepatocystis Levaditi and Schoen, 1932 emend. Garnham, 1948, and Rayella Dasgupta, 1967.

Among rodents, Hepatocystis is found exclusively in some of the Asian squirrels. The commoner of the two species parasitizing these animals, H. vassali (Laveran, 1905), is divided into four subspecies based upon geographical distribution and minor morphological differences of the gametocytes (Garnham, 1966).

Rayella Dasgupta, 1967, was created to accommodate an Hepatocystis-like parasite of giant flying squirrels in parts of Asia. It differs from Hepatocystis by the morphology of the tissue stages which were described as small cystic schizonts occurring in groups in the liver. Hepatocystis is divided into species largely on the basis of the morphology of the asexual stages in the tissues (Garnham, 1966), and possibly the separation of Rayella solely on this character would not be generally accepted. Another difference, however, is the time necessary for the exflagellation of the microgametes of Rayella (8 mins) (Dasgupta and Chatterjee, 1969) which differs from the characteristically short time (2 mins) of Hepatocystis (Garnham, 1966, p. 21.).

67. Hepatocystis vassali vassali (Laveran, 1905);
Callosciurus flavimanus griseimanus (8) (Vassal, 1905,
 1907; Laveran, 1905b), C. vittatus (15) (Vassal, 1907),

- C. flavimanus ssp. (9) (van Peenen et al., 1968),
Callosciurus sp. (16) (Vassal, 1907) Vietnam; Callo-
sciurus findlaysoni (11) Tamias maclellandii kongensis
 (17) (Coatney et al., 1960) Thailand.
68. Hepatocystis vassali ratufae (Mulligan and Somerville,
 1947); Ratufa indica (5) India (Donovan, 1920; Mulligan
 and Somerville, 1947); R. bicolor gigantea (4) Thailand
 (Coatney et al., 1960); R. macroura (6) Ceylon (Dissanaike,
 1963); Ratufa spp. (7) Malaya (Wharton et al., 1963).
69. Hepatocystis vassali malayensis Field and Edeson, 1950;
Callosciurus vittatus miniatus (15A) C. nigrovittatus (14)
 (Green, 1931), C. caniceps ssp. (12) C. notatus (13A)
C. tenuis (13B) (Field and Edeson, 1949, 1950) Callosciurus
 sp. (16) (Wharton et al., 1963) Malaysia; C. prevosti
 (13) London Zoo (quoted by Coatney and Roudabush, 1949).

The record from the London Zoo is based on autopsy reports of squirrels. Since C. prevosti is a Malaysian species (Moore and Tate, 1965), I assume the subspecies of the parasite was malayensis. Coatney and Roudabush, 1949, list a second record from the London Zoo in which "Sciurus stramineus guayanus" was reported with an infection of a malaria parasite sensu lato. It seems certain the parasite was Hepatocystis sp., but the country of origin of the host and its correct name are now in doubt.

70. Hepatocystis vassali yokogawai Wu, 1953; Callosciurus flavimanus taiwanensis (10) (Wu, 1953; Manwell and Kuntz, 1966), Tamias maritimus maritimus (18) (Wu, 1953), Taiwan.

71. Hepatocystis sp. Dunn, Lim and Yap, 1968; Lariscus insignis (19) Malaysia.

Field and Edeson (1949) reported Hepatocystis in two specimens of L. insignis in Malaya, but this was later found to be a mistake (Field and Edeson, 1950). The rare infection reported by Dunn et al., (1968) may be H. v. malayensis in a new host. The same workers listed a burrowing myomorph, Rhizomys sumatrensis, as a host of Hepatocystis but this record is now thought to have been a clerical error (Garnham, personal communication).

72. Rayella rayi (Tokura and Wu, 1961); Petaurista inornatus (34) Mukteswar, India (Ray, 1949, 1960); P. p. grandis (37) Taiwan (Tokura, and Wu, 1961; Manwell and Kuntz, 1966; Lien and Cross, 1968); P. magnificus (63) Darjeeling, India (Dasgupta, 1965, 1967).

73. Rayella hylopetei Dasgupta, Pal, Chatterjee and Chatterjee, 1971; Hylopetes alboniger (38) Darjeeling, India.

BABESIIDAE

Babesiidae includes the piroplasms of rodents, which are here all rather arbitrarily placed in the genus Babesia Starcovici, 1893. It seems certain that more than one genus is represented, but the differential criteria are far from clear. Neitz (1956) listed ten synonyms of Babesia which were originally named because of differences in the size, shape and method of division of the form in the red-cell. Shortt (1962) pointed out that these morphological characters tended to overlap with one another, and Neitz, Shortt and others had no doubts that the group will have to be revised as soon as more life-cycles in the invertebrate host (ticks) become known. Neitz (1965) later suggested that the complement fixation test may prove to be a means of recognising species of Babesia, whereas Gourlay et al., (1970) believed that disc electrophoresis of soluble proteins would prove to be a useful aid in identification.

Russian workers (Cheissin, 1965; Krylov, 1965) place some piroplasms in the genus Nuttallia on the basis of division into 4 in the red-cell, and an absence of transovarial transmission in the tick; this view is gaining support (Nowell, 1969; McMillan and Brocklesby, 1971). Other piroplasms of rodents, however, appear to be closely related to Theileria, dividing as schizonts (Koch's blue bodies) in the internal organs (see Neitz, 1956). Examples are the parasites found by Tsur et al. (1960) in a gerbil of Israel

by van Peenen and Atalla (1963) in an Egyptian gerbil, and possibly one of the piroplasms described by Fay and Rausch (1969) from Alaskan voles. At the moment, it seems that the only way to achieve a semblance of order is to lump the piroplasms of rodents together in the genus Babesia, and await a revision of the whole group which will doubtless follow as more complete life-cycles become known. Levine (1971) synonymizes a number of piroplasms of rodents, and suggests that the number of true species may be very small indeed. In the present work the names have largely been retained, because it is feared that a sweeping synonymy in the absence of knowledge of life-cycles may lead to good species being doubted, or even being later overlooked.

The confusion extends to the higher taxa. In the present work, Piroplasma is considered as a class of the Sporozoa because of observations on the fine structure of piroplasms, which are now known to have organelles typical of the subphylum (see Levine, 1971), Earlier some workers (e.g. Cheissin, 1965) suggested piroplasms should be considered as blood-inhabiting amoebae and be classified in the superclass Sarcodina of the subphylum Sarcomastigophora, but this notion has now lost support.

In light infections in rodents, piroplasms are easily mistaken for malaria parasites. Both inhabit erythrocytes, and young rings of Plasmodium are similar to Babesia. The true schizogony of Plasmodium in the blood is, however, thought to be absent in Babesia, and although pigment is said to be produced by at least one piroplasm

(Molyneux and Bafort, 1970), it is not visible, as in malaria parasites, with the light microscope.

74. Babesia muris (Fantham, 1905); Rattus norvegicus (89)
England.

This parasite was seen in three laboratory white rats. Possibly the infection was acquired from ticks in the bedding of the cage, and the parasite may have been Babesia microti, the common piroplasm of British small mammals. If so, the name muris has priority over microti.

75. Babesia quadrigemina (Nicolle, 1907); Ctenodactylus gundi (39) Tunisia.

76. Babesia avicularis Wenyon, 1909; Lemniscomys barbarus (82) Sudan.

77. Babesia microti (França, 1912); Microtus arvalis (59) Portugal (França, 1912); Arvicola terrestris (56) (Coles, 1914), Microtus agrestis (58) (Coles, 1914, Baker et al., 1963; Shortt and Blackie, 1965), Apodemus sylvaticus (76) (Coles 1914; Jacobs, 1953; Shortt and Blackie, 1965), Micromys minutus (74) (Young, quoted by Cox, 1970), Clethrionomys glareolus (54) (Jacobs, 1953; Shortt and Blackie, 1965), England; Microtus pennsylvanicus (63)

Mass., U.S.A. (Tyzzer, 1938), New York, U.S.A. (Kirner, et al., 1958); Microtus californicus (60) Peromyscus californicus (45) Neotoma lepida (51) California, U.S.A. (van Peenen and Duncan, 1968).

In a laboratory study on the course of infection in laboratory rodents of strains of this piroplasm from several English hosts, Shortt and Blackie (1965) found no differences in infectivity or morphology. This suggests that piroplasms of small rodents in England, and possibly other parts of Europe, are represented by one species, B. microti. For reasons which are not clear, the name B. colesi Levine, 1971 was proposed for the piroplasm of A. sylvaticus in England. It was first named Nuttallia muris Coles, 1914 and later moved to Babesia by Reichenow (1953). Levine saw that Babesia muris was a homonym of Fantham's (1905) piroplasm of the white rat and gave the new name to the parasite of Apodemus. I view B. colesi as a synonym of B. microti.

78. Babesia decumani (Macfie, 1915); Rattus norvegicus (89) Ghana (Macfie, 1915), Vietnam (Capponi et al., 1955); R. bowersi (91) R. sabanus (92) R. musschenbroeki (93) Malaysia (Yap Loy Fong et al., 1963).

Capponi et al. (1955) did not identify the piroplasm they found in Vietnam. Largely for convenience, and because their parasite appears morphologically indistinguishable from Macfie's, and was in the same host, the record is included here. B. decumani may be a synonym of B. muris (Fantham, 1905).

79. Babesia sp. Bruce, Harvey, Hamerton, Davey and Bruce,
1915; "edible rat", (see 119) Malawi.
80. Babesia rossica (Yakimoff and Saphronowitsch, 1917); voles,
(64) 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). Zasuchin (1956) assigned it to Gonderia.

81. Babesia rigolleti (Léger and Bédier, 1922); Graphiurus murinus (117) Senegal.

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

82. Babesia golundae (Léger and Bédier, 1923); Myliomys lowei (79) Republic of Senegal.
83. Babesia myoxi (Franchini, 1924); Muscardinus avellanarius (115) Italy.
84. Babesia sp. Nauck, 1927; Sciurus vulgaris (2) China.
85. Babesia kolzovi (Zasuchin, 1930); Citellus pygmaeus (30) S. Vostok, U.S.S.R. (Zasuchin, 1930, 1931);

Citellus fulvus (27) U.S.S.R. (Zasuchin, 1936).

86. Babesia eliomysi (Galli-Valerio, 1930); Eliomys quercinus (115A)
87. Babesia ratti Schwetz and Collart, 1930; Arvicanthis niloticus (81) Mastomys natalensis (95) Democratic Republic of the Congo.

Schwetz and Collart thought this parasite was possibly the same as B. avicularis Wenyon, and B. decumani Macfie, 1915. Levine (1971) listed the parasite only of Arvicanthis as a synonym of B. avicularis.

88. Babesia citelli Becker and Roudabush, 1933; Citellus tridecemlineatus (31) Iowa, U.S.A.
89. Babesia gerbilli (Tivlov, 1934); Rhombomys opimus (72) S. Vostok, U.S.S.R. (Zasuchin et al., 1934).
90. Babesia volgense (Zasuchin, 1936); Citellus fulvus (27) Kazakstan, U.S.S.R.

A redescription of this parasite is given by Zasuchin (1956).

91. Babesia cricetuli (Sprinholz-Schmidt, 1937); Cricetulus furunculus (53).

92. Babesia jakimovi (Sprinholz-Schmidt, 1937); Tamias
sibiricus asiaticus (33) U.S.S.R.

 93. Babesia tucotucui Carini, 1941; Ctenomys sp. (117A)
Brazil.

 94. Babesia wrighti Tomlinson, Smith and Fogg, 1948;
Citellus variegatus buckleyi (32) Texas, U.S.A.

 95. Babesia lemniscomysi (Rousselot, 1949); Lemniscomys
striatus (83)

 96. Babesia rhombomys (Celischtschev, 1950); Rhombomys
opimus (72) Kazakstan, U.S.S.R.

 97. Babesia rodhaini van den Berghe, Vincke, Chardôme and
van den Bulcke, 1950. Grammomys surdaster (78) Demo-
cratic Republic of the Congo.
- The type locality of B. rodhaini is Sungu on the River Lufufui,
40 km. from Kamina (van den Berghe, et al., 1950). Strains from
another locality, the River Kasapa, near Lumbumbashi (Elisabethville),
were morphologically the same, but differed in infectivity to experi-
mental rodents (Rodhain and Vincke, 1952).
98. Babesia graingeri Heisch, 1952; Euxerus erythropus

fulvior (24) Kenya.

99. Babesia taterilli (Rousselot, 1953); Taterillus gracilis (68) Republic of Mali (Rousselot, 1953), Republic of Haute Volta (Landau, unpublished).

100. Babesia merionis (Rousselot, 1953); Meriones tristrami (70) Iran.

Intact and splenectomized white rats, splenectomized mice and intact hamsters were refractory to infection by the inoculation of blood.

101. Babesia sp. Bruce-Chwatt and Gibson, 1955; Praomys tullbergi (97) W. Nigeria.

102. Babesia sp. Bruce-Chwatt and Gibson, 1955; Thryonomys swinderianus (119) W. Nigeria.

According to Bray (1964), the piroplasm in Nigerian Thryonomys is different from the one found in the same host in Liberia.

103. Babesia dani (Tsur, Hadani and Pipano, 1960); Meriones tristrami (70) Israel.

Feldman-Muhsam (1962) had already named this parasite Nuttalia adleri in a paper presented at an international conference on entomology in Vienna, but before the proceedings of the conference

were published, Tsur et al. (1960) had given the name N. danii which has priority.

This piroplasm, first seen by Adler (1930), should probably be assigned to the genus Theileria (see Neitz, 1956). Tsur et al. (1960) described "schizont-like" division stages, resembling Koch's blue bodies, in smears of internal organs, as well as divisions stages in the blood. Barnett and Brocklesby (1969) considered it to be a link between the Babesiidae and Theileriidae.

104. Babesia sp. van Peenen and Atalla, 1963; Meriones lybicus lybicus (69A) Egypt.

As with B. danii, division stages of this parasite similar to Koch's blue bodies of Theileria spp. were seen in tissue smears of experimentally infected Meriones; they were found in smears of the kidney 18 days after inoculation. Van Peenen and Atalla (1963) suggest that this piroplasm was B. danii adapted to a different host.

105. Babesia sp. van Peenen and Atalla, 1963; Gerbillus campestris (64A) Egypt.

Although this piroplasm looked like the one of M. lybicus from the same locality, Meriones was found to be refractory to infection. This report of two biologically distinct piroplasms of related hosts in a single locality, coupled with the records of B. danii (103) of M. tristrami in Israel, B. tadzhikstanica (106)

of M. erythrourus (99) in the U.S.S.R. and B. taterilli (99) of T. gracilis in Mali, suggests that there is a closely related complex of species or subspecies of piroplasms in gerbillines. They warrant a comparative study.

106. Babesia tadzhikstanica (Krylov and Zanina, 1963); Meriones erythrourus (69) Tadzhikstan, U.S.S.R.

This parasite was first named Smithia tadzhikstanica, but Russian workers now prefer to place it in the genus Nuttalia which in their view differs from Babesia, Smithia etc. by the absence of transovarial transmission in the arthropod host (Cheissin, 1965; Krylov, 1965). The development of B. tadzhikstanica in the tick Hyalomma anatolicum has been described in detail by Krylov (1965).

Barnett and Brocklesby (1969) suggested that this piroplasm of a Russian gerbil was probably the same as Babesia danii (89) a parasite of Meriones tristrami in Israel.

107. Babesia bandicootia Manwell and Kuntz, 1964;
Bandicota indica nemorivaga (104)

108. Babesia sp. Bray, 1964; Thryonomys swinderianus (119)
Liberia.

109. Babesia sp. Gunders (Bray, 1964); Praomys tullbergi

(97) Liberia.

110. Babesia musculi (Muratov, 1966); Mus musculus (100)
Tadzhikistan, U.S.S.R.

Muratov (1966) compared the morphology of B. musculi with that of B. tadzikstanica, a parasite of jirds in the same locality, and found they were different; moreover, although mice were susceptible to the parasite from jirds, B. musculi failed to infect Meriones. Unfortunately, because he was unable to compare it with some other piroplasms of rodents, Muratov named the piroplasm of mice conditionally; by Article 15 of the International Code of Zoological Nomenclature (1964) the name musculi is not, therefore, available, and Muratov's parasite, if it is considered to be a good species, will have to be re-named.

111. Babesia sp. van Peenen and Duncan, 1968; Citellus beecheyi (26) California, U.S.A.

112. Babesia spp. Fay and Rausch, 1969; Microtus oeconomus (62) M. pennsylvanicus (63) Clethrionomys rutilus (55)
Alaska, U.S.A.

Fay and Rausch found two quite different piroplasms in Alaskan voles. One, from C. rutilus, had typical division stages in erythrocytes but the other, from M. oeconomus, appeared seldom, if ever, to divide in red-cells

and was not infective to C. rutilus. Cyclical transmission of the latter parasite to M. oeconomus was obtained in the laboratory through the tick Ixodes angustus.

113. Babesia hylomysci Bafort, Timperman and Molyneux, 1970;
 Hylomyscus stella (99) Kinshasa, Katanga, Democratic
 Republic of the Congo.

114. Babesia meri (Gunders, in press); Psammomys obesus
 (71) Israel.

115. Babesia sp. (Gunders and Sarfatti, 1971); Nesokia indica
 (105) Israel.

116. Babesia sp. Adam and Landau (unpublished); Praomys
 jacksoni (96) Brazzaville, Republic of Congo.

117. Babesia, sp. Adam and Landau (unpublished); Thryonomys
 swinderianus (119) Brazzaville, Republic of Congo.

118. Babesia sp. Bruce-Chwatt and Gibson (unpublished);
 Cricetomys gambianus (73) Agege, W. Nigeria.

119. Babesia sp. Bruce-Chwatt and Gibson (unpublished);
 Stochomys longicaudatus (98) Agege, W. Nigeria.

120. Babesia sp. Landau (unpublished); Hybomys univittatus
 (84) Central African Republic.
- 121 Babesia sp. Landau (unpublished); Praomys jacksoni (96)
 Central African Republic.

The unnamed piroplasms of Praomys in Nigeria (101), Liberia (109), Brazzaville (116), and the Central African Republic may be the same.

DACTYLOSOMIDAE

Anthemosoma was 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, dimorphic forms thought to be gametocytes were found, and a resemblance to the dactylosomes of fishes and amphibia was recognised. The new genus, Anthemosoma Landau, Boulard and Houin, 1969, was therefore placed in the family Dactylosomidae (Piroplasmae: Piroplasmida). Levine, (1971) felt this position was wrong and that Anthemosoma was perhaps closer to the malaria parasites than the piroplasms. From the fine-structure, however, Anthemosoma seems to resemble

Babesia (Vivier and Petitprez, 1969), and it is perhaps best to leave Anthemosoma in Dactylosomidae until its life-cycle is known.

122. Anthemosoma garnhami Landau, Boulard and Houin,
1969; Acomys percivali (103) Omo Valley, Ethiopia.

INDEX OF RODENTS WHICH ARE KNOWN HOSTS OF BLOOD-INHABITING 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. As in the check-list, an asterisk before the name of a haemogregarine indicates the host-cell is an erythrocyte.

SCIUROMORPHA

Fam. Sciuridae (squirrels)

Subfam. Sciurinae (ground and tree squirrels)

Tribe Sciurini (European and American tree squirrels)

1. Sciurus carolinensis (grey squirrel)

Hepatozoon griseisciuri (34)

2. Sciurus vulgaris (red squirrel)

Hepatozoon sciuri (14)

Babesia sp. (84)

Tribe Funambulini (African tree squirrels and allied

Asian genera).

3. Funambulus pennanti (palm squirrel or 5-striped Indian squirrel)

Hepatozoon funambuli (4)

Tribe Ratufini

4. Ratufa bicolor gigantea (giant squirrel)

Hepatocystis vassali ratufae (68)

5. Ratufa indica (Indian giant squirrel or Malabar squirrel)

Hepatocystis vassali ratufae (68)

6. Ratufa macroura (grizzled giant squirrel)

Hepatocystis vassali ratufae (68)

7. Ratufa spp.

Hepatocystis vassali ratufae (68)

This record is from Malaya where Medway (1969) lists only two species of Ratufa, R. bicolor and R. affinis, each with several sub-species.

Tribe Callosciurini (Oriental tree squirrels)

Moore and Tate's (1965) detailed study of museum material of squirrels of the Indo-Chinese sub-region gives by far the best guide to this difficult tribe, and although the composition of their species differs from earlier concepts, in their comprehensive work they group the species in a logical way producing order from chaos.

The largest genus of the tribe is Callosciurus, in which Ellerman (1940) recognises 320 forms. For the Indo-chinese subregion, Moore and Tate list 59, and for the Malaysian sub-region Medway (1969) gives 26. The range of the genus does not include the Indian sub-region, and Callosciurus is not found west of the Garo-Rajmahal Gap. In the Indo-chinese and Malaysian subregions, the great rivers form the boundaries of the ranges of many of the subspecies.

According to Moore and Tate, in the Indo-chinese subregion there

are 8 species with one artemis of 4 allopatric species (erythraeus, ferrugineus, flavimanus and findlaysoni) spread throughout the region, and a second (pygerythrus, phayrei, caniceps and inornatus) which is less widespread and largely sympatric with the first.

For the Malaysian subregion, Moore and Tate list only 5 species, prevosti, notatus, nigrovittatus, albescens and melanogaster. Medway gives three of these (two spelt differently), prevostii, notatus and nigrovittatus, but also lists flavimanus and caniceps which Moore and Tate recognise in the Indo-chinese but not in the Malaysian subregion. This lack of agreement, coupled with a very large number of subspecific names, makes it impossible confidently to assign some old records of Hepatocystis in Asian squirrels to hosts with current modern names.

8. Callosciurus flavimanus griseimanus

Hepatocystis vassali vassali (67)

Laveran (1950b) and Vassal (1905, 1907) called this squirrel Sciurus griseimanus, now a synonym of C. f. griseimanus (Moore and Tate, 1965; Ellerman and Morrison-Scott, 1966).

9. Callosciurus flavimanus ssp.

Hepatocystis vassali vassali (67)

10. Callosciurus flavimanus taiwanensis

Hepatocystis vassali yokogawai (70)

Moore and Tate (1965) recognise 19 subspecies of C. flavimanus (the belly-banded squirrel) distributed throughout the Indo-chinese subregion east of the Sittang and Irrawaddy rivers, except Thailand,

Cambodia and part of Laos (along the Mekong river) where C. findlaysoni occurs.

Wu (1953) and Manwell and Kuntz (1966) record Hepatocystis in several squirrels in Taiwan which they identified as C. erythraeus and subspecies roberti, centralis and thaiwanensis. Moore and Tate, however, consider the range of C. erythraeus to be limited to west of the Irrawaddy and its tributary the river Nmai. They view thaiwanensis as a subspecies of flavimanus, and list centralis and roberti as synonyms of subspecies thaiwanensis. Unlike Ellerman and Morrison-Scott (1966), Moore and Tate do not support the division of the species in Taiwan into subspecies.

11. Callosciurus finlaysoni ssp. (Siamese squirrel)

Hepatocystis vassali vassali (67)

Although Moore and Tate (1965) accept C. finlaysoni as a good species, they do so only provisionally, and with some serious reservations. They list 15 subspecies distributed in Thailand, Cambodia and along the Mekong river in Laos. The record above is from Thailand, and the squirrel was thought to be close to the subspecies boucourti (Coatney et al., 1960).

12. Callosciurus caniceps ssp. (grey-bellied squirrel)

Hepatocystis vassali malayensis (69)

13. Callosciurus prevosti

Hepatocystis vassali malayensis (69)

13A Callosciurus notatus

Hepatocystis vassali malayensis (69)

13B Callosciurus tenuisHepatocystis vassali malayensis (69)14. Callosciurus nigrovittatus (black-striped squirrel)Hepatocystis vassali malayensis (69)

Moore and Tate give the name of this Malaysian squirrel as shown, and not C. nigrovittatus as given by others (Field and Edeson, 1950; Harrison and Traub, 1950; Medway, 1969).

15. Callosciurus vittatusHepatocystis vassali vassali (67)

This record, from Indo-China, is from a rodent not listed for the subregion by Moore and Tate; it is not possible now to be sure of the host's identity.

15A Callosciurus vittatus mineatusHepatocystis vassali malayensis (69)16. Callosciurus sp.Hepatocystis vassali vassali (67)Hepatocystis vassali malayensis (69)

In the first of these records (Vassal, 1907) the host was called Sciurus sp. The report was from Annam (Vietnam), and the animals examined would now be known as Callosciurus.

17. Tamias maclellandii kongensis (striped tree squirrel)Hepatocystis vassali vassali (67)18. Tamias maritimus maritimusHepatocystis vassali yokogawai (70)

In this report (Wu, 1953) the host was given as "Tamias

macellandi formosanus". This is presumably the same as Sciurus maclellandi formosanus, listed by Moore and Tate (1965) as a synonym of T. m. maritimus.

19. Lariscus insignis (striped ground squirrel)

Hepatocystis sp. (71)

20. Atlantoxerus getulus (Barbary ground squirrel)

Hepatozoon getulum (18)

21. Xerus rutilus

Hepatozoon sp. (52)

22. Xerus inauris

Hepatozoon sp. (53)

23. Euxerus erythropus (red-legged ground squirrel)

Hepatozoon sp. (19)

24. Euxerus erythropus fulvior

Babesia graingeri (98)

Unlike a number of early workers, Rosevear (1969) considers Euxerus to be separate from Xerus; he believes fulvior to be possibly a valid race of X. microdon, a synonym of Euxerus erythropus.

Tribe Xerini incertae sedis

25. Spermophilopsis leptodactylus (long-tailed ground squirrel)

Hepatozoon sp. (31)

Tribe Marmotini (northern ground squirrels)

26. Citellus beecheyi (gopher)

Hepatozoon citellicolum (6)

Babesia sp. (111)

27. Citellus fulvus (large toothed suslik)

Babesia kolzovi (85)

Babesia volgense (90)

28. Citellus guttatus (ground squirrel)

Hepatozoon mereschkowskii (9)

29. Citellus musicus (ground squirrel)

Hepatozoon mereschkowskii (9)

30. Citellus pygmaeus (little suslik)

Babesia kolzovi (85)

31. Citellus tridecemlineatus

Babesia citelli (88)

32. Citellus variegatus buckleyi (black-backed rock-squirrel)

Babesia wrighti (94)

33. Tamias sibiricus asiaticus (Siberian chipmunk)

Babesia jakimovi (92)

Zasuchin (1956) listed the host as Eutamias asiaticus
orientalis. Ellerman and Morrison-Scott (1966) give Eutamias
 as a subgenus of Tamias. They consider only one species (T.
sibiricus) to be present in the U.S.S.R. and from their synonymy
 the host is assumed to be the subspecies asiaticus.

Subfam. Petauristinae (flying squirrels)

34. Petaurista inornatus (flying squirrel)

Rayella rayi (72)

35. Petaurista magnificus (Himalayan flying squirrel)

Hepatozoon sp. (40)

36. Petaurista petaurista (giant flying squirrel)

Hepatozoon sp. (20)

Plasmodium booliati (64)

37. Petaurista petaurista grandis

Plasmodium watteni (65)

Rayella rayi (72)

38. Hylopetes alboniger (particoloured flying squirrel)

Rayella hylopetei (73)

SCIUROMORPHA incertae sedis

Fam. Ctenodactylidae (gundis)

39. Ctenodactylus gundi (gundi)

Babesia quadrigemina (75)

40. Pectinator spekei

Hepatozoon plicatum (11)

The host of H. plicatum in the Somali Republic was identified as a marmot (Arctomys (= Marmota) marmota), a rodent which is absent from Africa. Brumpt (1946) suggested that the host was perhaps a hyrax mistaken for a rodent, a plausible idea since some Italians in Africa call the hyrax "marmotta". But Rosevear (personal communication) points out that, in spite of its smaller size the animal most likely to have been confused

with a marmot is the gundi Pectinator. This ctenodactylid is known only from the Somali peninsular (Ellerman, 1940) and, like the marmot, lives in companies in rocky terrain. Only the re-discovery of the parasite will settle which animal was the host.

THERIDOMYOMORPHA

Fam. Anomaluridae (African scaly-tailed flying squirrels)

Subfam. Anomalurinae

41. Anomalurus derbianus

Plasmodium anomaluri (63)

Plasmodium sp. (66)

For more than a century, this animal was known as A. fraseri; the neglected name derbianus was then shown by Ellerman et al., (1953) to have priority over fraseri (see Rosevear, 1969).

41A Anomalurus peli

Plasmodium spp. (66)

Subfam. Zenkerellinae

42. Idiurus macrotis (pygmy flying squirrel)

Hepatozoon sp. (45)

Hepatozoon sp. (46)

MYOMORPHA

Fam. Cricetidae

Subfam. Cricetinae

Tribe Hespermyini (New World mice)

43. Oryzomys capito (rice rat)
Hepatozoon sp. (50)
44. Peromyscus boylii (Boyle's white-footed mouse)
Hepatozoon sp. (37)
45. Peromyscus californicus (deer mouse)
Babesia microti (77)
46. Peromyscus maniculatus (Gambel's white-footed mouse)
Hepatozoon leptosoma (35)
47. Peromyscus truei (Gilbert's white-footed mouse)
Hepatozoon sp. (37)
48. Akodon fuliginosus (vole mouse)
Hepatozoon akodoni (16)
49. Graomys medius
Hepatozoon graomysi (28)
50. Holochilus brasiliensis
Hepatozoon sp. (49)
51. Neotoma lepida
Babesia microti (77)

Tribe Cricetini (hamsters)

52. Cricetus cricetus
Hepatozoon criceti (8)

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

53. Cricetulus furunculusBabesia cricetuli (91)

Tribe Microtini (voles)

54. Clethrionomys glareolus (bank vole)Hepatozoon erhardovae (39)Babesia microti (77)55. Clethrionomys rutilusHepatozoon erhardovae (39)Babesia sp. (112)

The haemogregarine of this vole was reported from Germany, a country not listed by Ellerman and Morrison-Scott (1966) in the distribution of C. rutilus; possibly the range of the rodent is wider than supposed.

56. Arvicola terrestris (water vole)Hepatozoon sp. (32)Babesia microti (77)57. Pitymys savii (pine mouse)Hepatozoon pitymysi (17)58. Microtus agrestis (short-tailed field vole)Hepatozoon microti (13)Babesia microti (77)59. Microtus arvalisHepatozoon lavieri (29)Babesia microti (77)

Another species of Hepatozoon, H. arvalis (10), was reported from an Ethiopian rodent wrongly identified as M. arvalis (Martoglio, 1913). The true identify of the host will never now be known with certainty, but it was possibly Arvicanthis niloticus (Brumpt, 1946).

The piroplasm was recorded from M. incertus from Portugal. This rodent is now considered to be a subspecies of M. arvalis restricted to Switzerland (Ellerman and Morrison-Scott, 1966); since the true range of H. lavieri is unknown, the report is listed here with the subspecies of the host undetermined.

60. Microtus californicus

Hepatozoon sp. (36)

Babesia microti (77)

61. Microtus montebelli

Hepatozoon arvicolae (25)

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 mention of the specific epithet hatengumi appears in Ellerman's comprehensive lists. It seems likely that the host was M. montebelli, the only Japanese field vole Ellerman gives.

62. Microtus oeconomus (root vole or northern vole)

Hepatozoon sp. (33)

? Babesia sp. (112)

63. Microtus pennsylvanicus

Hepatozoon sp. (23)

Babesia microti (77)

Babesia sp. (112)

64.? Microtus sp.

Hepatozoon microti (13)

Babesia rossica (80)

The hosts were described as voles (Yakimoff and Saphronowitsch, 1917; Dubinin, 1953).

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

64A Gerbillus campestris

Babesia sp. (105)

65. Tatera indica (Indian gerbil)

*Hepatozoon gerbilli (3)

Gerbillus indicus, the host in the original report (Christophers, 1905) is now known as Tatera indica.

66. Tatera indica ceylonica

*Hepatozoon gerbilli (3)

67. Tatera leucogaster

*Hepatozoon sp. (38)

Keymer (1964, 1966) called the host Tatera afra, but the record is from Zambia, which is outside the range of this species (Davis, 1966). Dr G.B. Corbet of the British Museum (Natural History) kindly re-examined Keymer's deposited specimens, and identified them as T. leucogaster.

68. Taterillus gracilis (bushy-tailed gerbil)Babesia taterilli (99)

Rousselot (1953) identified the type host of this parasite from Mali 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).

69. Meriones erythrourus (jird)Hepatozoon sp. (30)Babesia tadzhikstanica (106)

Latyshev (1949) called the host of the haemogregarine 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; possible erythrourus and libycus are distinct, although it has been suggested that erythrourus and its races may represent subspecies of libycus (Ellerman, 1940).

69A. Meriones lybicus lybicusBabesia sp. (104)70. Meriones tristramiBabesia merionis (100)Babesia dani (103)71. Psammomys obesusBabesia meri (114)

72. Rhombomys opimus (great gerbil)*Hepatozoon gerbilli (3)Babesia gerbilli (89)Babesia rhombomys (96)

Subfam. Cricetomyinae

Petter (1964) removed Cricetomys from the Muridae and created this new sub-family to accommodate the Gambian pouched rat.

73. Cricetomys gambianus (giant rat or Gambian pouched rat)*Hepatozoon cricetomysi (26)Babesia sp. (118)

Fam. Muridae

Subfam. Murinae (Old World rats and mice)

74. Micromys minutus (harvest mouse)Babesia microti (77)75. Apodemus flavicollis (yellow-necked mouse)Hepatozoon sylvatici (15)76. Apodemus sylvaticus (long-tailed field mouse)Hepatozoon sylvatici (15)Babesia microti (77)77. Thamnomys rutilans (shining thicket-rat)

Plasmodium berghei yoelii (55)

P. b. killicki (56)

P. berghei (Nigeria) (57)

P. vinckei chabaudi (60)

P. v. lentum (61)

P. vinckei ssp. (59)

78. Grammomys surdaster (thicket-rat)

Plasmodium berghei berghei (54)

?P. vinckei vinckei (58)

Babesia rodhaini (97)

The systematics of the thicket-rats present a number of problems which some workers solve by lumping them together in the single genus Thamnomys, sometimes divided into subgenera (e.g. Davis, 1965). Rosevear (1969), however, suggests that 3 or 4 closely related genera of thicket-rats may eventually be recognised. He views the genus Grammomys as separate from Thamnomys on the basis of (i) the absence of "gutter-hairs" found, for example, in the pelage of Thamnomys rutilans, (ii) "indisputable differences" in cuspidation and (iii) differences in the crania.

79. Mylomys lowei (western 3-toed grass rat)

Babesia golundae (82)

The host of B. golundae from Senegal was given as Golunda campanae. This genus is Indian (Ellerman and Morrison-Scott, 1966). It seems most likely that the infected rodent was an African groove-toothed swamp rat, and was probably confused with Pelomys

campanae. Although modern authorities are not in agreement, Pelomys is generally considered to be absent from West Africa where closely related forms are now placed in Mylomys represented in that part of the continent by the single species M. lowei (see Rosevear, 1969). This is, therefore, the probable identity of the host of B. golundae. An objection to this identification is the lack of reports of Mylomys in Senegal, but determined trapping in the neighbouring Ivory Coast has recently shown M. lowei to be common in that country (Rosevear, 1969), and it is likely that Mylomys occurs in Senegal.

80. Mylomys dybowski (eastern 3-toed grass rat)

Hepatozoon sp. (7)

The host of this parasite was called Pseudomys cunninghami. This is an Australasian genus (Simpson, 1945), and the rodent was probably Mylomys cuninghamei, now considered by Rosevear (1969) to be a synonym of M. dybowski.

81. Arvicanthis niloticus (harsh-furred rat)

Hepatozoon arvicanthis (21)

Babesia ratti (87)

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

Martoglio's (1913) record of Hepatozoon arvalis (10) in a

rodent in Ethiopia, mistaken for Microtus arvalis, was possibly from a misidentified Arvicanthis (Brumpt, 1946).

82. Lemniscomys barbarus

Babesia avicularis (76)

Wenyon (1909) reported B. avicularis from a Sudanese rodent "Avicularis zebrae", later corrected to Arvicanthis zebra (Wenyon, 1926). Ellerman (1941) does not list zebra as a form of Arvicanthis, and Wenyon's rodent was probably Lemniscomys barbarus zebra. Lemniscomys is closely allied to Arvicanthis, and their separate identities were not recognized 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.

83. Lemniscomys striatus

Babesia lemniscomysi (95)

84. Hybomys univittatus (one-striped rat)

Hepatozoon sp. (51)

Babesia sp. (120)

85. Hybomys trivirgatus (three-striped rat)

Hepatozoon sp. (47)

86. Aethomys ?kaiseri

Hepatozoon sp. (41)

There are about 16 forms of A. kaiseri among which there are possibly two species; they are not yet sorted out satisfactorily (Davis, 1965).

87. Rattus assimilis (allied rat)
 Hepatozoon muris (2)
88. Rattus conatus (dusky field rat)
 Hepatozoon muris (2)
89. Rattus norvegicus (brown rat; laboratory rat)
 Hepatozoon muris (2)
 Babesia decumani (78)
 Babesia muris (74)
90. Rattus rattus (black rat)
 Hepatozoon muris (2)
91. Rattus bowersi
 Babesia decumani (78)
92. Rattus sabanus (giant long-tailed rat)
 Babesia decumani (78)
93. Rattus musschenbroeki (little spiny rat)
 Babesia decumani (78)
94. Rattus sp. ("grey rats")
 Hepatozoon epsteini (44)
95. Mastomys natalensis (multi-mammate rat)
 Babesia ratti (87)
 Hepatozoon sp. (43)

The identification of this rodent presents a number of problems. The host of the piroplasm was given as Mastomys ugandae. This was a subspecies of M. coucha (Ellerman, 1941) a rodent which is now known as either Mastomys natalensis (e.g. Rosevear, 1969) or

Praomys natalensis (e.g. Davis, 1965).

96. Praomys jacksoni

Plasmodium berghei berghei (54)

Babesia sp. (116, 121)

97. Praomys tullbergi

Hepatozoon sp. (48)

Plasmodium vinckei ssp. (59)

Babesia sp. (101, 109)

Praomys provides some of the most difficult problems of taxonomy of any of the African Muridae, rivalled only by Mastomys. Rosevear (1969) gives an up-to-date account of the difficulties from which it may be concluded that Praomys and Mastomys are likely to undergo extensive revision as new taxonomic procedures evolve.

98. Stochomys longicaudatus

Babesia sp. (119)

99. Hylomyscus stella

Babesia hylomysci (113)

100. Mus musculus (house mouse)

Hepatozoon musculi (5)

Babesia musculi (110)

An unnamed piroplasm was recorded by Bruce et al. (1911) in a white laboratory mouse (Mus musculus) in unusual circumstances. Mice were being used for the maintenance of a strain of spirochaete isolated from the blood of a bushbuck. Seven days after an inoculation of infected mouse blood, a recipient

mouse was found to be infected with the spirochaete and a piroplasm. The piroplasm was seen on four successive days and then disappeared. Possibly it was a parasite of the bushbuck and the concomitant infection with the spirochaete may have enabled it to gain a temporary foothold in an abnormal host.

101. Leggada bella (pygmy mouse)

Plasmodium berghei berghei

102. Lophuromys flavopunctatus

Hepatozoon sp. (42)

103. Acomys percivali (spiny mouse)

Anthemosoma garnhami (122)

104. Bandicota indica nemorivaga (Indian bandicoot rat)

Babesia bandicootia (107)

105. Nesokia indica

Babesia sp. (115)

Subfam. Dendromurinae (African tree mice)

106. Dendromus ?insignis

*Hepatozoon dendromus (27)

Subfam. Otomyinae

107. Otomys kemp

*Hepatozoon sp. (42)

Fam. Dipodidae (jerboas)

Subfam. Dipodinae

A record by Rodhain et al. (1913) of Hepatozoon (12) in the erythrocytes of a dipodine ("Jaculus ?johnstoni") from Sankisia in the Democratic Republic of the Congo must have been from a wrongly identified host. The distribution of Jaculus does not extend below the Sahara desert (Ognev, 1948), and it is difficult now to be sure which was the rodent examined. It might have been a gerbil mistaken for a jerboa. Unlike the haemogregarines of almost all other myomorphs, those of both jerboas and gerbils are in erythrocytes, not leucocytes.

108. Dipus sagitta (rough-legged or 3-toed jerboa)

*Hepatozoon alactaguli (24)

Dipus sowerbyi, the host given in the report by Zasuchin (1936), is listed by Ellerman and Morrison-Scott (1966) as a subspecies of D. sagitta.

109. Jaculus gordonii

*Hepatozoon balfouri (1)

110. Jaculus orientalis (greater Egyptian jerboa)

*Hepatozoon balfouri (1)

111. Jaculus jaculus (lesser Egyptian jerboa)

*Hepatozoon balfouri (1)

112. Allactaga major (great jerboa)

*Hepatozoon alactaguli (24)

113. Allactaga sibirica (5-toed jerboa)

*Hepatozoon alactaguli (24)

Jettmar (1932) called the host Alactagulus mongolica, which Ellerman and Morrison-Scott (1966) give as a synonym of Allactaga sibirica.

114. Alactagulus pumilio (little earth hare)

*Hepatozoon alactaguli (24)

A. acontion of the original report (Zasuchin, 1931) is considered as a synonym of A. pumilio by Ellerman and Morrison-Scott (1966), but not by Ognev (1948) who lists pumilio as a synonym of pygmaeus; the nomenclature is confused.

MYOMORPHA incertae cedis

Fam. Gliridae

Subfam. Glirinae (dormice)

115. Muscardinus avellanarius (common dormouse)

Babesia myoxi (83)

115A Eliomys quercinus

Babesia eliomysi (86)

CAVIOMORPHA

Fam. Ctenomyidae

117A Ctenomys sp. (tucu-tucu)

Babesia tucotucui (93)

Fam. Caviidae (guinea pigs or cavies)

Subfam. Caviinae

There appear to be no true records of protozoa in the blood-cells of guinea-pigs. Theileria caviae Corrêa, 1966 of Cavia porcellus in Brazil was later shown to be Histoplasma capsulatum (Corrêa and Pacheco, 1967). Macfie (1914) described two intra-erythrocytic "parasites" from laboratory-bred guinea-pigs in Nigeria. One, which Macfie called Paraplasma flavigellum, was shown not to be a protozoon (Wenyon and Low, 1914), but the illustrations in Macfie's paper suggest that the other, Paraplasma cobayae, might possibly have been a piroplasm.

HYSTRICOMORPHA

Fam. Hystricidae (Old World porcupines)

Subfam. Atherurinae (brush-tailed porcupines)

118. Atherurus africanus (brush-tailed porcupine)

Plasmodium atheruri (62)

Fam. Thryonomidae

119. Thryonomys swinderianus (cane rat or cutting grass)

Babesia spp. (102, 108, 117)

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 Thryonomys. Bruce's unnamed parasite from Malawi was probably from this rodent.

DISCUSSION

The remarkable diversity of habits and habitats of rodents is well described by Simpson (1955) who wrote: "The total range of adaptations in the order is extraordinary and is probably not equalled by any other one order of organisms. Some are amphibious or nearly aquatic (e.g. the coypu) and some (e.g. Kangaroo mice) may never even touch water in their whole lives. Some are among the smallest of all mammals, some as large as bears Some plow like moles, some crawl, some hop on hind legs only, some gallop, some swim as well as ducks, many climb trees, some glide on wing-like membranes. They live from the polar regions to the equator and from the highest mountains to below sea level." This striking exploitation of ecological niches accompanied by such widely varying habits brings rodents into contact with many different blood-sucking arthropods. This provides an array of alternate vertebrate and invertebrate hosts required by the vector-borne blood-sporozoa which, in turn, appear to have well exploited the niches provided.

With such diversity, it is difficult to draw many firm conclusions about the true hostal and geographical distribution of the parasites dealt with in the present work. This is made even more difficult because the separate identities of most of the parasites have not been established by experiments in the laboratory, and there remain many rodents which have never been properly examined or, indeed, examined at all. Some patterns of distribution nevertheless emerge and a

brief review, although certain to be inadequate, points the way to new work.

Hepatozoon is the commonest blood sporozoan of sciurormorphs 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, which, because of their habits, permit the parasite to undergo development within the invertebrate largely unaffected by extremes of ambient temperature. Gametocytes of haemogregarines of all squirrels and most myomorphs are in leucocytes. The exceptions are the species parasitic in jerboas and gerbils, and in Cricetomys, Dendromus and Otomys which inhabit erythrocytes.

Piroplasms of rodents are less evenly distributed both hostally and geographically than the haemogregarines. There are many records of Babesia in myomorphs, especially gerbils, but comparatively few from sciurormorphs. No records were found of Babesia in rodents other than Rattus in the Far East, and only two from S. America. Dunn et al. (1968) looked at the blood of Malaysian rodents but recorded no infections of piroplasms, and Lainson (personal communication) has examined hundreds of Brazilian cricetids without encountering Babesia. This unexplained rarity of piroplasms of rodents in some places is important when considering the source of infection of rare cases of piroplasmosis of man such as those recorded from Yugoslavia (Skrabalo and Deanovic, 1957), the U.S.A. (Scholtens et al., 1969; Benson 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 (see Garnham, 1966), it has never been recorded in an African rodent.

As with Hepatocystis, Plasmodium is not known in New World rodents. True malaria parasites of mammals other than man are poorly represented in that continent; two species, P. simium and P. brasilianum, are known from S. American monkeys, and an unnamed malaria parasite has been reported in deer from Texas (Kuttler et al., 1967). Murine rodents, among which are 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 African murines of the Lower Guinea Forest, (ii) two African and two Asian flying 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, arboreal rodents would appear to be the ones most likely to harbour malaria parasites.

In Africa, the distribution of the malaria parasites of murine

rodents is apparently restricted to the periphery of the Lower Guinea or Congo Forest, which extends from Katanga in the east, to Nigeria in the west. The principal vertebrate hosts are Thamnomys and Grammomys, semi-arboreal rodents which flourish in secondary forest formed by the farming activities of man, or in forest galleries.

A number of negative surveys have been carried out in the Upper Guinea Forest west of the Dahomey Gap (see Killick-Kendrick, 1971). In this part of Africa, arboreal murids appear to be scarce or difficult to catch, and although Thamnomys and Grammomys occur, they 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 Upper Guinea block some time well into the late Pleistocene (see Moreau, 1963, 1964). The vicissitudes of this part of the African forest must also have affected the sylvatic anophelines, and possibly malaria parasites are not present in murids in this most westerly part of the continent.

ACKNOWLEDGEMENTS

I am indebted to Professor P.C.C. Garnham F.R.S. for advice and encouragement in the preparation of this paper, and to Dr Elizabeth Canning and Dr J.R. Baker for helpful discussions, particularly on the systematics of piroplasms. I wish also to thank Professor R.S. Bray, Mr D.W. Brocklesby, Dr F.E.G. Cox, Dr A. de Jong and Professor D.N. Zasluchin for bringing some of the less obvious reports to my notice.

I owe Mr D.R. Rosevear of the British Museum (Natural History) special thanks for advice on the taxonomy of rodents. I wish to thank Miss Joan Freeman and Dr Canning for help with translations. Mr Rosevear, Dr Canning and Dr Cox kindly read the paper in draft.

Permission to include unpublished records was generously given by Mr J.P. Adam, Professor L.J. Bruce-Chwatt, Dr R. Lainson, Dr Irène Landau, Mr M. Markus, Dr B.R. Manawadu, Dr D. Minter and Dr W.M. Tadros. Support from the Medical Research Council is gratefully acknowledged.

REFERENCES TO PART V.

- ADAM, J.P., LANDAU, I., & CHABAUD, A.G. (1966). Découverte dans la région de Brazzaville de rongeurs infecté par des Plasmodium. C. r. hebd. Séanc. Acad. Sci., Paris, 263: 140-141.
- ADLER, S. (1930). The results of splenectomy in white mice as indicated by their reaction to Bartonella muris. Trans. R. Soc. trop. Med. Hyg. 24: 75-82.
- BAFORT, J. (1967). La transmission cyclique de Plasmodium vinckei. Annls Soc. belge Méd. trop. 47: 271-276.
- BAFORT, J. (1968). Primary exoerythrocytic forms of Plasmodium vinckei. Nature, Lond. 217: 1264-1265.
- BAFORT, J., TIMPERMAN, G. & MOLYNEUX, D.H. (1970). Babesia hylomysci n. sp., a new Babesia from the Congo, transmissible to mice. Annls Soc. belge Méd. trop. 50: 301-318.
- BAKER, J.R., CHITTY, D., & PHIPPS, E. (1963). Blood parasites of wild voles, Microtus agrestis, in England. Parasitology 53: 297-301.
- BALFOUR, A. (1905). A haemogregarine of mammals, H. jaculi (H. balfouri Laveran). J. trop. Med. Hyg. 8: 241-244.
- BALFOUR, A. (1906). A haemogregarine of mammals and some notes on trypanosomes in the Anglo-Egyptian Sudan. J. trop. Med. Hyg. 9: 81-92.
- BARNETT, S.F. & BROCKLESBY, D.W. (1969). Some piroplasms of wild mammals. Symp. zool. Soc. Lond. No 24, 159-176.
- BECKER, E.R. & ROUDABUSH, 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: 527-531.

- BENSON, G.D., GALDI, V.A., ALTMAN, R., & FIUMARA, N.J. (1969). Human babesiosis. Massachusetts: Morbidity and Mortality 18: 277-278.
- van den BERGHE, L., PEEL, E., CHARDOME, M. & LAMBRECHT, F.L. (1958). Le cycle asexué de Plasmodium atheruri n. sp. du pore-épïc Atherurus africanus centralis au Congo belge. Annls Soc. belge Méd. trop. 38: 971-976.
- van den BERGHE, L., VINCKE, I.H., CHARDOME, M., & van den BULCKE, M. (1950). Babesia rodhaini n. sp. d'un rongeur du Congo belge transmissible à la souris blanche. Annls Soc. belge Méd.trop. 30: 83-86.
- 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: 238-315.
- BRUCE, D., HAMERTON, A.E., BATEMAN, H.R., MACKIE, F.P. & LADY BRUCE. (1911). The occurrence of piroplasmosis in a white mouse. Rep. sleep Sickn. Comm. R. Soc. 11: p 189.
- BRUCE, D., HARVEY, D., HAMERTON, A.E., DAVEY, J.B., & LADY BRUCE. (1915). Trypanosomes and other parasites of animals in Nyasaland. Piroplasm of edible rat. Rep. sleep. Sickn. Comm. R. Soc. 16: p. 206
- BRUCE-CHWATT, L.J., & 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: 9-10.
- BRUMPT, E. (1946). Contribution à l'étude d'Hepatozoon muris. Utilisation dux énodiaagnostic pour l'identification des espèces d'hémogrégarines. Annls. Parasit. hum. comp. 21: 1-24.
- CAPPONI, M., SUREAU, P., & DESCHIENS, R. (1955). Présentation d'un hematozoaire observé dans le sang de Mus norvegicus au centre Vietnam. Bull. Soc. Path. exot. 48: 649-651.
- CARINI, A. (1941). Piroplasmidea do sangue de um roedor do Brasil. Arq. Biol. S. Paulo 25: 71-72.

- CARINI, A., & MACIEL, J. (1915). Sur une hémogregarine et un trypanosome d'un Muridé. (Akodon fuliginosus) Bull. Soc. Path. exot. 8: 165-169.
- ČERNA, Z. (1957). Further founds of Hepatozoon microti Coles, 1914 in Czechoslovakia (In Czech). Acta Soc. Zool. Bohem. 21: 95-96.
- CHEISSIN, E.M. (1965). Taxonomics of piroplasmae and some peculiarities of their development in the vertebrate and invertebrate hosts. Acta Protozool. 3: 103-109.
- CHRISTOPHERS, S.R. (1905). Haemogregarina gerbilli. Sci. Nem. Off. med. san. Dep. N.S., No 18, Calcutta Govt. of India, 15 pp.
- CLARK, M.G. (1958). Hepatozoon griseiscuiri n. sp.; a new species of Hepatozoon from the grey squirrel (Sciurus carolinensis Gmelin, 1788), with studies on the life-cycle. J. Parasit. 44: 52-63.
- COATNEY, G.R., ELBEL, R.E., & KOCHARATANA, P. (1960). Some blood parasites found in birds and mammals from Loei Province, Thailand. J. Parasit. 46: 701-702.
- COATNEY, G.R., & ROUDABUSH, R.L. (1949). A catalogue of the species of the genus Plasmodium, and an index of their hosts. In Malariology, vol. 1, ed by M.F. Boyd, Lond., Saunders, pp. 29-53.
- COLES, A.C. (1914). Blood parasites found in mammals, birds, and fishes in England. Parasitology 7: 17-61.
- CORREA, W.M. (1966). Theileria caviae: nova espécie descrita cobaias. Cienc. cult., Sao Paulo 18: 143.
- CORREA, W.M., & PACHECO, A.C. (1967). Naturally occurring histoplasmosis in guinea pigs. Canad. J. comp. Med. vet. Sci. 31: 203-206.
- COX, F.E.G. (1970). Parasitic protozoa of British wild mammals. Mammal Review 1: 1-28.

- DASGUPTA, B. (1965). Blood parasites in the Himalayan flying squirrel. Trans. R. Soc. trop. Med. Hyg. 59: 716-717.
- DASGUPTA, B. (1967). A new malarial parasite of the flying squirrel. Parasitology 57: 467-474.
- DASGUPTA, B., & CHATTERJEE, A. (1969). Further observations on the malaria parasite of the Himalayan flying squirrel. Parasitology 59: 733-736.
- DASGUPTA, B., & MEEDENIYA, K. (1958). The vector of Hepatozoon sciuri. Parasitology 48: 419-422.
- DASGUPTA, B., PAL, N., CHATTERJEE, A., & CHATTERJEE, D. (1971). A new malaria parasite in Hylopetes alboniger Hodgson. Parasitology 62: 195-197.
- DAVIS, D.H.S. (1965). Classification problems of African Muridae. Zoologica afr. 1: 121-145.
- DAVIS, D.H.S. (1966). Contribution to the revision of the genus Tatera in Africa. Annls Mus. R. Afr. cent., series 8, Zoological Sciences 144: 49-65.
- DISSANAIKE, A.S. (1963). On some blood parasites of wild animals in Ceylon. Ceylon vet. J. 11: 73-86.
- 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: 717-721.
- DUBININ, V.B. (1953). Parasites of mice (rodents) and their changes in the Volga delta (in Russian). Parazitologicheskii Sbornik 15: 252-
- DUNN, F.L., LIM, B.L., & YAP, L.F. (1968). Endoparasite patterns in mammals of the Malayan rain forest. Ecology 49: 1179-1184.
- ELLERMAN, J.R. (1940, 1941). The families and genera of living rodents. I: Rodents other than Muridae. II: Family Muridae. London: British Museum (Natural History).
- ELLERMAN, J.R. & MORRISON-SCOTT, T.C.S. (1966). Check-list of the Palearctic and Indian Mammals, 2nd edit., London: British Museum (Natural History).

- ELLERMAN, J.R., MORRISON-SCOTT, T.C.S. & HAYMAN, R.W. (1953).
Southern African Mammals, 1758-1951: a reclassification.
 London: British Museum (Natural History).
- ERHARDOVA, B. (1955). Hepatozoon microti Coles, 1914
U nasich drobných ssavců Ces. Biol. 4: 307-311
- FANTHAM, H.B. (1905). "Piroplasma muris, n. sp." Note on
 a microscopic exhibit. Proc. zool. Soc. London 2:
 491.
- FANTHAM, H.B. (1906). Piroplasma muris Fant. from the blood
 of the white rat, with remarks on the genus Piroplasma.
Q. J. microsc. Sci. 50: 493-516.
- FAY, F.H. & RAUSCH, R.L. (1969). Parasitic organisms in
 the blood of arvicoline rodents in Alaska. J. Parasit.
 55: 1258-1265.
- FELDMAN-MUHSAM, B. (1962). On Nuttallia adleri of Meriones
tristrami. Proc. int. Congr. Ent. Vienna, No 11, 3:
 91-94.
- FIELD, J.W., & EDESON, J.F.B. (1949). A note on presumed
 exoerythrocytic development of Plasmodium vassali
 in the liver of the Malayan squirrel.
Trans. R. Soc. trop. Med. Hyg., 42, 569-572.
- FIELD, J.W. & EDESON, J.F.B. (1950). A malaria parasite of
 the Malayan squirrel. Bull Inst. med. Res. Fed. Malaya.
 No 2 of 1949, 16 pp.
- 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: 861-862.
- FOGGIE, A. (1962). Studies on tick pyaemia and tick-borne
 fever. Symp. zool. Soc. Lond. No 6, 51-58.
- FRANCA, C. (1912). Sur la classification des piroplasmes et
 description de deux formes de ces parasites. Arch. Inst.
Bact. Cam. Pest. 3: 11-18.

- FRANCHINI, G. (1924). Piroplasmose chez un petit loir (Myoxus avellanarius). Bull. Soc. path. exot. 17: 881-884.
- FRANCHINI, G. (1932). Su di una leucocito-gregarina dello scoiattolo (Sciurus vulgaris). Archo ital. Sci. Med. Colon. 13: 385-389.
- FRANDSEN, J.C., & GRUNDMAN, A.W. (1961). Endoparasitism in isolated populations of rodents of the Lake Bonneville Basin, Utah. J. Parasit. 47: 391-396.
- FURMAN, D. P. (1966). Hepatozoon balfouri (Laveran, 1905).. Sporogonic cycle, pathogenesis and transmission by mites to jerboa. J. Parasit. 52: 373-382.
- GARNHAM, P.C.C. (1948). The developmental cycle of Hepatocystes (Plasmodium) kochi in the monkey host. Trans. R. Soc. trop. Med. Hyg. 41: 601-616.
- GARNHAM, P.C.C. (1964). The subgenera of Plasmodium in mammals. Annls Soc. belge Méd. trop. 44: 267-272.
- GARNHAM., P.C.C., LANDAU, I., KILLICK-KENDRICK, R. & ADAM, J.P. (1967). Répartition et caracteres différentiels des Plasmodiums de muridés. Bull. Soc. Path. exot. 60: 118-127.
- GILLIES, M.T. & de MEILLON, B. (1968). The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region). 2nd Ed., Johannesburg: S. African Institute for Medical Research.
- GOURLAY, R.N., BROCKLESBY, D.W., & SELLWOOD, S. (1970). Disc electrophoresis as an aid in the identification of piroplasms. Vet. Rec. May 23rd. 1970: 629-630.
- GREEN, R. (1934). Ann. rep. Inst. med. Res., Kuala Lumpur for 1933, Appendix 4, pp 75-100.
- GUNDERS, A.E. (1971).. Nuttallia meri, n. sp. - a new piroplasm from Psammomys obesus in the lower Jordan Valley. Parasitology 63: 431-437.
- GUNDERS, A.E., & SARFATTI, M. (1971). Nuttallia sp. from Nesokia indica. J. Protozool. (Supplement), 18: 37.

- HARRISON, J.L. & TRAUB, R. (1950). Rodents and insectivores from Selanger, Malaya. J. Mammalogy, 31: 337-346.
- HEISCH, R.B. (1952). New piroplasms of a genet cat and a ground-squirrel from Kenya. Ann. trop. Med. Parasit. 46: 150-154.
- HERMAN, C.M. & PRICE, D.L. (1954). Hepatozoon in grey squirrels, Sciurus carolinensis. J. Protozool. 1: 11-12.
- HOARE, C.A. (1972). The trypanosomes of mammals. Oxford: Blackwell.
- HOOGSTRAAL, H. (1959). Observations on some blood protozoa of Egyptian vertebrates with special reference to Hepatozoon balfouri (Laveran, 1905). PhD. Thesis London University.
- HOOGSTRAAL, H. (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: 231-248.
- JACOBS, B. (1953). Some blood parasitic protozoa of small wild mammals in England. Trans. R. Soc. trop. Med. Hyg. 47: 10-11.
- JETTMAR, H.M. (1932). Studien über Blutparasiten ostasiatischer wilder Nagetierre. Z. Parasitkde 4: 254-284.
- KAKABADZE, M.B & ZASUCHIN, D.N. (1969). Hepatozoon epsteini sp. n. - a parasite of rats. Progress in Protozoology, Leningrad, Nauka 3: pp 337-338.
- KEYMER, I.F. (1964). Hepatozoon species in wild African mammals. Vet. Rec. 76: p 641.
- KEYMER, I.F. (1966). Studies on blood protozoa of wild mammals from Central Africa. PhD. thesis, London University.
- KILLICK-KENDRICK, R. (1971). The collection of strains of murine malaria parasites in the field, and their maintenance in the laboratory by cyclical passage. In Isolation and Maintenance of Parasites in vivo, Eds A.E.R. Taylor and R. Muller, Symposia of the British Society for Parasitology,

vol 9, Oxford: Blackwell, pp. 39-64.

KILLICK-KENDRICK, R. & BELLIER, L. (1971). Blood parasites of scaly-tailed flying squirrels in the Ivory Coast. Trans. R. Soc. trop. Med. Hyg. 65: 430-431.

KILLICK-KENDRICK, R., SHUTE, G.T. & LAMBO, A.O. (1968). Malaria parasites of Thannomys rutilans (Rodentia, Muridae) in Nigeria. Bull. Wld Hlth Org. 38: 822-824.

KIRNER, S.H., BARBEHENN, K.R. & TRAVIS, B.V. (1958). A summer survey of the parasites of two Microtus pennsylvanicus populations. J. Parasit. 44: 103-105.

KLEINE, F.K. (1910). Trypanosomenbefunde am Tanganyika und andere Beobachtungen. Dt. med. Wschr. 36: 1400

KRAMPITZ, H.E. (1957). Ricerche sugli emoparassiti dei micro-mammiferi selvatici della Sicilia. Riv. Parassit. 18: 219-233.

KRAMPITZ, H.E., SACHS, R., SCHALLER, G.B. & SCHINDLER, R. (1968). Zur Verbreitung von Parasiten der Gattung Hepatozoon Miller, 1908 (Protozoa, Adeleidea) in ostafrikanischen Wild säugetieren. Z. ParasitKde. 31: 203-210.

KRYLOV, M.V. (1965). The development of Nuttallia tadjikistanica Krylov and Zanina, 1962 in the tick Hyalomma anatolicum. (In Russian). Acta Protozool. 3: 369-382.

KRYLOV, M.V. & ZANINA, Z.L. (1963). Smithia tadjikistanica sp. n. from the red-tailed gerbil. (In Russian). Proc. Inst. Zool. Parasit. Acad. Sci. Tadzhik SSR. 24: 169-170.

KUSAMA, S., KASAI, K. & KOBAYASHI, R. (1919). The leucocytogregarine of the wild rat with special reference to its life-history. Kitasato Archs exp. Med. 3: 103-122.

KUTTLER, K.L., ROBINSON, R.M. & ROGERS, W.P. (1967) Exacerbation of latent erythrocytic infections in deer following splenectomy. Can. J. comp. Med. 31: 317-319.

- LANDAU, I. (1965). Description de Plasmodium chabaudi n. sp. parasite de rongeurs africains. C. r. hebd. Séanc. Acad. Sci., Paris 260: 3758-3761.
- LANDAU, I., ADAM, J.P. & BOULARD, Y. (1969a). A malaria parasite of the brush-tailed porcupine in Calomys and white mice. Trans. R. Soc. trop. Med. Hyg. 63: 8-9.
- LANDAU, I., BOULARD, Y. & HOUIN, R. (1969b). Anthemosoma garnhami n.g. n. sp., premier Dactylosomidae connu chez un mammifère. C. r. hebd. Séanc. Acad. Sci., Paris 268: 873-875.
- LANDAU, I. & CHABAUD, A.G. (1965). Infection naturelle par deux Plasmodium du rongeur Thamnomys rutilans en République Centre Africaine. C. r. hebd. Séanc. Acad. Sci., Paris 261: 230-232.
- LANDAU, I. & KILLICK-KENDRICK, R. (1966). 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: 1113-1116.
- LANDAU, I., MICHEL, J.C. & ADAM, J.P. (1968). Cycle biologique au laboratoire de Plasmodium berghei killicki n. sub. sp. Annls Parasit. hum. comp. 43: 545-550.
- LANDAU, I., MICHEL, J.C., ADAM, J.P. & BOULARD, Y. (1970). 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. 64: 315-323.
- LANGUILLON, J. (1957). Note sur deux parasites sanguicoles de mammifères du Cameroun. Bull. Soc. Path. exot. 50: 362-363.
- LATYSHEV, N.I. (1949). Some parasitological discoveries among mammals of the Murgab River (Turkmenia) (in Russian) Vopr. Draev. Obsh. i Ekper. Parazitol. 4: 83-86. (quoted from Clark, 1958).

- LAVERAN, A. (1905a). Sur une hémogregarine des gerboises.
C. r. hebd. Séanc. Acad. Sci., Paris 141: 295-298.
- LAVERAN, A. (1905b). Haemocytozoa. Essai de classification.
Bull. Inst. Pasteur 3: 809-817.
- LAVIER, G. (1921). Hémogrégarines, Grahamella, spirochète
et trypanosome du campagnol indigène Microtus arvalis
Pallas. Bull. Soc. Path. exot. 14: 569-576.
- 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: 348-354.
- LEGER, M. & BEDIER, E. (1922). Plasmodium du lérot,
Myoxus murinus. C. r. Séanc. Soc. Biol. 87:
1336-1337.
- LEGER, M. & BEDIER, E. (1923). Piroplasme d'un muride,
Golunda campanae, du Sénégal. C. r. Séanc. Soc. Biol.
88: ~~424-425~~.
- LEVADITI, C. & SCHOEN, R. (1932). Sur un parasite sporulé
du singe. C. r. Séanc. Soc. Biol. 109: 343-346.
- LEVINE, N.D. (1971). Taxonomy of the piroplasms.
Trans. amer. micros. Soc. 90: 2-33.
- LIEN, J.C. & CROSS, J.H. (1968). Plasmodium (Vinckeia)
watteni sp. n. from the Formosan flying squirrel,
Petaurista petaurista grandis. J. Parasit. 54:
1171-1174.
- MACFIE, J.W.S. (1914). Notes on some blood parasites
collected in Nigeria. Ann. trop. Med. Parasit.
8: 439-468.
- MACFIE, J.W.S. (1915). Babesiasis and trypanosomias
at Accra, Gold Coast, West Africa. Ann. trop.
Med. Parasit. 9: 457-494.
- MACKERRAS, M.J. (1959). The haematozoa of Australian
mammals. Austr. J. Zool. 7: 105-135.

- MANWELL, R.D. & KUNTZ, R.E. (1964). A new Babesia from the Indian bandicoot. J. Parasit. 50: 390-393.
- MANWELL, D. & KUNTZ, R.E. (1966). Hepatocystis in Formosan mammals with a description of a new species. J. Protozool. 13: 670-672.
- MARCHIAFAVA, E. & CELLI, A. (18885). Weitere Untersuchungen über die Malariainfektion. Fortschr. Med. 3: 787-792.
- MARTOGLIO, F. (1913). Contributo alla conoscenza della leucocytogregarine. Annali Ig. sper. 23: 161-170.
- McMILLAN, W.G. & BROCKLESBY, D.W. (1971). The fine structure of intra-erythrocytic stages of Nuttallia (Babesia) microti. Res. vet. Sci. 12: 185-186.
- MEDWAY, LORD. (1969). The wild mammals of Malaya and offshore islands including Singapore. London: Oxford University Press.
- 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 51 pp.
- MIYAIRI, K. (1932). Zur Entwicklungsgeschichte einer Haemogregarine (richtiger Karyloysus) aus der Feldmaus Arvicola hatanezumi Sasaki. Proc. Imp. Acad. Tokyo. 8: 328.
- MOLYNEUX, D.H. (1969). The morphology and life-history of Trypanosoma (Herpetosoma) microti of the field-vole, Microtus agrestis. Ann. trop. Med. Parasit. 63: 229-244.
- MOLYNEUX, D.H. & BAFORT, J.M. (1970). The fine structure of Babesia microti in the natural host, Microtus agrestis. Z. Tropenmed. Parasit. 21: 393-401.

- MOORE, J.C., & TATE, G.H.H. (1965). A study of the diurnal squirrels, Sciurinae, of the Indian and Indochinese subregions. Fieldiana: Zoology 48: 1-351.
- MOREAU, R.E. (1963). Vicissitudes of the African Biomes in the late Pleistocene. Proc. zool. Soc. London 141: 395-421.
- MOREAU, R.E. (1964). The distribution of tropical African birds as an indicator of past climatic changes. In African ecology and human evolution ed. by F.C. Howell and F. Bourlière; London: Methuen, pp 28-42.
- MOUCHET, J., GARIOU, J. & RIVOLA, E. (1957). Observations sur le biologie d'Anopheles smithi var rageaui Mattingly and Adam, 1954. Vecteur d'un Plasmodium de mammifères aux environs de Yaoundé (Sud Cameroun). Bull. Soc. Path. exot. 50: 157-164.
- MULLIGAN, H.W., & SOMERVILLE, T. (1947). Malaria in the Malabar squirrel. Indian J. Malar. 1: 329-335.
- MURATOV, E.A. (1966). Blood parasite of the house mouse (Mus musculus Lin.) belonging to the genus Nuttallia França. (In Russian). Dokl. Akad. Nauk tadzik. S.S.R. 9: 34-37.
- NAJERA ANGULO, L. (1937). Sur le parasitisme de Trypanosoma blanchardi et Hepatozoon lusitanicus n. sp. en Espagne 12th Congress on International Zoology, Lisbon, 1935. 3: 1921-1947.
- NAUCK, E.G. (1927). Ueber Befund in Blut splenektomierter Nager. Arch. Sciffs-Tropenhyg. 31: 322-329.
- NEITZ, W.O. (1956). Classification, transmission, and biology of piroplasms of domestic animals. Ann. N.Y. Acad. Sci. 64: 56-111.
- NICOLLE, C. (1907). Sur un piroplasmose nouvelle d'un rongeur. C. r. Séanc. Soc. Biol. 63: 213-216.
- NOLLER, W. (19). Die Blutprotozoen des Hamsters (Cricetus frumentarius Pall.) und ihre Übertragung. (Vorl. Mitt.). Arch. Protistenk. 25: 377-385

- NOWELL, F. (1969). The blood picture resulting from Nuttallia (= Babesia) rodhaini and Nuttallia (= Babesia) microti infections in rats and mice. Parasitology 59: 991-004.
- OGNEV, S.I. (1948). Mammals of the U.S.S.R. and adjacent countries Vol. VI, Rodents. Bulletin of the Academy of Sciences of the U.S.S.R. English translation by A. Birron and Z.C. Cole, Israel Program for Scientific ranslations, Jerusalem, 1963.
- PARKER, J.C. (1968). Parasites of the gray squirrel in Virginia. J. Parasit. 54: 633-634.
- PATTON, W.S. (1906). On a parasite found in the white corpuscles of the blood of palm squirrels. Sci. Mem. Off. med. san. Dep. N.S., No 24, Calcutta: Govt. of India, 13 pp.
- van PEENEN, P.F.D. & DUNCAN, J.F. (1968). Piroplasms (Protozoa: Sarcodina) of wild mammals in California. Bull. Wildlife Diseases Assoc. 4: 3-8.
- van PEENEN, P.F.D., HOOGSTRAAL, H., DUNCAN, J.F. & RYAN, P.F. (1968). Hematozoa from mammals of South Vietnam. J. Protozool. 15: 608-614.
- PEREZ-ALVA, S. & GIUNTINI, J. (1957). Etude morphologique de Bartonella bacilliformis au microscope électronique. Bull. Soc. Path. exot. 50: 188-194.
- PETERS, D., & WIGAND, R. (1955). Bartonellacea. Bact. Rev. 19: 150-159.
- PETTER, F. (1964). Affinités du genre Cricetomys. Une nouvelle sous-famille de rongeurs Cricetidae, les Cricetomyinae. C. r. hebd. Séanc. Acad. Sci. Paris 258: 6516-6518.
- PHILIP, C.B. (1957). In Bergey's manual of determinative bacteriology, ed. by R.S. Breed, E.G.D. Murray, & N.R. Smith, 7th ed. Baltimore: Williams and Wilkins, p. 934.

- PORTER, A. (1908). Leucocytozoon musculi sp. n., a parasitic protozoon from the blood of white mice. Proc. zool. Soc. Lond. 2: 703-716.
- PRINGLE, G. (1960). Two new malaria parasites from East African vertebrates. Trans. R. Soc. trop. Med. Hyg. 54: 411-414.
- 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: 241-244.
- RAY, H.N. (1960). Exo-erythrocytic schizogony of a malaria parasite in the himalayan flying squirrel, Petaurista inornatus (Geoffrey). Proc. natn. Inst. Sci. India. 26B, Suppl. 1, 1-5.
- RIOUX, J.A. & GOLVAN, Y.T. (1969). Epidémiologie des leishmanioses dans le sud de la France, Paris: l'Institut de la Santé et de la Recherche Médicale.
- RODHAIN, J. (1952). Un deuxième Plasmodium parasite de rongeur sauvages au Katanga. Annls Soc. belge Méd. trop. 32: 275-280.
- RODHAIN, J., PONS, C., VANDENBRANDEN, R. & BEQUAERT, J. (1913). Notes sur quelque hématozoaires du Congo belge. Arch. ProtistKde 29: 259-278.
- RODHAIN, J., & VINCKE, I. (1953). Sur une deuxième souche de Babesia rodhaini du rongeur africain Thamnomys surdaster. Acta trop. 10: 264-266.
- ROMANA, C. (1945). Infección de un Graomys medius Thomas por diversos haemoparasitos. An. Inst. Med. reg., Tucumán 1: 177-183.
- ROSEVEAR, D.R. (1969). Rodents of West Africa, London: British Museum (Natural History).
- ROUSSELOT, R. (1949). Piroplasma lemniscomysi, n. sp. Ann. Parasit. 24: 409-411.

- SKRABALO, Z. & DEANOVIC, Z. (1957). Piroplasmosis in man. Documenta Med. geogr. trop. 9: 11-16.
- SPLENDRE, A. (1918). Studi nell'interesse di una lotta biologica contro le arvocoli. Bolletino dei Ministeri per l'Agricoltura e per l'Industria il Commercio ed il Lavoro, Ser. B., Rome 2: 1-10.
- SPLENDRE, A. (1920). Sui parassiti delle arvicole (2nd part). Annali Ig. sper. 30: 560-582.
- SPRINHOLZ-SCHMIDT, A.J. (1937). Zur Frage der Blutparasiten der Nagetiere in Fernen Osten. Arch. Protistenk. 88: 445-450.
- STARCOVICI, 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: 1-8.
- TARTAKOWSKY, M. (1913). Katalogue der exponaten der Landwirtschaftlichen Ausstellung. Petersburg. (quoted from Hoogstraal, 1959).
- TIVLOV, V.E. (1934). In Zassuchin, Tivlov and Schulz (1934)
- TOKURA, N., & WU, CHEN LAN, (1961). A malaria parasite, Hepatocystes of the flying squirrel in Taiwan. Endem. Dis. Bull. Nagasaki Univ. 3: 174-179
- 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: 254-258.
- TSUR, I., HADANI, A., & PIPANO, E. (1960). Nuttallia dani n. sp. - a haemoprotozoon from the gerbil (Meriones tristrami shawi). Refuah vet. 17: 244-236.
- TYZZER, E.E. (1938). Cytoecetes microti n.g., n. sp., a parasite developing in granulocytes and infective for small rodents. Parasitology 30: 242-257.

- ROUSSELOT, R. (1953). Notes de parasitologie tropicale. Parasites du sang des animaux. Paris: Vigot Frères.
- SANDOSHAM, A.A., YAP, L.F., & OMAR, I., (1965). A malaria parasite, Plasmodium (Vinckeia) booliati sp. nov. from a Malayan giant flying squirrel. Med. J. Malaya. 20: 3-7.
- SANGIORGI, G. (1912). Leucocyt _____ musculi.
Zentbl. Bakt. ParasitKde 66: 287-
- 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: 810-813.
- 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: 529-532.
- SERGEANT, E. (1921). Haemogregarine (Hepatozoon) de l'ecureuil de Getulie. Archs. Inst. Pasteur Afr. nord. 1: p.56.
- SHINGAREVA (1910). Quoted by Zasuchin (1936).
- SHORTT, H.E (1962). Ticks and pioplasms. Symp. zool. Soc. Lond. No 6, 157-174.
- 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: 37-42
- SIMPSON, G.G (1945). The principles of classification and a classification of mammals. Bull. Am. Mus. nat. Hist. 85: 1-350.
- SIMPSON, G.G. (1959). The nature and origin of supraspecific taxa. Cold Spring Harbor Symposium on Quantitative Biology, 24: 255-271.

- TYZZER, E.E. (1939). Haemobartonella n.g. (Bartonella olim pro parte). H. microti n. sp. of the field vole Microtus pennsylvanicus. Am. J. Hyg. 30: 141-157.
- TYZZER, E.E. (1942). A comparative study of Grahamellae, Haemobartonellae and Eperythrozoon in small animals. Proc. Am. phil. Soc. 85: 359-398.
- VASSAL, J.J. (1905). Sur un hématozoaire endoglobulaire nouveau d'un mammifère. Annls Inst. Pasteur, Paris 19: 224-232.
- VASSAL, J.J. (1907). Nouvelle contribution à l'étude de l'hématozoaire de l'écureuil (Haemomoeba vassali Lav.) Annls Inst. Pasteur, Paris 21: 851-857.
- VINCKE, I.H. (1954). Natural history of Plasmodium berghei. Indian J. Malar. 8: 245-256.
- VINCKE, I.H. & LIPS, M. (1948). Un nouveau Plasmodium d'un rongeur sauvage du Congo, Plasmodium berghei n. sp. Annls Soc. belge Méd. trop. 28: 97-104.
- VIVIER, E. & PETITPREZ, A. (1969). Observations ultrastructurales sur l'hématozoaire Anthemiosoma garnhami et examen de critères morphologiques utilisables pour la taxonomie chez les sporozoaires. Protistologica 5: 363-379.
- WEINMAN, D. (1957). Family III. Bartonellaceae Gieszczykiewicz, 1939. In Bergey's manual of determinative bacteriology eds, R.S. Breed, E.G.D. Murray, and N.R. Smith, 7th ed., Baltimore: Williams and Wilkins, p. 169.
- WELLMAN, C. & WHERRY, W.B. (1910). Some new internal parasites of the California ground squirrel (Otospermophilus beecheyi). Parasitology 3: 417-422.

- WENYON, C.M. (1909). Report of the travelling pathologist and protozoologist. 3rd Rept. Wellcome Trop. Res. Labs, Khartoum, 1908, 152.
- WENYON, C.M. (1926). Protozoology, vols I and II, London: Ballière, Tindall and Cox.
- WENYON, C.M. & 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: 369-372.
- 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. Wld Hlth Org. 29: 357-374.
- WOOD, A.E. (1955). A revised classification of the rodents. J. Mammal. 36: 165-187.
- WOOD, S.F. (1962). Blood parasites of mammals of the Californian Sierra Nevada foothills, with special reference to Trypanosoma cruzi Chagas and Hepatozoon leptosoma n. sp. Bull. Soc. Calif. Acad. Sci. 61: 161-176.
- WU, C.L. (1953). Contributions to the study on the mammalian malaria in the Formosa (Taiwan). Acta med. Fakuoka 23: 1979-1994.
- YAKIMOFF, W.L. & SAPHRONOWITSCH, R.A. (1917). Parasite du sang des animaux en Transcaucasie. II: Theileria chez le campagnol. Bull. Soc. Path. exot. 10: 98-100.
- YAKIMOFF, W.L. & SCHOKHOR, N.J. (1917). Leucocytozoon musculi A. Porter à Petrograd. Bull. Soc. Path. exot. 10: 100-101.
- YAP LOY FONG & SANDOSHAM, A.A. (1963). Babesia decumani (Macfie) and other blood parasites in a little spiny rat, Rattus musschenbroeki (= R. whiteheadi). Singapore med. J. 4: 171-172.

- ZASUCHIN, D.N.(1930). Blood parasites of rodents from Southern Vostok. R.S.F.S.R. II: Blood parasites from the steppe marmot. (In Russian). Rev. Microbiol. Epidemiol. Parasit., 9: 555-570.
- ZASUCHIN, D.N. (1931). Blood parasites of rodents from Southern Vostok. R.S.F.S.R. III: Grahamia and Haemogregarina (In Russian) Rev. Microbiol. Epidemiol. Parasit., 10: 97.
- ZASUCHIN, D.N. (1936). Blood parasites found in rodents and some related mammals in Southern Vostok, R.S.F.S.R. (In Russian). Rev. Microbiol. Epidemiol. Parasit., 15: 45.
- ZASUCHIN, D.N. , TIVLOV, V.E., & SCHULZ, R.E. (1934). Endo - and ectoparasites of Rhombomys opimus Licht. Rev. Microbiol. Epidemiol. Parasit. 13: 335-338.

APPENDIX 1
TRYPANOSOMES AND HAEMOGREGARINES
OF AFRICAN FLYING SQUIRRELS

Introduction

During the search for malaria parasites of flying squirrels in the Ivory Coast (see Part III), two trypanosomes and two haemogregarines were found. Brief notes on the incidence and morphology of these parasites are given in this appendix; full accounts will be prepared when all the material has been examined.

TRYPANOSOMES OF AFRICAN FLYING-SQUIRRELS

Trypanosomes were in the thick films of heart blood of 10/15 Anomalurus peli and 5/6 A. derbianus. Parasitaemias were all low. The descriptions given below are of the trypanosomes in the thin blood films of A. peli No 70/37 and A. derbianus No 70/34. The parasite of A. peli was identified as Trypanosoma denysi, while that of A. derbianus is previously undescribed*. No trypanosomes were seen in thick and thin films of heart blood from 16 Idiurus macrotis.

* I thank Dr C.A. Hoare F.R.S. for advice on the identity of the trypanosomes.

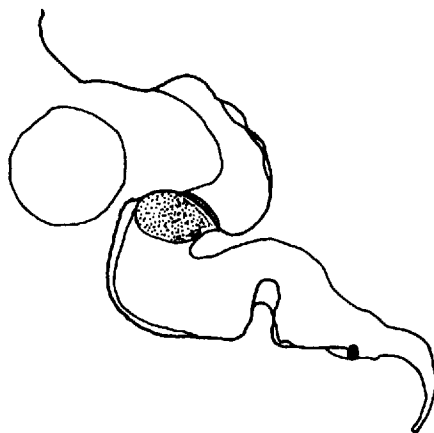
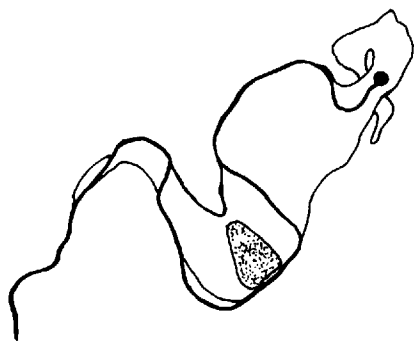
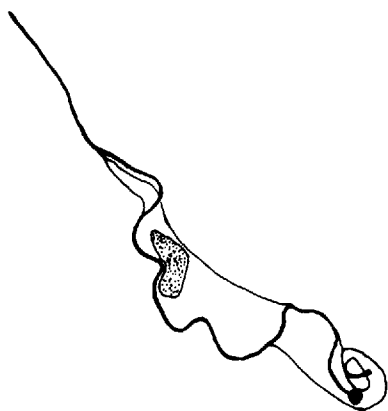
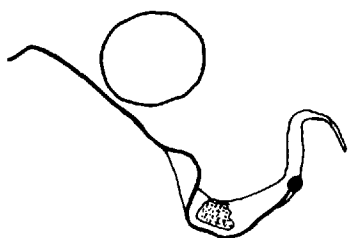
T. denysi in A. peli (fig. 9)

The longest forms of this large trypanosome measured more than 50 μ m; they were 5 μ m wide. The undulating membrane was moderately well developed and a free flagellum was always present. The kinetoplast was round, subterminal and almost always marginal; it was never close to the nucleus which lay in the anterior half of the body. No dividing stages were seen, but about a third of the trypanosomes were slender, smaller forms which were, presumably, growing stages.

Hoare (in press) synonymizes T. anomaluri Schwetz, 1933, with T. denysi Rodhain, Pons, Vandenbranden and Bequaert, 1912. Both reports are from the Congo - Kinshasa. The hosts in both instances can be presumed to be A. derbianus (see Hoare, in press). Schwetz's description is better than that of Rodhain et al. (1912, 1913), and in all respects except one the parasite of A. peli is indistinguishable from his parasite. In Schwetz's (1933) description an almost constant morphological character was a twist at the end of the body posterior to the kinetoplast. This was never seen in any of the trypanosomes of A. peli, but great significance is not attached to Schwetz's observations since it may be the result of the way the blood films were prepared. On the present evidence, the parasite of A. peli

Figure 9

Trypanosoma denysi in the blood of Anomalurus
peii No 70/37 (X 2,100).



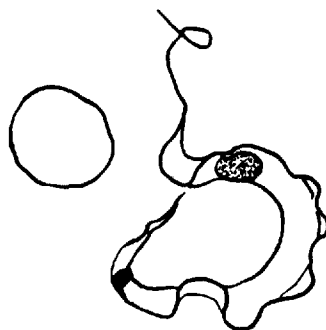
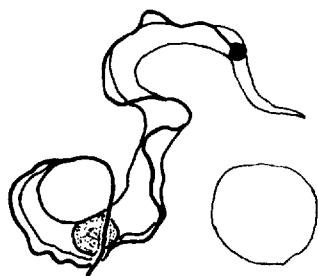
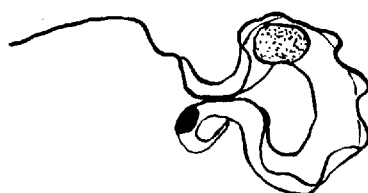
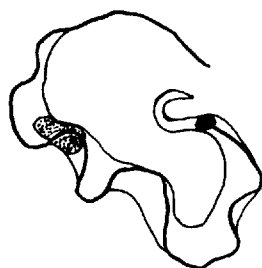
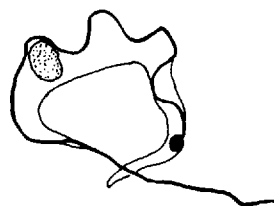
is identified as T. denysi in a new host and new locality. As pointed out by Hoare (in press) it is not possible confidently to assign T. denysi to one of the subgenera of mammalian trypanosomes. It has the morphology of the *Stercoraria* in which there are three subgenera: Megatrypanum, Herpetosoma and Schizotrypanum. It bears no resemblance to the cruzi - like trypanosomes of the last subgenus, but has the general appearance of a member of Megatrypanum with the kinetoplast in the position of the species in Herpetosoma (Hoare, 1964, 1966, in press).

Trypanosoma sp. in A. derbianus (fig 10)

This trypanosome had the morphology of the subgenus Herpetosoma Doflein, 1901 (see Hoare, 1966). The mean length of six trypanosomes was 40.8 μm (33.5 - 47.5 μm); the width was 1.5 - 2.2. μm . The undulating membrane was well developed and thrown into folds; a free flagellum was always present. The kinetoplast was large, round and subterminal, and usually lay centrally, filling the width of the pointed posterior end. The nucleus lay in the anterior half of the body at a point where it began to narrow. No dividing stages were seen.

Figure 10

Trypanosoma sp. in the blood of Anomalurus
derbianus No 70/34 (X 2,100).



This trypanosome differed from T. denysi by its smaller size and narrower body, its monomorphism and, most strikingly, by the larger size and central position of its kinetoplast. It is thought to be a new species.

HAEMOGREGARINES OF AFRICAN FLYING SQUIRRELS

Gametocytes of haemogregarines were found in smears of heart blood of 4/16 Idiurus macrotis. There were two species, both parasites of leucocytes. There were none in the blood of 15 A. peli and 6 A. derbianus.

The commoner haemogregarine (later found by the writer in 10/16 I. macrotis in the West Cameroons) was in all four infected animals; it is referred to here as the short haemogregarine. One of the four animals (No 70/58) was also infected with a second haemogregarine, here referred to as the long haemogregarine. In the thick blood film of this animal three immature schizonts were found. Because of the size of the forming gametocytes of the most mature schizont (described below) these were thought to be the division stages of the long haemogregarine. No schizonts have yet been found in sections of the heart, lungs, liver, spleen or kidney of the host.

In thick blood films gametocytes of both haemogregarines

had left the host cells, and even in thin films rapidly dried with gentle heat the majority of the parasites were lying free. The speed with which the gametocytes left the cell is rare with mammalian haemogregarines, though common in many haemogregarines of reptiles.

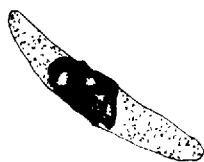
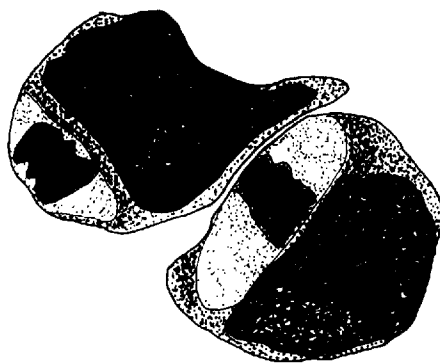
The short haemogregarine of *I. macrotis* (fig 11)

This was a parasite of monocytes. The intracellular gametocytes were broad elongate bodies with a lightly vacuolated cytoplasm. The nucleus was a darkly staining beaded mass, frequently rectangular, lying at about the centre of the body. Free forms were more slender than the intracellular forms, and tended to have one end of the body more pointed than the other. The mean size of 10 extracellular gametocytes was $11.9 \times 2.8 \mu\text{m}$ ($10.5\text{--}13.5 \times 2.0\text{--}3.4 \mu\text{m}$); the mean length of their nuclei was $6.3 \mu\text{m}$ ($5.7\text{--}7.0 \mu\text{m}$).

There appear to be no published records of haemogregarines of pygmy flying squirrels, although several true sciurormorphs are hosts of these parasites (see Part V). Because of the special habits of Idiurus and its lack of a close relationship to the true squirrels, it is assumed that the short haemogregarines is new. To be sure of the genus to which it should be assigned it is necessary to know the site of schizogony (see Wenyon, 1926).

Figure 11

Intracellular and free gametocytes of Hepatozoon
sp. (short form) in the blood of Idiurus macrotis
No 70/58 (X2,100).



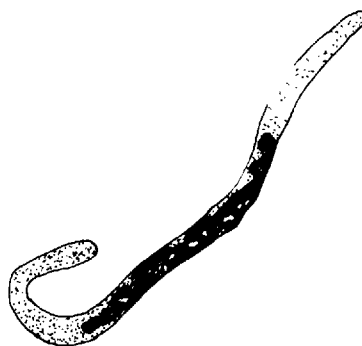
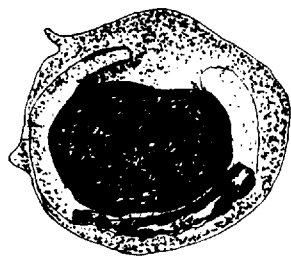
But since the morphology of its gametocytes is similar to the well-studies species of Hepatozoon Miller, 1908 of rodents, the short haemogregarine is considered to be a new species of this genus.

The long haemogregarine of I. macrotis (fig 12)

The extracellular gametocytes of this parasite were so long and slender that they were at first mistaken for micro-filariae. A closer examination revealed that they had only a single nucleus and were protozoa. A resemblance to the long haemogregarines of some reptiles and amphibia was recognized, and the thin film was searched completely in an attempt to find intracellular parasites. Two were found in monocytes (fig 12). They measured $32.7 \times 2.0 \mu\text{m}$ and $37.0 \times 1.8 \mu\text{m}$; the length of their nuclei was $12.5 \mu\text{m}$ and $13.0 \mu\text{m}$ respectively. The nucleus of both intra and extracellular forms stained deeply and had a beaded appearance. It usually lay towards one end of the body. Once intracellular forms were recognized, other infected cells were found but, in all except the two described above, only the nucleus of the parasite was clearly seen. All infected cells were monocytes. Ten free gametocytes in the thin blood film measured $33.2 \times 1.5 \mu\text{m}$ ($27.5 - 38.5 \times 1.2 - 1.7 \mu\text{m}$); the mean length of their nuclei

Figure 12

Intracellular and free gametocytes of Hepatozoon
sp. (long form) in the blood of Idiurus macrotis
No 70/58 (X 1,800).

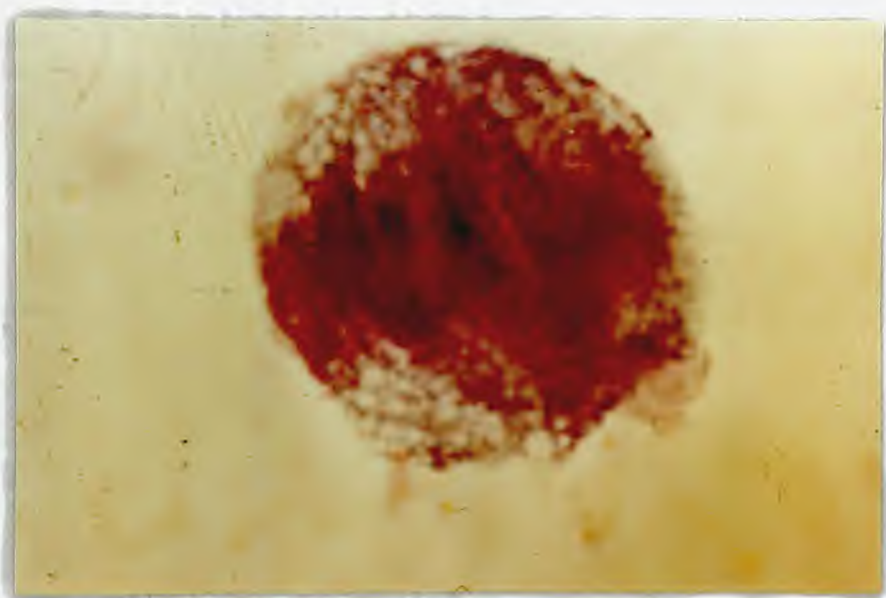


was 13.8 μm (10.5 17.5 μm).

The three immature schizonts (mentioned above) in the thick film of heart blood of Idiurus No 70/58 had a mean diameter of 29 μm . They all had a much vacuolated cytoplasm, typical of schizonts of haemogregarines, and in one (Plate 11) the beginnings of the formation of gametocytes were visible. This schizont contained 30-40 elongate nuclei lying parallel to each other. Since the size of the partly formed gametocytes was too big for them to have been the short haemogregarine of the same rodent, it is assumed that the schizonts were developmental stages of the long form.

The long haemogregarine of Idiurus has by far the longest gametocytes of any species infecting mammals. Although its morphology is not typical, it seems best for the moment to view this distinctive parasite as a new species of Hepatozoon, the only haemogregarine of mammals. As with so many haemogregarines, its true generic status will remain doubtful until more stages of the life-cycle are seen.

PLATE 11



Almost mature schizont of Hepatozoon in a thick
blood film from the heart of Idiurus macrotis

No 70/58 (X 2,000).

REFERENCES IN APPENDIX 1

- HOARE, C.A. (1964). Morphological and taxonomic studies on mammalian trypanosomes. X. Revision of the systematics. J. Protozool., 11, 200-207.
- HOARE, C.A. (1966). The classification of mammalian trypanosomes. Ergebn. Mikrobiol. Immunforsch. exp. Ther., 39, 43-57.
- HOARE, C.A. (in press). The trypanosomes of mammals. Oxford: Blackwell.
- RODHAIN, J., PONS, C., VANDENBRANDEN, F., & BEQUAERT, J. (1912). Les trypanoses animales au bas-Katanga et leur rapport avec les glossines (3e note). Trypanosoma denysi n. sp., parasite de l'ecureuil volant. Bull. Soc. Path. exot., 5, 608-611.
- RODHAIN, J., PONS, C., VANDENBRANDEN, F., & BEQUAERT, J. (1913). Rapport sur les travaux de la mission scientifique du Katanga, Octobre 1910, à Septembre, 1912. Brussels.
- SCHWETZ, J. (1933). Trypanosomes rares de la région de Stanleyville (Congo Belge) Annls Parasitol. hum. comp., 11, 287-296.
- WENYON, C.M. (1926). Protozoology. 2 vols, London: Ballière, Tindall & Cox.

Appendix 2

SUBSIDIARY MATTER

Candidate's published work in 1970 & 1971

In the joint publications listed below (Nos. 4 - 8) the candidate initiated the work in paper 4; collaborated fully in the work in papers 5, 6 and 7; and played a minor role in the work in paper 8.

1. KILLICK-KENDRICK, R. (1970). The life-cycles of two murine malaria parasites from Nigeria (Abstract). J. Parasit. Suppl., 56: No 4, Sect. II, Part 2, 435-436
2. KILLICK-KENDRICK, R. (1971). The collection of strains of murine malaria parasites in the field, and their maintenance in the laboratory by cyclical passage. In Isolation and Maintenance of Parasites in vivo, A.E.R. Taylor & R. Muller (Eds), Symp. Brit. Soc. Parasit., 9, 39-64.
3. KILLICK-KENDRICK, R. (1971). The low pathogenicity of Trypanosoma brucei to cattle (Correspondence). Trans. R. Soc. trop. Med. Hyg., 65, 104.
4. KILLICK-KENDRICK, R., & BELLIER, L. (1971). Blood parasites of scaly-tailed flying squirrels in the Ivory Coast (Laboratory demonstration). Trans. R. Soc. trop. Med. Hyg., 65, 430-431.
5. HEISCH, R.B., KILLICK-KENDRICK, R., GUY, M.W., & DORRELL, J. (1970). The development of trypanosomes, leishmaniae and ascitic tumour cells in the testicles of laboratory animals. Trans. R. Soc. trop. Med. Hyg., 64, 679-682.
6. DRAPER, C.C., KILLICK-KENDRICK, R., HUTCHISON, W.M., SIIM, J. CHR., & GARNHAM, P.C.C. (1971). Experimental toxoplasmosis in chimpanzees. Brit. med. J., 1971, 2, 375-378.
7. CROLL, N.A., & KILLICK-KENDRICK, R. (1971). Epidemiological significance of Strongyloides in sputum (Correspondence). Trans. Soc. trop. Med. Hyg., 65, 688-689.
8. GARNHAM, P.C.C., WARREN, M. & KILLICK-KENDRICK, R. (1971). The action of terramycin on the primary exoerythrocytic schizonts of Plasmodium vivax and Plasmodium cynomolgi ceylonensis. J. trop. Med. Hyg., 74, 32-35.

Experimental Toxoplasmosis in Chimpanzees

C. C. DRAPER, R. KILLICK-KENDRICK,
W. M. HUTCHISON, J. CHR. SIIM,
P. C. C. GARNHAM

Summary

Two chimpanzees were given by mouth large numbers of viable oocysts of *Toxoplasma gondii* obtained from the faeces of experimentally infected cats. Before the experiment the first chimpanzee had a positive dye test reaction (1:250), an indication that it had undergone an earlier infection of toxoplasmosis; the serum antibody titres remained unchanged, no evidence of illness was found, and oocysts did not appear in its faeces during the subsequent six weeks. The second chimpanzee showed a negative dye test reaction before infection, and this converted to positive on the 7th day, rose to a peak on the 35th day, and remained high for six months. This animal appeared unwell during the first week, and on the 7th day its blood proved infective to mice; on the 40th day the lymph nodes became enlarged and biopsy specimens of a node and muscle in the 11th week were also infective to mice. No oocysts were passed in the faeces. The presumed cycle in the

Ross Institute, London School of Hygiene and Tropical Medicine,
London W.C.1.

C. C. DRAPER, D.M., Senior Lecturer
Department of Zoology and Applied Entomology, Imperial College,
Ascot, Berks, SL5 7DE

R. KILLICK-KENDRICK, M.PHIL., Research Assistant
P. C. C. GARNHAM, C.M.G., M.D., F.R.S., Professor Emeritus
Department of Biology, University of Strathclyde, Glasgow C.1

W. M. HUTCHISON, B.SC., PH.D., Senior Lecturer
Department of Toxoplasmosis and Viral Diseases, Statens Serum-
institut, Copenhagen, Denmark

J. CHR. SIIM, M.D., Director

chimpanzee and in man and the relationships between *Toxoplasma* and *Isospora* are discussed.

Introduction

Hutchison *et al.* (1970) elucidated the coccidian nature of *Toxoplasma gondii* by feeding specific pathogen-free cats with material infected with the cysts of this organism and observing typical stages of a coccidian parasite in the intestinal mucosa and faeces of the animals at various intervals after ingestion of the cysts. This work was confirmed by Sheffield and Melton (1970) and by Frenkel *et al.* (1970) in the United States, and by other workers in Germany, Holland, and Brazil. In all instances the cat was the experimental host and the infective forms were oocysts of a characteristic isosporan morphology (with two sporocysts each containing four elongated sporozoites).

Though toxoplasmosis is known to be transmitted in various ways, such as by placental passage, contamination of wounds, and consumption of infected uncooked meat, these new observations indicate that the natural biological cycle involves the swallowing of oocysts and the subsequent development of the parasite in the small intestine.

T. gondii is such a common human parasite that it is obviously important to determine the significance of these stages in the life history of the organism in natural infections in man. Though such infections are usually inapparent and rarely accompanied by disease it is clearly unjustifiable to use human volunteers to observe the reaction of man to the ingestion of oocysts. Accordingly, we fed oocysts to two chimpanzees, animals which are well known to exhibit a response to protozoan parasites similar to that shown by man, particularly if they have been previously splenectomized. Only two chimpanzees were available; it would have been better to have used a larger number but this was impracticable because of the high cost of these animals. Nevertheless, the results obtained suggest the sequence of events which may follow the ingestion of *toxoplasma* oocysts by man.

Our observations were directed to the following points: (1) clinical response, (2) passage of oocysts, (3) recovery of the organism in blood or tissues, (4) serological reaction, and (5) persistence of cysts in organs of animals after a long period (this work will be the subject of a later note.)

Materials and Methods

Oocysts of *T. gondii* were prepared by feeding tissue cysts of the Beverley strain to specific pathogen-free cats and recovering oocysts from their faeces by flotation (Hutchison, 1967). No other protozoan parasites were present in the faeces and the viability of the oocysts was checked by intraperitoneal inoculations into white mice. Two immature female chimpanzees about 15 kg in weight were used for the experiments. The older, "Bonnie," had undergone splenectomy one and a half years previously and soon afterwards had had a sporozoite-induced infection of *Plasmodium vivax*; about six months previously she had had a blood-induced infection of *Plasmodium falciparum*. The other chimpanzee, "Justine," had had a splenectomy and a sporozoite-induced infection of *P. vivax* about one year previously. In both animals low parasitaemias from relapses of the *P. vivax* infections were occasionally seen.

Oocysts were given to the chimpanzees by making a suspension in a milk substitute which was readily drunk. Thereafter all faeces found in their cages were collected every one or two days and were examined for excreted oocysts by flotation. The separations were allowed to stand for two weeks at room temperature and each was examined microscopically for oocysts before being administered to a group of four mice. Each mouse in the group received orally 1 ml of a suspension containing approximately 1/16 of the separated material. After five weeks all mice were killed and their brains were examined for tissue cysts; dye tests were performed on samples of sera.

Several specimens of serum were taken from each chimpanzee before attempted infection and thereafter they were bled at about weekly or, later, fortnightly intervals. The coded sera were stored at -20°C and were later examined for specific antibodies by the dye test of Sabin and Feldman (1948) as standardized by Aagaard (1960), and by a complement fixation test. Some sera were also examined by an indirect fluorescent antibody test, following in general the method of Kagan and Norman (1970) and using a Nordic rabbit antihuman serum conjugate. All serum titres are given as reciprocals of the dilutions.

Each time the chimpanzees were bled after infection 0.25 ml of whole blood, treated with Sequestrene, was at once inoculated intraperitoneally into each of four mice. These had previously been bled and their serum had been examined by the dye test and found negative; they were kept separately from the chimpanzees. The mice were looked at every few days and the brains of any that were sick or dead were examined for tissue cysts. After periods ranging from 5 to 12 weeks the surviving mice were bled and dye tests were performed; the

brains of any that showed a serum conversion from negative to positive were examined to confirm the diagnosis.

Total white blood cell counts were also done on the blood from the chimpanzees.

Results

Chimpanzee Bonnie was used for the first experiment. Several specimens of sera taken before attempted infection had shown the presence of pre-existing antibodies to toxoplasma, titres of 250 in the dye test and of 16 to 32 in the complement fixation test being obtained. She was then fed about 1.5 million oocysts. Some of the actual oocyst suspension was also later force-fed to mice, all of which became sick or died after two to three weeks and tissue cysts were seen in their brains. Her faeces were examined for five weeks but no oocysts were seen, though numerous other protozoan cysts, and nematode ova and larvae, were found. These are common in chimpanzees and could not have come from the inoculum as this contained only toxoplasma oocysts. No isolations of toxoplasma were made from weekly blood samples taken over seven weeks, and neither in these samples nor in later ones, taken up to 16 weeks after attempted infection, was any antibody change measured by the dye and complement fixation tests. There were no significant variations in the white blood cell counts and the animal remained well throughout. We assume that it had suffered a previous infection with toxoplasma and was immune to re-infection.

A different course of events was observed with Justine, in whose serum there were no pre-existing toxoplasma antibodies. She was fed a total of about 2.5 million oocysts in two doses on consecutive days; the first dose was a new preparation and the other was material remaining from the first experiment two months earlier. Tissue cysts were found in the brains of mice dead or sick two to three weeks after having been fed oocyst suspensions from both occasions. The chimpanzees' faeces were examined for a period of six weeks but no toxoplasma oocysts were seen though, as in Bonnie, many other organisms were found. However, an isolation was made from blood taken one week after infection; two of four inoculated mice showed a dye test conversion to positive and tissue cysts were found in the brain of one of them. Weekly blood samples from Justine were tested for up to nine weeks after infection but no other isolations were made.

During the first week the animal appeared listless and off its food; there was no diarrhoea. Some temporary enlargement of

the superficial lymph nodes was noticed about six weeks after infection, but there was no appreciable variation in the white blood cell counts over a period of 13 weeks. Retinoscopy at 11 weeks showed no evidence of chorioretinitis or other pathological changes in the eyes. Eleven weeks after the animal had received the oocysts biopsy specimens were taken of an inguinal lymph node and of muscle from the thigh, and a lumbar puncture was done to obtain cerebrospinal fluid. About 1 g of the node and muscle were separately ground up in isotonic saline and 0.25 ml of each suspension and a similar amount of cerebrospinal fluid was inoculated into three groups of five mice. The first two groups showed dye test conversions and tissue cysts were found in their brains, while the third group, which received cerebrospinal fluid, remained negative.

The serological findings in Justine are shown in Fig. 1. The titres of the antibodies demonstrated by the dye and indirect fluorescent antibody tests started to rise one week after infection, when the isolation of toxoplasma from blood was made, and continued to rise steadily until plateaux of 6,250 and 4,096 respectively were reached by the 35th day. The first rise in complement fixation antibodies was detected on the 20th day. The dye and indirect fluorescent antibody test antibodies had similar titres throughout the observations, whereas the complement fixation antibodies ran parallel at a lower level.

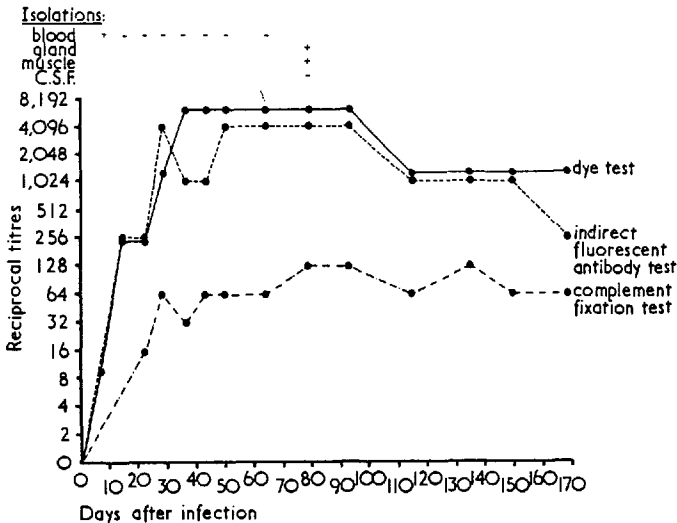


FIG. 1.—Serum antibody titres in chimpanzee Justine after feeding with toxoplasma oocysts.

Discussion

The serum of the first chimpanzee (Bonnie) gave positive dye and complement fixation tests before the administration of oocysts, and the subsequent serological reactions suggest that there was no response to the new infection. Premunition is thought to be the usual response to a previous infection with toxoplasma and such an animal would be resistant to challenge.

The serum of the second chimpanzee (Justine) gave negative antibody reactions before the experiment began and then converted to positive in a typical response later. In spite of the massive dose of oocysts the clinical reaction was slight and similar to the latent or mild form which the infection usually assumes in man. The failure of the animal to produce oocysts is again typical of what apparently happens in other animals (except cats) after infection either by cyst-infected meat or by oocysts (see Fig. 2).

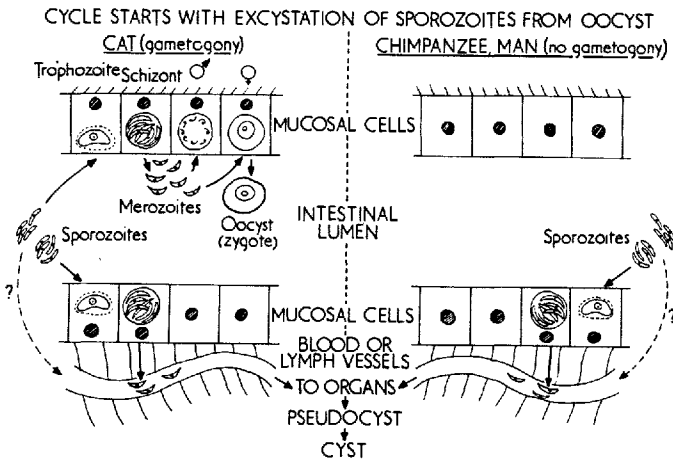


FIG. 2—Possible developmental cycles of toxoplasma in different hosts.

It might be thought that the inability of animals other than cats and various wild felines (Frenkel, 1970) to produce oocysts is due to their unsuitability as normal or biological hosts for

T. gondii. The oocyst is the product of the sexual cycle and there are many examples in other sporozoan parasites, such as malaria, of the extinction of the sexual cycle in abnormal hosts, though the asexual cycle may be profuse. Some support is given to this theory by the course of the infection in the second chimpanzee. The sporozoites from the oocysts must have invaded the intestine and presumably have given rise to at least one cycle of schizogony in the mucosal cells. It is suggested that the merozoites of this generation, instead of continuing schizogony and gametogony in the intestine, invade the portal circulation or lymphatics to reach the lymphatic nodes (see Fig. 2). Here they enter mesodermal cells to produce pseudocysts after multiplication by endodyogeny. It is possible, however, that the sporozoites proceed directly to such a site.

Smetana (1933) showed that the sporozoites of another coccidian parasite (*Eimeria stiedae*) travel directly from the duodenum via the blood stream to the bile ducts where they start their endogenous development; also Sheffield and Melton (1970) showed that sporozoites, freed from the oocysts of *T. gondii*, are capable of undergoing immediate asexual development in tissue cultures of monkey kidney cells. Lotze *et al.* (1964) reported the metastatic growth of the coccidian parasite *Eimeria arloingi* in the mesenteric lymph nodes 13 days after lambs and goats had received oocysts.

Our own observations showed, firstly, that the blood of the chimpanzee contained *T. gondii* on the 7th day after ingestion of oocysts, and, secondly, that lymph nodes and muscle removed in the 11th week were also infective. We suggest therefore that the possible course of infection in man and other animals is invasion of the intestine by the sporozoites, followed either by a preliminary cycle of schizogony in the mucosa or by transport to the neighbouring lymph nodes where they multiply by endodyogeny into pseudocysts. If the former occurs then the merozoites are assumed to penetrate into blood vessels in the intestinal wall and thereby reach lymph nodes and other tissues. This so-called aberrant cycle involving the production of pseudocysts and tissue cysts is, however, peculiar to toxoplasma and is of totally different character from the rare metastatic development of *E. arloingi* where division of the parasites takes place by schizogony as in the primary seat in the intestinal mucosa. The existence of the aberrant cycle is the chief reason for retaining toxoplasma as a separate genus. Nevertheless, it would be interesting to inoculate portions of intestine containing mature schizonts of various species of *Eimeria* or *Isospora* into homologous or heterologous hosts which have been given immunosuppressant therapy. Perhaps, then a generalized "toxoplasma" type of infection would be established.

Valid species of *Isospora* occur in man and cats in addition

to many other vertebrate animals, and their oocysts require careful differentiation from those of *T. gondii*. Two species of *Isospora* are found in man, *I. belli* and *I. hominis*. The oocysts of *I. belli* are a characteristic shape and are larger than those of *T. gondii*. There is, moreover, some evidence that a previous infection of *I. belli* confers no immunity to toxoplasma. Garnham and Killick-Kendrick (1965) reported a laboratory infection of *I. belli* in a man whose serum, when examined four years later, showed a titre of 1,280 in the indirect fluorescent antibody test for toxoplasma; this indicates a recent infection of toxoplasmosis and an absence of any cross-immunity derived from the earlier infection with *I. belli*. Oocysts of *I. hominis*, on the other hand, are similar in size to those of *T. gondii*, and it is not yet known if there is a cross-immunity between these two parasites.

The presence of several species of *Isospora* in cats, in addition to natural infections of *T. gondii* (Jacobs, 1967), may give rise to confusion. Zaman (1970) compared the morphology of oocysts of toxoplasma with those of *I. felis*, *I. rivolta*, and the large type of *I. bigemina*, and it is clear that toxoplasma is different from these species of *Isospora*. But the oocysts of the small type of *I. bigemina* are similar to, if not identical with, oocysts of toxoplasma, and the possibility that they may be one and the same cannot be discounted on morphological grounds alone. Infections of cats with oocysts of the large type of *I. bigemina* have been shown by Owen (personal communication) to be accompanied by sera giving a negative dye test.

We owe many thanks to Miss J. Dorrell, of the Ross Institute, for much assistance with the observations on the chimpanzees. The dye and complement fixation tests were done by Miss Ebba Moller and Miss Else Lauritzenen, and the indirect fluorescent antibody tests were done by Mr. S. Thaver. We are grateful to Mr. M. Hime for performing the biopsies and the retinoscopy, and we acknowledge also useful discussions with Professor L. J. Bruce-Chwatt. The chimpanzees were purchased from funds kindly made available by the World Health Organization.

References

- Aagaard, K. (1960). In *Human Toxoplasmosis*, ed. J. Chr. Siim, p. 206. Copenhagen, Munksgaard.
- Frenkel, J. K. (1970). *Journal of Infectious Diseases*, **122**, 553.
- Frenkel, J. K., Dubey, J. P., and Miller N. L. (1970). *Science*, **167**, 893.
- Garnham, P. C. C., and Killick-Kendrick, R. (1965). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **59**, 359.
- Hutchison, W. M. (1967). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **61**, 80.

- Hutchison, W. M., Dunachie, J. F., Siim, J. Chr., and Work, K. (1970). *British Medical Journal*, **1**, 142.
- Jacobs, L. (1967). *Advances in Parasitology*. London, Academic Press.
- Kagan, I. G., and Norman, L. (1970). In *Manual of Clinical Microbiology*, ed. J. Blair, E. H. Lennette, and J. P. Truant, Chapt. 51. Bethesda, American Society for Microbiology.
- Lotze, J. C., Shalkop, W. T., Leek, R. G., and Behin, R. (1964). *Journal of Parasitology*, **50**, 205.
- Sabin, A. B., and Feldman, H. A. (1948). *Science*, **108**, 660.
- Sheffield, H. G., and Melton, M. L. (1970). *Science*, **167**, 892.
- Smctana, H. (1933). *Archives of Pathology*, **15**, 330.
- Zaman, V. (1970). *South East Asian Journal of Tropical Medicine and Public Health*, **1**, 329.

Reprinted from
ISOLATION AND MAINTENANCE
OF PARASITES *IN VIVO*

SYMPOSIA OF THE
BRITISH SOCIETY FOR PARASITOLOGY

VOLUME 9

edited by
ANGELA E. R. TAYLOR
and
R. MULLER

Blackwell Scientific Publications
Oxford and Edinburgh
1971

THE COLLECTION OF STRAINS OF MURINE MALARIA PARASITES IN THE FIELD, AND THEIR MAINTENANCE IN THE LABORATORY BY CYCLICAL PASSAGE

R. KILLICK-KENDRICK

*Department of Zoology and Applied Entomology, Imperial College
Field Station, Ascot, Berks.*

The subspecies of the two murine malaria parasites, *Plasmodium berghei* and *P. vinckei*, are known from only four countries of West and Central Africa: Congo-Kinshasa; the Central African Republic (C.A.R.); Congo-Brazzaville; and Nigeria* (Fig. 1). Strains have been collected from mosquitoes and rodents or from rodents alone in each of these countries.

In Katanga Province of Congo-Kinshasa, *P. berghei berghei* Vincke and Lips, 1948 has been found in a number of localities including forest galleries at Kisanga and Kasapa; near Lumbumashi (Elisabethville); at Kanzenze, near Kowezi; on the eastern edge of the Kundelungu Plateau; at Sandoa, Kamina and Kamena; and just north of Kalemi (Albertville) (Vincke, 1954; Vincke *et al.*, 1966; Bafort, 1969). The collecting sites are in forest galleries at high altitude (1,000–1,700 m above sea level) notable for the low ambient temperatures throughout much of the year (Vincke, 1954; Yoeli, 1965; Vincke *et al.*, 1966).

The type locality of *P. vinckei vinckei* Rodhain, 1952, also in Katanga Province, is a forest gallery on the River Kinga 9 km south of the village of Kamena, where the only two known strains of this subspecies were isolated from wild-caught mosquitoes (Rodhain, 1952; Bafort, 1967). Bafort (1969) gives an account of the topography of the locality, which lies 1,100 m above sea level with notably low temperatures within the gallery.

In the C.A.R. strains of *P. berghei yoelii* Landau and Killick-Kendrick, 1966 and *P. vinckei chabaudi* Landau, 1965 have been isolated from rodents collected at several places not far from the Station Expérimentale de la Maboké, 150 km from Bangui, including the village of Bébé, between Bébé and Boukoko, and between Bébé and Bagandou (Landau and Chabaud, 1965; Landau and Killick-

* British and French workers recognize the parasites from the different countries as subspecies of *P. berghei* and *P. vinckei* (Landau and Killick-Kendrick, 1966a, b; Garnham *et al.*, 1967; Garnham 1967, 1968; Landau *et al.*, 1968, 1970) but Bafort *et al.* (1968) and Bafort (1970) doubt the wisdom of dividing *P. berghei* into subspecies and question the differential criteria.

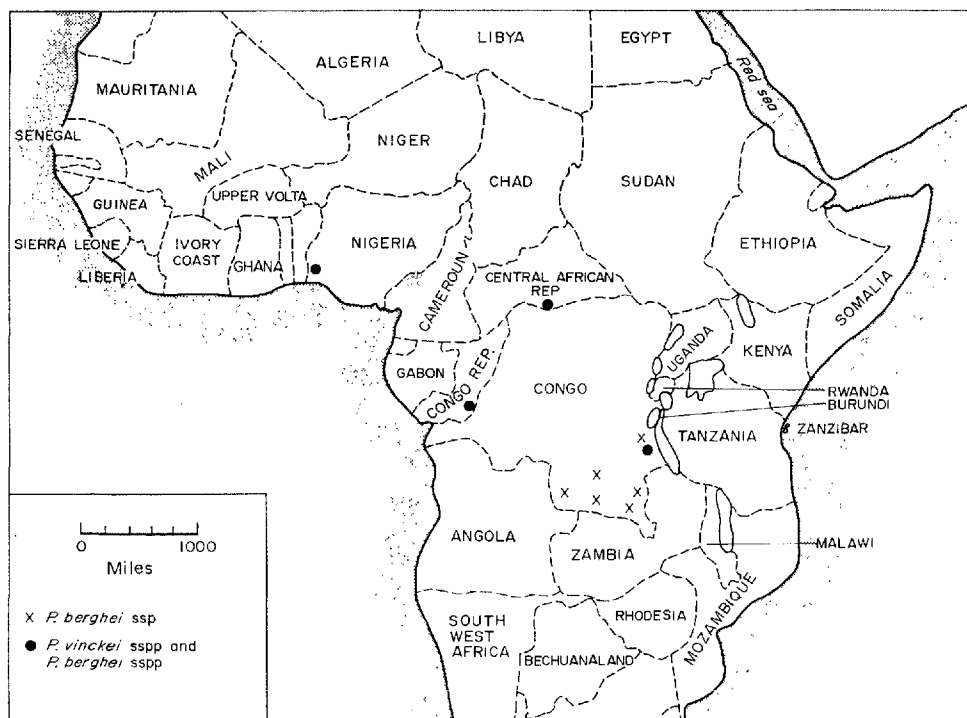


FIG. 1. Map of Africa showing the known distribution of murine malaria parasites.

Kendrick, 1966b). These villages lie in secondary forest on the edge of high forest at an altitude of 600 m; ambient temperatures are higher than in Katanga. Widespread surveys have not been done in the C.A.R., and there is no reason to suppose that the distribution of *P.b. yoelii* and *P.v. chabaudi* is limited to these few places.

The type locality of *P. berghei killicki* Landau, Michel and Adam, 1968 and *P. vinckei lentum* Landau, Michel, Adam and Boulard, 1970 is a forest gallery near the village of N'ganga Lingolo, 15 km from the capital of the Congo-Brazzaville. Strains were isolated from rodents caught on a wooded slope on the right bank of a small stream on the western border of this small, very degraded forest (Adam *et al.*, 1966; Landau, 1966). Apart from the lower altitude (600 m) and a higher ambient temperature, this locality is said to resemble the ones in Katanga. Although the forest is a reserve, it is being rapidly destroyed by farming activities. Adam (personal communication) is examining rodents from other parts of the country in an attempt to discover other enzootic localities.

In the Western State of Nigeria, as yet unnamed subspecies of *P. berghei* and *P. vinckei* have been isolated from rodents trapped in secondary forest and farmland along the edge of the Ilaro Forest Reserve near the village of Ilobi (longitude 3°03 E., latitude 6°45 N). This is the lowest of the lowland localities, lying

at an altitude of only 100 m (Bruce-Chwatt and Gibson, 1955; Killick-Kendrick *et al.*, 1968).

The distribution of murine malaria parasites thus appears to be restricted to the periphery of the Lower Guinea or Congo Forest, the degraded fringes of which lie in Katanga in the east and Nigeria in the west (see Moreau, 1964). Unpublished results of surveys in other parts of Africa by Gunders and Bray (Liberia), Michel (Ivory Coast), Cross (Sierra Leone), Davis (South Africa), Keymer (Malawi) and Matson (Rhodesia) have so far failed to reveal collecting sites other than in the Lower Guinea Forest. The principal vertebrate hosts in the enzootic localities are thicket-rats, *Grammomys* and *Thamnomys*,* semi-arboreal rodents which live in forest galleries or in secondary forest formed by farming activities (Plate 1). If these and other semi-arboreal rodents are intensively collected and properly examined in unsurveyed parts of the Lower Guinea Forest, new collecting sites will probably be discovered, but evidence is slowly accumulating that malaria parasites may be absent in murine rodents in other parts of the continent.

COLLECTION OF STRAINS FROM RODENTS

INCIDENCE OF INFECTION

In the C.A.R. and Congo-Brazzaville, the only murine host of *Plasmodium* yet discovered is *Thamnomys rutilans*. Remarkably high rates of infection have been recorded in the C.A.R., where Landau and Chabaud (1965) found every adult thicket-rat was infected with *P.v. chabaudi* and about half were additionally infected with *P.b. yoelii* but infections were never seen in young thicket-rats aged about 2 months.

In Brazzaville, the incidence of infection is lower. From the examination of a single thick blood-film from each animal, Adam *et al.* (1966) found 10 out of 24 *Thamnomys* collected in March-May 1966 infected with *P.v. lentum*; one rat was also infected with *P.b. killicki*. A second strain of the latter parasite was isolated from a *Thamnomys*, also with a double infection, collected in September, 1966 (Landau *et al.*, 1968).

Unpublished observations by Bruce-Chwatt and Gibson and later work by Killick-Kendrick *et al.* (1968) show that *Thamnomys rutilans* is again the principal vertebrate host in Western State, Nigeria. More than 2,500 Nigerian rodents have been systematically examined by the inoculation of blood into white mice. Only 1 out of 1,607 *Praomys tullbergi* was found infected with a *P. vinckei*-like parasite (see Bruce-Chwatt and Gibson, 1955), whereas 6 out of

* Rosevear (1969) suggests that 3 or 4 closely related genera of thicket-rats may eventually be recognized, but at the moment he separates only *Thamnomys* and *Grammomys*. The latter genus lacks gutter-hairs in the pelage, and in Rosevear's view also differs from the former in cuspidation and cranial features.

18 *Thamnomys rutilans* were infected with what was almost certainly the same parasite; 1 *Thamnomys* was also harbouring a second parasite which is thought to be a new subspecies of *P. berghei* (Killick-Kendrick *et al.*, 1968). During November 1968 Lambo (personal communication) examined blood films from 7 *Thamnomys* from Ilobi and found malaria parasites in 5; in 1969 he and Mrs. Otitoju found 2 out of 6 infections in January, 0 out of 24 in April and 3 out of 16 in May. It thus seems possible that transmission in western Nigeria may be at a peak in November, about a month after the end of the rainy season.

In the early work in Nigeria, blood from good samples of *Lemniscomys striatus*, *Hybomys trivirgatus*, *Stochomys longicaudatus* and *Lophuromys sikapusi* failed to give rise to infections in mice, and it may be assumed that these species are seldom, if ever, infected in nature. Some other rodents in the locality, notably *Leggada musculoides* have, however, not been examined and further work may yet reveal other hosts.

Of the four known localities, the infection rate in rodents is the lowest in Katanga where three murines *Grammomys surdaster*, *Praomys jacksoni* and *Leggada bella*, have been found infected (Vincke, 1954). Unlike the lowland localities, patent infections in Katangan rodents have only seldom been seen (Vincke, 1950). Subpatent infections of *P.b. berghei*, diagnosed by the inoculation of blood into mice or white rats, were found in 7 out of 61 *Grammomys* and 5 out of 99 *Praomys* (Vincke, 1954). Infected animals were found in both the wet and dry seasons. During Bafort's (1969) work on the River Kinga, blood from 48 rats was inoculated into white mice and one strain of *P.b. berghei* was recovered from a *Grammomys*. No natural infections of *P.v. vinckei* patent or subpatent, have yet been encountered in rodents in Katanga.

The low incidence of infection in Katangan rodents contrasts sharply with the reports from the lowland localities, and is difficult to understand. There may be other hosts not yet discovered, or the presence of piroplasms in Katangan rodents (see van den Berghe *et al.*, 1950; Rodhain and Vincke, 1953; Bafort *et al.*, 1970), may afford some protection against malaria. A cross-immunity between murine malaria parasites and *Babesia rodhaini*, a piroplasm of *Grammomys*, has been demonstrated (Cox and Milar, 1968). In practical terms, the low incidence means that strains of the Katangan parasites (*P.b. berghei* and *P.v. vinckei*) are more usually isolated from wild-caught mosquitoes than from rodents. In the lowland localities, however, where the vectors are not known, the best approach to the collection of strains is the same in each place; to paraphrase Mrs. Beeton, first catch your thicket-rat.

TRAPPING THICKET-RATS

Suitable traps, used by French workers in Brazzaville and British workers in Nigeria, are available from Manufrance, St. Etienne, France (catalogued as



PLATE I. Habitats of the thicket-rat *Thamnomys rutilans* at Ilobi, Western State, Nigeria. A. Cleared farmland and secondary forest on fallow farmland. B. Cocoa plantation. (Photographs by Dr. F. R. S. Kellett.)

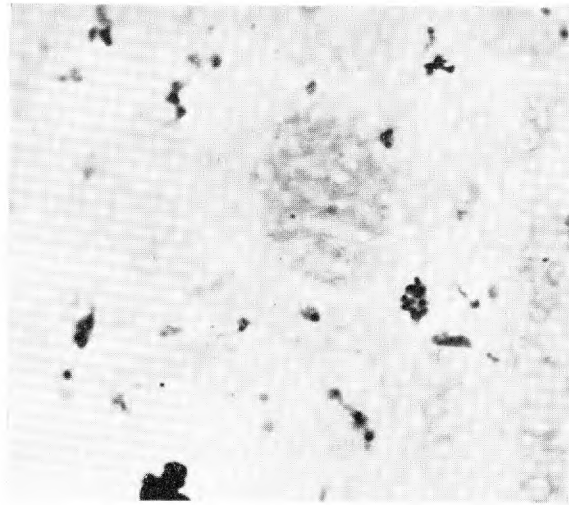


PLATE II. A. Nest of *Thamnomys rutilans* in a cocoa tree in the plantation shown in Plate I
 B. Nigerian *P. vinckei* in a stained thick-blood film from a naturally infected *Thamnomys rutilans* ($\times 1,500$).

"16-7870 Ratier"). They are made of galvanized wire, spaced at intervals of 1 cm, forming a rectangular tunnel $40 \times 12 \times 14$ cm. At each end are spring-operated doors which are released by the movement of a vertical locking-wire bearing the bait. *T. rutilans* takes apple, banana, or palm-nuts readily, whereas Bafort (1969) caught *G. surdaster* and *Praomys* with groundnuts or cassava.

In Nigeria, *T. rutilans* was caught only in traps set in bushes, and among the 2,000 rodents caught on the ground by Bruce-Chwatt and Gibson, none was a thicket-rat. *G. surdaster*, on the other hand, appears to be less arboreal in habit than *T. rutilans* and may be caught on the ground (Bafort, 1969).

The presence of nests of *T. rutilans* is an infallible guide to a good trapping site. In Nigeria the nests are built in creepers, and banana and cocoa trees, 1.5-2.5 m from the ground (Plate IIA). The preferred habitat is fallow farm land and secondary forest. The nests are oval and measure about 18×12 cm. They have an outer layer of leaves untidily woven from the bush in which the nest is built. The inner part of the nest is made of long strips of stems of bushes looking like dried grass; there is usually no obvious entrance. During the day *T. rutilans* is found in the nest which it quickly leaves as soon as the nest is disturbed. In the C.A.R., Genest and Petter (in press) found that the nests of *T. rutilans* lacked the outer leaves always found in Nigeria.

There is apparently no published description of the nests of *G. surdaster* although it has been said that this thicket-rat nests in holes in trees (Yoeli, 1965; Yoeli *et al.*, 1965).

In parts of Katanga and the C.A.R. where thicket-rats are hunted for the pot, large numbers of living rats may be bought from hunters. In villages north of Ibadan in Nigeria, Happold (personal communication) obtains *Thamnomys* from schoolchildren who catch the rats in traps made of tins.

Apart from *T. rutilans* and *G. surdaster*, there are several other thicket-rats which appear not to have been examined for malaria. These, and other murine rodents with arboreal and semi-arboreal habits such as *Thallomys*, *Dephomys*, *Hylomyscus* and *Oenomys*, should be considered as possible hosts of malaria parasites.

Many African murids are difficult to identify, and it is advisable to eviscerate all specimens and fix them in 10% formalin so that field identifications may be confirmed by specialists.

EXAMINING WILD RATS FOR MALARIA PARASITES

Patent infections are diagnosed by the examination of thick and thin blood films of caudal blood (obtained by cutting off the tip of the tail), after staining in Giemsa's stain diluted with buffered water at pH 7.2 (see Shute and Maryon, 1966; Garnham, 1966). Parasites may be difficult to recognize in thick films, but as long as they are stained 3-24 hours after preparation, reasonable lysis in staining takes place, and readable films are produced (Plate IIB). In humid

conditions films should be dried with gentle heat; they must be protected (e.g. in a plastic bag) against the predations of flies and ants.

Subpatent infections are diagnosed by the intra-peritoneal inoculation of up to 1 ml of blood, obtained by heart puncture under ether anaesthesia or from the retro-orbital plexus, into laboratory-bred rodents. Such subinoculation of blood or other tissue into a susceptible host as a diagnostic test is termed 'isodiagnosis' by Sergent (1963).

If wild African rodents are caught in conditions where it is not possible to prepare and examine blood films, a rough survey can be done simply by killing and opening the animals and looking at their spleens. In chronic low-grade infections this organ, and to a lesser degree the liver, becomes pigmented, though not necessarily enlarged. Spleens of animals with primary parasitaemias are not pigmented, but it is unlikely that all infections will be at this stage. If pigmented spleens are seen, the preliminary survey should be followed by attempts to demonstrate parasites and establish strains in the laboratory.

An efficient routine of examination using a combination of methods is to collect the animals alive, and, firstly, to examine stained thick and thin blood films. Rodents with negative films are then anaesthetized with ether, and blood is collected by cardiac puncture using heparin as an anticoagulant. The next step is to open the animal and examine its spleen. If it is pigmented the blood is inoculated into white mice, blood films from which are regularly examined. If the spleen looks normal, the blood is discarded and the animal is assumed to be uninfected. A great advantage of this combination of methods is that large numbers of mice are not needed, yet subpatent infections are still detected.

If colonies of laboratory animals are not available, subpatent infections can be diagnosed by splenectomizing rodents and awaiting a recrudescence of parasitaemia. This method may reveal parasites with a low infectivity to laboratory-bred rodents which would be overlooked by isodiagnosis.

Serological techniques have yet to be employed in surveys of murine malaria but new methods of collecting small samples of blood on filter paper overcome the problems of collecting sera in the field, and will probably lead to the Fluorescent Antibody Test being used for large-scale surveys. Once again, the results of such work must be considered as a preliminary guide to indicate which species of rodents in which locality should be more intensively studied.

COLLECTION OF STRAINS FROM MOSQUITOES

At the time of writing, the only proven invertebrate host of murine malaria parasites in nature is *Anopheles durenii millescampsi* in Katanga. During the early work, the Belgian investigators suspected they were dealing with a variety of the little known *A. durenii* (see Vincke, 1954) which was eventually named *A. durenii*

var. *millecampsi* by Lips (1960). Gillies and de Meillon (1968) raised the variety to a subspecies.*

This is a shade-loving, sylvatic mosquito found only in Katanga; adults are confined to forest galleries, and the larval habitat is the margin of swiftly flowing streams within the galleries. During the wet season (December-April) adult mosquitoes may be very abundant, but at other times of the year, Vincke (1950, 1954) was unable to find them.

Astonishingly high rates of infection with *P.b. berghei* have been reported in *A.d. millecampsi* by several workers. Yoeli and Boné (1967) dissected many specimens of this subspecies collected at Kisanga near Lumbumbashi (Elisabethville) and recorded 'general infection rates' of 14.4 to 17.2 %. In an earlier account of this work, Yoeli *et al.* (1964) reported an oocyst rate of 24 % (81/334) and a sporozoite rate of 10.5 % (35/334). Vincke (1950) examined the salivary glands of *A.d. millecampsi* collected throughout the rainy seasons of 1944-46 and found an overall sporozoite rate of 7%. Later, the same worker (1954) plotted the sporozoite rates month by month of mosquitoes collected at Kasapa and Kisanga early in 1950. The first positive glands were found in January, peak rates of 8 and 11.2% were recorded in February and the last infected mosquitoes were collected in April.

Such high rates are not always found. At Kamena, Bafort (1967) dissected 500 wild-caught *A.d. millecampsi* and recorded a sporozoite rate of only 1.26%. In a more full account of this work Bafort (1969) reported an overall rate of 1.42% (14/984), and recorded oocysts on the midgut of only 1 out of 91 mosquitoes. When sporozoite rates are high, numerous strains are easily isolated. Vincke (1954), for example, isolated 367 strains of *P.b. berghei* from *A.d. millecampsi* collected in 4 separate regions of Katanga.

In striking contrast to the extensive entomological investigations in Katanga, almost nothing is known of the invertebrate hosts of murine malaria parasites in other parts of Africa. Because of its limited distribution, *A.d. millecampsi* is obviously not a host in localities other than Katanga. Vincke (1967), drawing on a lifetime's experience of African mosquitoes, postulated that the vector of the parasites of the C.A.R. would be found to be one of the exophilic, zoophilic species which are often very abundant in restricted areas which are difficult to find. In Nigeria it has been suggested that the vector at Ilobi is probably one of three anophelines, *A. cinctus*, *A. coustani* or *A. obscurus* which occur there, but which are never taken in catches when man is the bait (Killick-Kendrick *et al.*, 1968). Many years ago, Barber and Olinger (1931) saw oocysts on the midguts of 2 specimens of wild-caught *A. obscurus* collected in southern Nigeria, but in the laboratory they were unable to infect this species with malaria parasites of man; perhaps the oocysts were of a murine malaria parasite.

* By article 10(b) of the International Code of Zoological Nomenclature (1964), the correct citation is *Anopheles durenti millecampsi* Gillies and de Meillon, 1968.

In Brazzaville, Adam (personal communication) found very long sporozoites, typical of the murine malaria parasites of Congo-Brazzaville (Landau *et al.*, 1970), in the salivary glands of a single lightly infected *A. cinctus* collected in the gallery forest at N'ganga Lingolo, where about half the thicket rats harbour malaria parasites. Although the identity of the sporozoites was not confirmed by the inoculation of rodents, the striking morphology suggests that *A. cinctus* may be the vector in Brazzaville. Support for this comes from the close relationship between *A. cinctus* and *A. durenii*, both of which lie in the *ardensis* section of series *Neomyzomyia* in the subgenus *Cellia* (see Gillies and de Meillon, 1968). Until the vectors in the lowland localities are identified with certainty, the isolation of strains from sylvatic mosquitoes is a method of use only in Katanga. But workers in other places will follow the trail blazed by Vincke, and it should not be long before murine malaria parasites are collected from anophelines in other parts of Africa.

COLLECTION OF *A.d. MILLECAMPSI*

During the rainy season from December to April, *A.d. millecampsi* may be found in great numbers on the trunks of trees close to small streams in forest galleries in the highlands of Katanga; up to 80% of the females may be engorged with blood from rodents (Vincke, 1950, 1954). Damp, moss-covered trees, especially *Syzygium cordatum*, are particularly favoured. Only two anophelines are found on the trees 1–2 m from the ground. One, *A. implexus*, is only occasionally seen and is easily identified by its large size and the long tufts of narrow scales projecting from the abdominal segments (Gillies and de Meillon, 1968), while the other, *A.d. millecampsi*, is more abundant than *A. implexus*, and is readily recognized by its silvery scales and prominent markings on its legs and wings (Yoeli, 1965; Yoeli and Boné, 1967).

Resting mosquitoes are caught with an aspirator or test-tube. The best time of collection is in the early hours of the morning (06.30–10.00 a.m.) ; in the afternoon and evening it is thought they retreat into tree-holes (Yoeli and Boné, 1967).

The abundance of *A.d. millecampsi* is strictly seasonal—no adults are seen in the dry season—and varies from place to place. In Kundelungu, for example, where several strains of *P.b. berghei* were isolated from rodents, only 40 females of *A.d. millecampsi* (one of which was infected) were collected throughout one wet season. In other places, one experienced collector can catch four times this number in a single morning (Vincke, 1954).

The transport of delicate sylvatic mosquitoes from the field to the laboratory in tropical conditions presents a special problem. Yoeli and his Belgian colleagues packed cartons of engorged females, each containing 50 mosquitoes, into sealed insulated boxes containing a damp sponge; a 5–10% solution of glucose was provided on a dental wick (Yoeli *et al.*, 1964, 1965; Yoeli, 1965).

Three quarters of 7,840 engorged females packed in this way survived a 2–4 day journey from Katanga to New York (Yoeli and Boné, 1967). For shorter journeys, mosquitoes live well in a pre-cooled thermos flask containing ice-cubes in a plastic bag.

EXAMINATION OF WILD-CAUGHT MOSQUITOES

Salivary glands and midguts, in that order, are dissected out by the conventional methods (see Shute and Maryon, 1966; Garnham, 1966), into a suitable physiological fluid (see the section on routine cyclical maintenance). Cover-slips are applied and the preparations are examined with the microscope. Phase contrast illumination is of great help in detecting light infections in glands.

ESTABLISHMENT OF STRAINS IN THE LABORATORY

Strains are established in the laboratory by inoculating rodents with either sporozoites from wild-caught mosquitoes or blood from wild rodents.

When isolating strains from naturally infected *A.d. millescamps* it is better to inoculate sporozoites from the salivary glands rather than from crushed oocysts. This was well demonstrated by Yoeli and Boné (1967), who found that all (14/14) rats given single pairs of infected glands became infected with *P.b. berghei*, but infections arose in only 3 out of 14 inoculated with infected midguts. Since oocysts continue to ripen, and sporozoites to invade the salivary glands after the collection of the mosquitoes, it is preferable to keep the mosquitoes for a few days before dissection.

It is not always realized that the first inoculation into a laboratory rodent is a sudden, new, selective pressure on the parasite and only populations able to adapt quickly to an unusual host will survive. For this reason, perhaps the best host to use for this first taming of a wild parasite is *Grammomys surdaster*, a natural vertebrate host of *P.b. berghei*. A colony of these thicket-rats was established in the laboratory by Yoeli *et al.* (1963). *Thammomys rutilans*, the vertebrate host in the lowland localities, has not yet been bred in the laboratory. Possibly laboratory-bred *Praomys natalensis*, which are more easily bred than *G. surdaster* (Davis and Oettle, 1958), would be as good as the thicket-rat but they appear not to have been used for primary isolations.

If these exotic animals are not available, the choice lies between mice, white rats, and hamsters. Of these, only mice are susceptible to *P. vinckei* spp. on primary isolation, but all three have been used for the establishment of *P. berghei* spp. in the laboratory.

Sporozoites of *P.b. berghei* from wild-caught *A.d. millescamps* may fail to infect white mice, and better results are reported when rats are used. Vincke (1950) inoculated infected glands into rats and mice and recorded infections in 8 out of 10 white rats but 0 out of 15 white mice. Yoeli and Boné (1967) successfully

infected all of 14 white rats (2–3 weeks old) by inoculating each with the glands of a single mosquito, whereas Bafort (1969) similarly inoculated 5 white mice and only one became infected. Some inbred white mice have been shown to be relatively insusceptible to *P.b. berghei* (Most *et al.*, 1966; Vincke and Bafort, 1968) and if these animals are used, outbred lines, or inbred lines of known susceptibility, should be selected.

In experimental infections it has been shown that usually more tissue schizonts develop in the livers of mice and rats given sporozoites intravenously than in those inoculated intraperitoneally (Wéry and Killick-Kendrick, 1967; Wéry, 1968). This suggests that strains from wild-caught mosquitoes are best established in rodents by inoculating the sporozoites intravenously. A novel way of isolating strains from *A.d. millescampsi*, collected when freshly engorged with blood, is to inoculate laboratory animals without delay with the contents of the midgut (Vincke and Lips, 1948, 1950).

All the strains from parts of Africa other than Katanga have been isolated from wild rodents by the intraperitoneal inoculation of blood into white mice (Bruce-Chwatt and Gibson, 1955; Landau, 1965; Landau and Chabaud, 1965; Adam *et al.*, 1966; Killick-Kendrick *et al.*, 1968). Volumes of blood up to 1 ml are readily tolerated. Blood is obtained either with a Pasteur pipette from the retro-ocular plexus, or with a syringe and needle from the heart of the anaesthetized animal. When ether is used, some animals succumb; Penthrane (Abbot Laboratories) is better but it is less volatile than ether and is administered by bubbling air through the liquid. The anticoagulant of choice is heparin B.P., which is free from phenolic preservatives; 20 i.u./ml of blood is enough to prevent clotting.

On primary isolation, initial parasitaemias may be low and fleeting, and inoculated rodents must be examined regularly. Spontaneous recoveries result in the loss of strains. Prepatent periods may be prolonged with untamed strains, and rodents should not be abandoned less than 3 weeks after inoculation.

In Africa, the modern balanced diets for laboratory animals are not generally available, and a mixture of locally obtainable foods is normally given. It is essential that the diet of inoculated animals contains para-aminobenzoic acid, since this is a requirement of malaria parasites without which parasitaemias are much depressed (Hawking, 1953; Adler, 1958). It may conveniently be given as a supplement in the drinking water (0.05%).

When inoculating mice with blood from wild rodents, concomitant infections may present problems. Unexplained deaths of mice within 48 hours of inoculation, which may be caused by viruses, sometimes occur. Shute, Lambo and I (unpublished) lost a number of mice in this way in Nigeria, and Bafort (1969) records similarly rapid deaths in mice in Katanga after the inoculation of either blood from rodents or salivary glands from wild *A.d. millescampsi*.

Another better-known concomitant infection, which is common in African rodents, is the erythrocytic rickettsia, *Eperythrozoon coccoides*. When laboratory rodents are inoculated with malaria parasites contaminated with *E. coccoides* parasitaemias are much depressed (Peters, 1965; Ott *et al.*, 1967). This depression does not occur when malaria parasites are inoculated into mice previously infected with *E. coccoides* (Suntharasamai and Marsden, 1969) but once a strain becomes mixed with the rickettsia, both are passaged together. If cyclical passages are carried out in the laboratory, and care is taken to use mice known to be free from *Eperythrozoon* the rickettsia is lost. Alternatively, strains may be cleaned by blood-passage through mice pre-treated with neo-arsphenamine BPC at a dose of 125 mg/kg (Peters, 1967; Voller and Bidwell, 1968). To ensure laboratory rodents are free from *Eperythrozoon* a control sample is splenectomized; this results in recrudescences of subpatent infections, which are then easily recognized in blood films stained in Giemsa's stain. If virulent strains of *P.b. berghei* or *P. v. vinckei* are being blood-passaged in the same laboratory and there are unexpected recoveries, this is probably a sign of *Eperythrozoon* in the stock mice used earlier.

Another erythrocytic rickettsia, *Haemobartonella muris*, has been shown to have a synergistic effect upon infections of *P.b. berghei* (Hsu and Geiman, 1952), but this organism is apparently seldom met when isolating new strains.

Many small African mammals are infected with yet another rickettsia-like organism which looks like *Anaplasma*. I believe this causes much prolonged prepatent periods and depressed parasitaemias, although its precise effect on malaria in rodents has not been determined; nevertheless, this organism is often seen when strains are difficult to handle.

Mixed infections of *P. berghei* spp. and *P. vinckei* spp. are frequently met in *T. rutilans* from the 3 lowland localities (Landau and Chabaud, 1965; Adam *et al.*, 1966; Killick-Kendrick *et al.*, 1968). Separation of the *P. berghei* spp. may be accomplished by taking advantage of the early appearance of their gametocytes, and the fact that their sporogonic cycles tend to be slightly shorter than those of *P. vinckei* spp. If a susceptible mosquito is fed on infected laboratory rodents when the first gametocytes are seen, and sporozoites are harvested as soon as the salivary glands of the mosquitoes become invaded, mice inoculated with the sporozoites will normally develop unmixed infections of *P. berghei*. This was the way in which the 17X strain of *P.b. yoelii* was separated from *P.v. chabaudi* (Landau and Killick-Kendrick, 1966b). Strain N67 of the Nigerian subspecies of *P. berghei* was similarly separated from *P. vinckei* ssp. by a cyclical transmission through *A. stephensi* (Killick-Kendrick *et al.*, 1968), but in this instance the latter parasite was not transmissible through the mosquito.

The differential susceptibility of white rats and hamsters to *P. berghei* spp. and *P. vinckei* spp. on primary isolation is another method which was successfully used to separate *P.b. killicki* from *P.v. lentum*; the former parasite infected

the rats, but the latter failed to become established in this host (Landau *et al.*, 1968).

It is seldom necessary to separate *P. vinckei* spp. from *P. berghei* spp., since mixed infections are known only from the lowland localities where *P. vinckei* is easily found free from the other species. If wild *T. rutilans* with mixed infections are kept under observation in the laboratory, there are many occasions when only *P. vinckei* is recovered when small samples of caudal blood are injected into white mice. To separate *P. v. chabaudi* from *P.b. yoelii*, advantage can be taken of the presence of highly infective gametocytes of the former parasite after the crisis in parasitaemia, many days after the height of infectivity of gametocytes of *P.b. yoelii* (Wéry, 1968).

MAINTENANCE BY CYCLICAL TRANSMISSION

TECHNIQUES

Rodents upon which mosquitoes are to be permitted to feed are immobilized by attaching adhesive tape to each leg and pinning the tape to a cork board with the animal on its back. When numbering animals, picric acid should not be used as a marker dye, since it appears to deter mosquitoes from feeding. Hair on the chest and abdomen is removed with electric clippers or a razor and the board bearing the rodent is then put inside the cage of mosquitoes, on a stand, so that it is held a few centimetres below the top of the cage. If mice are the gametocyte carriers, the maximum number of mosquitoes per mouse should not exceed 150, or the rodent will probably die before all hungry females have taken a blood-meal.

Unless mosquitoes are particularly hungry, the movements of the rodent may interfere with feeding. Sodium pentobarbitone (5 mg/10 gm body weight intravenously) is a suitable anaesthetic, but very anaemic animals may succumb to the normal dose, and since a slight overdose appears to cause a fall in body temperature, reducing the attraction of the rodent to the mosquitoes, many workers prefer not to use an anaesthetic.

The most commonly chosen mosquito for cyclical passage of murine malaria parasites in the laboratory is *Anopheles stephensi*, although the sporogony of two subspecies of *P. berghei* has been obtained in other anophelines (see Table I). *A. stephensi* is easily bred in the laboratory by the methods given by Shute and Maryon (1966). Care must be taken to avoid damaging adult mosquitoes and thereby shortening their life. The practice of permitting adults to emerge under netting covering larval bowls, and then collecting them with a suction tube is, therefore, not recommended. It is better, though more time consuming, to collect pupae either with a pipette or by the cold-water method of Weathersby (1963), and permit the adult mosquitoes to emerge in cages.

Female *A. stephensi* will be willing to feed 4–8 days after emergence. *A. stephensi* maintained on glucose alone for a long time appear to become progressively less interested in taking blood and do not, as might be expected, become more hungry. This, together with the belief that the susceptibility of mosquitoes to malaria parasites may decrease with age, has led to the routine of giving the infective meal within a week of emergence. When the infective feed is the first blood-meal taken, any unfed nulliparous females are easily seen and removed from the cage with either a suction-tube, or a test-tube containing cotton-wool dampened with chloroform.

During sporogony, mosquitoes are incubated at temperatures ranging from 19–26°C depending upon the subspecies of parasite (see Tables I and II). Humidity must be kept high (75–85%), since *A. stephensi* will not tolerate dry conditions. Two or three days after the infective feed, 20% glucose is provided, and an egg-bowl is put in the cage. Further blood meals appear to be unnecessary for the normal growth of the sporogonic stages (Wéry, 1968).

When sporogony is complete, the strain may be passaged simply by permitting the infected mosquitoes to feed upon laboratory rodents. This is an imprecise method which sometimes results in only a proportion of the rodents becoming infected. It is better to harvest the sporozoites by the mass dissection of salivary glands into cold physiological fluid, to prepare a suspension of sporozoites and inoculate a known dose.

Dissections are best carried out on perspex slides which prevent the drops of fluid, into which glands are dissected, spreading unduly. A practised dissector provided with a continual supply of stunned mosquitoes, with legs and wings removed, can dissect 60–70 mosquitoes per hour. Grinding the thoraces of mosquitoes is an alternative harvesting routine sometimes used (e.g. Vincke and Bafort, 1968); it saves a little time, but the suspension produced contains debris which may kill an animal when inoculated intravenously. Moreover, counts of sporozoites in such suspensions are of little use because immature sporozoites will almost certainly have been harvested.

Twenty percent rat serum in Ringer's fluid may be used to prepare suspensions of sporozoites, but since 1965, this has been largely replaced by commercially available tissue culture medium 199 (Morgan *et al.*, 1950). The survival of sporozoites of *P.b. yoelii* in this medium at 4°C was studied by Fink and Schica (1969). They found that all of 5 mice became infected when inoculated intravenously with 2,500 sporozoites even when they had been kept for 3 hours (at 4°C) in medium 199, but the prepatent periods were slightly prolonged. There is, however, an increasing suspicion that occasionally a batch of medium 199 is lethal to sporozoites, and there is a move to use media devised for the culture of insect tissues. Walliker (personal communication) is successfully using equal parts of Kitamura's medium and medium VP 12 (Varma and Pudney, 1969).

Throughout a mass dissection, the dissecting fluid and the tube containing the harvested glands should be kept cool by immersion in crushed ice, and there is a convention that the total time of dissection should not exceed 1 hour. Fink and Schica (1969) clearly showed a loss of infectivity of sporozoites of *P. b. yoelii* at temperatures higher than 4°C. When the dissection is completed, an even suspension of sporozoites is prepared by gently breaking the glands in a 'Teflon' tube homogenizer.*

For the simple maintenance of a strain, it is not essential to count the sporozoites. But the method is easy, and the prepatent periods following the inoculation of a known number of sporozoites give a guide to the success of sporogony, and the efficiency of harvesting. An improved Neubauer counting chamber is loaded with the suspension and allowed to stand in a damp chamber at room temperature for 10–15 mins. Sporozoites sink slowly, especially when only little fluid has been used and the suspension is thick, and unless time is allowed for them all to sink, a count through the depth of 0.1 mm is difficult and inaccurate. Sporozoites lying in the four outer blocks of 16 squares are counted (a total volume of 0.4 cu mm), the volume of the suspension is measured, and the number of sporozoites in 0.1 ml is estimated. Phase-contrast illumination greatly facilitates rapid, accurate counts, but it is necessary to use either a specially thin haemocytometer or a phase-contrast condenser with a working distance sufficiently long to permit good illumination on the top of the usual thick chamber. The most convenient magnification for counting sporozoites is X300 (X20 objective X15 oculars).

The best route of inoculation of sporozoites is intravenous. Livers of rats and mice given sporozoites intravenously are generally more heavily infected than animals inoculated with the same number intraperitoneally, but the density of infection varies less widely in animals infected by the latter route (Wéry and Killick-Kendrick, 1967; Wéry, 1968). Sporozoites are easily inoculated intravenously in white mice and young white rats; the caudal veins are first dilated by immersing the tail in warm water or warming the animals in an incubator or hot-box. A tube-holder† is useful to restrain mice. The tails of laboratory-bred *Grammomys* and *Praomys* are pigmented, and experience is necessary to inject them intravenously. Small doses may be given intra-cardially to these animals, and to hamsters.

PRESERVATION OF THE VIABILITY OF GAMETOCYTES

Continual blood-passages of murine malaria parasites result firstly in a diminution of the infectivity of gametocytes to mosquitoes, and secondly in the complete loss of gametocytes. It is essential, therefore, either to transmit newly established strains through mosquitoes without delay or to preserve them at low

* Size S20, obtainable from Camblab (Glass) Ltd., Milton Road, Cambridge.

† Obtainable from C. F. Palmer (London) Ltd., Effra Road, London S.W.2.

temperatures in solid carbon-dioxide (-78°C) or liquid nitrogen (-196°C). At these temperatures the ability to produce gametocytes is retained (Yoeli *et al.*, 1963; Vincke *et al.*, 1965). Either glycerol (7.5%, Molinari, 1961) or dimethyl sulphoxide (5%, Collins and Jeffery, 1963) is added to protect the parasites during freezing and thawing. The heat-sink of Goodwin and Thiel (1967) is a useful device for controlling the rate of freezing in liquid nitrogen.

It cannot be assumed that preservation at low temperatures keeps the strains totally unaltered, as it were, in suspended animation. The freezing process imposes selection upon the population, and it is thought that very few parasites survive. Changes may not be beneficial to establishing routine cyclical transmission. Wéry (1968) found that the virulence of *P.v. chabaudi* was enhanced by low-temperature preservation, making the strain more difficult to transmit cyclically.

Strains vary in the number of blood-passages tolerated before the viability of gametocytes is affected. Wéry (1968) showed that infection rates and numbers of oocysts of strain 17X of *P.b. yoelii* in experimentally infected *A. stephensi* fell markedly after only 7 blood passages in mice, but that a single cyclical passage restored the vitality of the gametocytes. Loss of viability is probably related to the experimental host. Michiels (1963), working with a strain of *P.b. berghei*, found that exflagellating male gametocytes were demonstrable in a strain maintained for 2 years in white rats, providing a line was obtained from an animal in the metacritical stage of the infection. In general, however, it is recommended that not more than 5 consecutive blood passages should be performed before either obtaining a cyclical passage, or storing the strain at low temperatures. With parasites which prove difficult to establish in mosquitoes, it is necessary either to have an infected wild rodent from which a line can be obtained for each new attempt, or to preserve numerous samples at low temperature. If attempts to infect mosquitoes fail, it is not long before a blood-passaged line becomes progressively less likely ever to be cyclically transmitted.

Strains which have lost gametocytes have been manipulated in a number of ways resulting in the reappearance of the sexual forms. Jadin *et al.* (1959) revitalized a strain of *P.b. berghei* which had undergone 191 consecutive blood passages in mice over a period of 3 years. Gametocytes appeared when the strain was passed through young hamsters or laboratory-bred *Grammomys*. Mosquitoes fed on these animals became infected, but the sporogony did not lead to the production of infective sporozoites (probably because the temperature at which the mosquitoes were incubated was too high). Some later years Bafort *et al.* (1966) found that preservation of an old strain of *P.v. vinckei* at -75°C appeared to have caused the reappearance of gametocytes. But the most remarkable report is that of Peters *et al.* (1969) who achieved the first cyclical transmission of a 20 year-old agametocytic strain of *P.b. berghei* (No. K173) after the production of gametocytes had been stimulated by the administration

of sub-curative doses of chloroquine. The drug was shown not only to cause the reappearance of gametocytes, but also to enhance their infectivity to mosquitoes (Ramkaran and Peters, 1969).

It is difficult to judge to what degree murine malaria parasites are changed by the selective pressures of life in the laboratory, and it is therefore of special interest to note that this old strain (No. K173) isolated in January, 1948 (Vincke and van den Bulcke, 1949), maintained by innumerable blood passages in laboratory rodents for over 20 years, stored for periods at sub-zero temperatures and then treated with chloroquine, differs in several ways from other more recently isolated strains of the same subspecies. It is more tolerant of high temperatures during sporogony, the oocysts are larger and the sporozoites are unusually long (see Bafort, 1970).

Tandon and Bhattacharya (1970) have recently reported the reappearance of gametocytes in an old strain of *P.b. berghei* after treatment with a corticosteroid.

CYCLICAL TRANSMISSION OF *P. BERGHEI* SSPP.

A summary of the conditions for the cyclical transmission of subspecies of *P. berghei* in the laboratory is given in Table I. The nominate subspecies has been the most intensively studied, and only little is known of the susceptibility of laboratory-bred mosquitoes to some of the other subspecies. The Nigerian subspecies is unusual in that sporogony with the production of infective sporozoites can take place at 28°C, although the number of sporozoites invading the glands is much less than at 24°C (Killick-Kendrick, 1970b).

No better mosquito than *A. stephensi* has been found as a laboratory host. Although sporogony of *P.b. berghei* is completed in *A. quadrimaculatus* and *A. gambiae* species A and B, the numbers of sporozoites in the salivary glands are fewer than in *A. stephensi*. *A. labranchiae atroparvus* is easily infected with *P.b. berghei*, but is a poor host of *P.b. yoelii* (Vincke *et al.*, 1966; Wéry, 1968). *A. aztecus* has the disadvantage of being difficult to breed in large numbers, and *A. annulipes* can be bred only by forced mating.

The muskrat is a good gametocyte carrier of *P. b. berghei* (Wellde *et al.*, 1966), and its large size may be a special advantage if very many infected mosquitoes are required. Its suitability as a host of other murine parasites has not been investigated.

Mosquitoes become most heavily infected with all subspecies of *P. berghei* if the infective feed is taken early in the infection when the very first gametocytes are present in the blood of the rodent host. In sporozoite-induced infections the first gametocytes may be seen very early since they arise directly from merozoites produced by exoerythrocytic schizonts (Killick-Kendrick and Warren, 1968). Variability in the production of gametocytes in a group of rodents is always seen, even in inbred lines, and suitable carriers have to be selected by the examination of thin blood-films.

TABLE I
The cyclical transmission of the subspecies of *P. berghei* in the laboratory

Subspecies of parasite	Suitable gametocyte carriers other than natural hosts	Best days of blood-induced infections for mosquitoes to be fed	Suitable laboratory mosquitoes	Unsuitable laboratory mosquitoes	Best temperatures for sporogony	Days glands first invaded	References
<i>P. b. berghei</i> (Katanga)	Some strains of white mice, white rats, hamsters, muskrats	4-5	<i>A. stephensi</i> <i>A. l. atroparvus</i> <i>A. annulipes</i> <i>A. gambiae</i> A, B <i>A. aztecus</i> <i>A. quadrimaculatus</i>	<i>A. albimanus</i> <i>Aedes aegypti</i> <i>Culex salinarius</i>	19-21°C	14	1-10
<i>P. b. yoelii</i> (C.A.R.)	mice, white rats, hamsters	4-5	<i>A. stephensi</i> <i>A. sudaicus</i>	<i>A. l. atroparvus</i> <i>A. b. balabacensis</i> * <i>A. quadrimaculatus</i> *	24°C	9	11, 12
<i>P. b. killicki</i> (Brazzaville)	mice, white rats	3-6	<i>A. stephensi</i>		22-24°C	10	13
<i>P. berghei</i> ssp. (Nigeria)	mice, white rats	3-10	<i>A. stephensi</i>		24°C	9	14

* Moore and Killick-Kendrick (unpublished).

References in TABLE I

- | | | |
|---|------------------------------------|---|
| 1. Perez-Reyes (1953) | 6. Yoeli <i>et al.</i> (1966) | 11. Landau and Killick-Kendrick (1966b) |
| 2. Yoeli and Most (1960) | 7. Wellde <i>et al.</i> (1966) | 12. Wéry (1968) |
| 3. Yoeli <i>et al.</i> (1964) | 8. Vincke <i>et al.</i> (1966) | 13. Landau <i>et al.</i> (1968) |
| 4. Vanderberg and Yoeli (1964) | 9. Vanderberg <i>et al.</i> (1968) | 14. Killick-Kendrick (1970a, b) |
| 5. Yoeli, Vanderberg <i>et al.</i> (1965) | 10. Kalucy and McMillan (1970) | |

In Table I, the days on which sporozoites first invade the salivary glands (at the best temperature for sporogony) are given as a guide to the day of harvest. Sporozoites continue to invade the glands for several days, and the best time of harvest is about 2 days after the first invasion. The infectivity of sporozoites tends to decline progressively after they have reached the glands (Wéry, 1968).

CYCLICAL TRANSMISSION OF *P. VINCKEI* SSPP.

This group of parasites is more difficult to transmit cyclically in the laboratory than the *P. berghei* group. A saving grace is the high infectivity of the sporozoites compared with those of *P. b. berghei* or *P. b. yoelii* (Wéry, 1968; Bafort, 1969).

Suitable gametocyte carriers other than the natural vertebrate hosts are few, and for some subspecies workers have had to resort to unusual hosts (see Table II). The time of the highest infectivity of gametocytes is not clear-cut, and for at least one subspecies, *P. v. chabaudi*, it is unusual. Wéry (1968) showed that gametocytes of this parasite were most infective at, or shortly after, the crisis in parasitaemia which occurs during the second or third week of the infection. These sexual stages look degenerate, but Wéry's careful observations show that this is nevertheless the best time to infect mosquitoes. He found that preservation of *P. v. chabaudi* at sub-zero temperatures increased the virulence and, since this resulted in only few mice surviving the crisis, the strain was then difficult to transmit cyclically. In such circumstances, passage of the strain into multi-mammate rats (*Praomys natalensis*) sometimes results in the production of a good crop of gametocytes, and the ability of the strain to infect mosquitoes is not lost.

Unlike *P. berghei* sspp, the subspecies of *P. vinckei* have not been cyclically transmitted in the laboratory through mosquitoes other than *A. stephensi*. Another difference is the tolerance of *P. vinckei* sspp. to a wide range of temperatures during sporogony (Wéry, 1968; Bafort, 1969), which is quite unlike *P. berghei* with the exception of the Nigerian subspecies. *P. vinckei* from Nigeria appears to be less adaptable than the subspecies from other places, and infective sporozoites have not yet been obtained in laboratory-bred mosquitoes.

FACTORS ADVERSELY AFFECTING ROUTINE CYCLICAL MAINTENANCE

Regular cyclical transmission of murine malaria parasites is less easily achieved than, for example, the transmission of *P. gallinaceum* through *Aedes aegypti*, and even when a system of routine cyclical maintenance is established, it commonly happens that for no apparent reason sporozoites suddenly become unobtainable or non-infective. There is sometimes no explanation, but there are several factors known adversely to affect the cyclical transmission of malaria parasites of rodents, which may be responsible.

TABLE II
The cyclical transmission of the subspecies of *P. vinckei* in the laboratory

Subspecies of parasite	Suitable gametocyte carriers other than natural hosts	Best days of blood-induced infections for mosquitoes to be fed	Suitable laboratory mosquitoes	Unsuitable laboratory mosquitoes	Best temperatures for sporogony	Days glands first invaded	References
<i>P. v. vinckei</i> (Katanga)	splenectomized or hairless mice	4-8	<i>A. stephensi</i>	<i>A. gambiae</i> <i>A. albimanus</i> <i>A. quadrimaculatus</i> <i>A. l. atroparvus</i>	20-21°C	13	1
<i>P. v. chabaudi</i> (C.A.R.)	mice, multimammate rats	10-18	<i>A. stephensi</i>	<i>A. aztecus</i> <i>A. freeborni</i> <i>A. sudaicus</i>	26°C	10	2, 3
<i>P. v. lentum</i> (Brazzaville)	<i>Hybomys univittatus</i>	5	<i>A. stephensi</i>		24-25°C	10	4
<i>P. vinckei</i> ssp. (Nigeria)	mice, multimammate rats	?	?	<i>A. stephensi</i> * <i>A. l. atroparvus</i> * <i>A. quadrimaculatus</i> * <i>A. gambiae</i> B†	?	?	5

* At 23-25°C sporozoites which were never infective first invaded the salivary glands of these mosquitoes on day 12 (Killick-Kendrick, 1970b).

† Insusceptible.

References in TABLE II

- | | | |
|--|--------------------------------|--------------------------------|
| 1. Bafort (1969) | 3. Wéry (1968) | 5. Killick-Kendrick (1970a, b) |
| 2. Landau and Killick-Kendrick (1966b) | 4. Landau <i>et al.</i> (1970) | |

The worker accustomed to the sporogony of malaria parasites of birds, monkeys or man is immediately struck by (i) the asynchronous growth of the oocysts of murine malaria parasites accompanied by the death of many oocysts, and (ii) the comparative paucity of sporozoites in the salivary glands of experimentally infected mosquitoes. The irregularity of oocyst growth is apparently a normal phenomenon unrelated to the use in the laboratory of hosts which are not natural vectors. Vanderberg and Yoeli (1964) noted aborted oocysts of *P. b. berghei* on the midguts of *A. d. millegampi*, the natural invertebrate host of this parasite. Overcrowding of oocysts also seems not to be the cause, since Vincke *et al.* (1966) found degenerated oocysts on stomachs of both heavily and lightly infected *A. quadrimaculatus*.

The lightly infected glands of *A. stephensi* infected with any murine malaria parasite, which contrast strikingly with the bulging glands oozing sporozoites commonly seen in infections of other species of *Plasmodium*, do however appear to be a consequence of using unnatural invertebrate hosts. Yoeli *et al.* (1964) found that the salivary glands of experimentally infected *A. d. millegampi* became much more heavily infected with sporozoites of *P. b. berghei* than did those of other mosquitoes.

The transmission of murine malaria parasites through mosquitoes in the laboratory is vulnerable to many interfering factors. The susceptibility of some strains or species of laboratory rodents may be so low that the production of gametocytes is quite inadequate and whatever the host, the viability of gametocytes falls after only few syringe-passages.

The temperature of incubation during sporogony is critical with some subspecies and must be carefully controlled. In general, the sporozoites, if they are still produced, lose their infectivity as temperatures increase.

A less easily controlled factor is the susceptibility of the mosquito. In studies on the sporogony of *P. cynomolgi*, Garnham (1970) and Rutledge *et al.* (1970) found that the susceptibility of a given species of mosquito varied according to geographical origin. Moreover, the susceptibility of mosquitoes in closed colonies did not remain constant. This last point may explain sudden difficulties in cyclically transmitting a parasite which has hitherto caused no problem.

There are observations suggesting that natural infections of a number of different micro-organisms in laboratory-bred mosquitoes interfere with the sporogony of malaria parasites. Bird *et al.* (1970) described several viruses in laboratory colonies of *A. stephensi*, one of which came to light when there was difficulty in transmitting *P. b. yoelii*. Trager (1959) found a virus which interfered with the growth of *P. lophurae* in ducks, and it seems possible that some viruses may similarly interfere with the development of malaria parasites in the invertebrate host. Many British workers now think that their colonies of *A. stephensi* have always had undetected viral infections which occasionally flare up and cause trouble.

Wéry (1968) reviewed the effect of bacteria in the midgut on the growth of oocysts, and pointed out that the addition of various antibiotics to the diet of infected mosquitoes has been shown both to inhibit and to stimulate growth (Terzian *et al.*, 1953; Micks and Ferguson, 1961). Wéry suggested that this may be explained by the effect on *Pseudomonas*, a bacterium thought to be beneficial to the development of oocysts (Jadin, 1965; Jadin *et al.*, 1966).

Garnham (1956) reported that mosquitoes infected with the microsporidian *Plistophora culicis* were poor hosts of malaria parasites, and Bray (1958) believed that the related parasite *Nosema stegomyiae* slowed down the growth of oocysts. Microsporidia normally enter a colony when naturally infected wild mosquitoes are brought into an insectarium and as long as this is prohibited, colonies will normally remain free from infection.

Controlled experiments to confirm that concomitant infections of viruses, bacteria or microsporidia are seriously detrimental to the sporogony of malaria parasites are difficult to carry out, and more work is needed before possible antagonisms between parasites are fully understood.

It is sometimes assumed that the biological and morphological characters of murine malaria parasites maintained by cyclical transmission remain constant, but the selection imposed in the laboratory by, for example, the use of unnatural hosts or periods of storage at low temperature, seems to result in changes which are not always beneficial. After 5 years in the laboratory, the type strain (No. 17X) of *P. b. yoelii* has become less easily handled than when first isolated, and no matter how carefully strains are handled, they may suddenly fail to produce infective sporozoites in a mosquito known to be a good host. In such circumstances, the only way to resume cyclical transmissions may be to strike another line from an earlier deep-frozen sample, or to obtain a line of the strain from another laboratory. It may even be necessary to begin again by isolating a new strain from a naturally infected host. It is a humbling fact that, as Laveran said, 'les résultats obtenus dans un laboratoire sont très imparfaits à côté de ceux que peut réaliser la nature'.

ACKNOWLEDGMENTS

I am indebted to Prof. P. C. C. Garnham, F.R.S. and Dr. Elizabeth Canning for help and encouragement, and to Dr. J. Bafort, Dr. C. C. Draper, Dr. I. Landau, Mr. A. I. Oxbrow, Mr. P. G. Shute and Dr. D. Walliker, all of whom have much experience of the cyclical transmission of murine malaria parasites, for many helpful discussions.

Support from the Medical Research Council is gratefully acknowledged.

REFERENCES

- ADAM, J. P., LANDAU, I. and CHABAUD, A. G. (1966). Découverte dans la région de Brazzaville de rongeurs infectés par des *Plasmodium*. *Comptes rendus hebdomadaire des séances de l'Académie des sciences* **263**: 140
- ADLER, S. (1958). The effect of a meat diet on the course of infections with *Plasmodium vinckei* in mice. *Bulletin of the Research Council of Israel Section B* **7E**: 9
- BAFORT, J. (1967). La transmission cyclique de *Plasmodium vinckei*. *Annales de la Société belge de médecine tropicale* **47**: 271
- BAFORT, J. (1969). Etude du cycle biologique du *Plasmodium v. vinckei* Rodhain 1952. *Annales de la Société belge de médecine tropicale* **49**: 533
- BAFORT, J. (1970). The variability of *Plasmodium berghei* Vincke and Lips, 1948. *Annales de la Société belge de médecine tropicale* **50**: 247
- BAFORT, J., TIMPERMAN, G. and DELBAR, T. (1968). Observations on tissue schizogony and sporogony of rodent malaria. *Annales de la Société belge de médecine tropicale* **48**: 535
- BAFORT, J., TIMPERMAN, G. and MOLYNEUX, D. H. (1970). *Babesia hylomysci* n.sp., a new *Babesia* from the Congo, transmissible to mice. *Annales de la Société belge de médecine tropicale* **50**: 301
- BAFORT, J., VINCKE, I. H. and TIMPERMAN, G. (1966). Observations on the gametocytogenesis of *Plasmodium vinckei*. *Nature, London* **211**: 439
- BARBER, M. A. and OLINGER, M. T. (1931). Studies on malaria in S. Nigeria. *Annals of Tropical Medicine and Parasitology* **25**: 461
- VAN DEN BERGHE, L., VINCKE, I. H., CHARDOME, M. and VAN DEN BULCKE, M. (1950). *Babesia rodhaini* n.sp. d'un rongeur du Congo belge transmissible à la souris blanche. *Annales de la Société belge de médecine tropicale* **30**: 83
- BIRD, R. G., DRAPER, C. C., GARNHAM, P. C. C., PUDNEY, M., SHUTE, P. G. and VARMA, M. G. R. (1970). Evidence of insect viruses in colonies of *Anopheles stephensi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **64**: 28
- BRAY, R. S. (1958). Studies on malaria in chimpanzees. V. The sporogonous cycle and mosquito transmission of *Plasmodium vivax schwetzi*. *Journal of Parasitology* **44**: 46
- BRUCE-CHWATT, L. J. and GIBSON, F. D. (1955). A *Plasmodium* from a Nigerian rodent. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **49**: 9
- COLLINS, W. E. and JEFFERY, G. M. (1963). The use of dimethyl sulfoxide in the low-temperature frozen preservation of experimental malaria. *Journal of Parasitology* **49**: 524
- COX, H. W. and MILAR, R. (1968). Cross protection immunization by *Plasmodium* and *Babesia* infections of rats and mice. *American Journal of Tropical Medicine and Hygiene* **17**: 173
- DAVIS, D. H. S. and OETTL, A. G. (1958). The multimammate mouse *Rattus (Mastomys) natalensis*: a laboratory-adapted African wild rodent. *Proceedings of the Zoological Society of London* **131**: 293
- FINK, E. and SCHICA, E. (1969). Influence of synthetic insect T.C. medium on the survival of malaria sporozoites in vitro (*Plasmodium berghei yoeli* and *P. cathemerium*). *Zeitschrift für Parasitenkunde* **32**: 93
- GARNHAM, P. C. C. (1956). Microsporidia in laboratory colonies of *Anopheles*. *Bulletin of the World Health Organization* **15**: 845
- GARNHAM, P. C. C. (1966). *Malaria parasites*. Oxford: Blackwell
- GARNHAM, P. C. C. (1967). Malaria in mammals excluding man. In *Advances in Parasitology* Dawes, B. (ed.) vol. 5, p. 139 London: Academic Press

- GARNHAM, P. C. C. (1968). Recent researches on rodent malaria parasites. *Israel Journal of Medical Sciences* 4: 1057
- GARNHAM, P. C. C. (1970). Primate malaria. In *Immunity to Parasitic Animals* Jackson, G. L., Herman, R. and Singer, I. (eds.) vol. 2, pp. 767-791 New York: Meredith Corporation
- GARNHAM, P. C. C., LANDAU, I., KILICK-KENDRICK, R. and ADAM, J. P. (1967). Répartition et caractères différentiels des *Plasmodiums* de muridés. *Bulletin de la Société de pathologie exotique* 60: 118
- GILLIES, M. T. and DE MEILLON, B. (1968). *The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region)*. 2nd ed. Johannesburg: S. African Institute for Medical Research
- GOODWIN, L. G. and THIEL, C. C. (1967). A portable slow-cooling device. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 61: 134
- HAWKING, F. (1953). Milk diet, *p*-aminobenzoic acid and malaria (*Plasmodium berghei*). Preliminary communication. *British Medical Journal* i: 1201
- Hsu, D. Y. M. and GERMAN, Q. M. (1952). Synergistic effect of *Haemobartonella muris* on *Plasmodium berghei* in white rats. *American Journal of Tropical Medicine and Hygiene* 1: 747
- JADIN, J. (1965). Les bactéries photosynthétiques pourpres peuvent-elles jouer un rôle dans la sporogonie des *Plasmodium*? *Académie Nationale de Médecine* 149: 470
- JADIN, J., VINCK, I. H., DUNJIC, A., DELVILLE, J. P., WERY, M., BAFORT, J. and SCHEEPERS-BIVA, M. (1966). Rôle des *Pseudomonas* dans la sporogonie de l'hématozoaire du paludisme chez le moustique. *Bulletin de la Société de pathologie exotique* 59: 514
- JADIN, J., YOELI, M. and PIERREUX, G. (1959). Réapparition du processus d'extraflagellation chez une souche de *Plasmodium berghei* régulièrement entretenue par passage mécanique. *Annales de la Société belge de médecine tropicale* 39: 847
- KALUCY, E. C. and McMILLAN, B. (1970). Transmission of *Plasmodium berghei* (NK65 strain) by *Anopheles annulipes* Walker. *Nature, London* 225: 97
- KILICK-KENDRICK, R. (1970a). Studies on the life-cycles of murine malaria parasites from Nigeria. *M. Phil. thesis. University of London*
- KILICK-KENDRICK, R. (1970b). The life-cycles of two murine malaria parasites from Nigeria. *Journal of Parasitology* 56: 435
- KILICK-KENDRICK, R., SHUTE, G. T. and LAMBO, A. O. (1968). Malaria parasites of *Thomomys rutilans* (Rodentia, Muridae) in Nigeria. *Bulletin of the World Health Organization* 38: 822
- KILICK-KENDRICK, R., and WARREN, M. (1968). Primary exoerythrocytic schizonts of a mammalian *Plasmodium* as a source of gametocytes. *Nature, London* 220: 191
- LANDAU, I. (1965). Description de *Plasmodium chabaudi* n.sp. parasite de rongeurs africains. *Comptes rendus hebdomadaire des séances de l'Académie des sciences* 260: 3758
- LANDAU, I. (1966). Comments on sporozoite-induced infections in rodent hosts. *Military Medicine, Supplement* 131: 919
- LANDAU, I. and CHABAUD, A. G. (1965). Infection naturelle par deux *Plasmodium* du rongeur *Thomomys rutilans* en République Centre Africaine. *Comptes rendus hebdomadaires des séances de l'Académie des sciences* 261: 230
- LANDAU, I. and KILICK-KENDRICK, R. (1966a). Note préliminaire sur le cycle évolutif des deux *Plasmodium* du rongeur *Thomomys rutilans* de la République Centrafricaine. *Comptes rendus hebdomadaire des séances de l'Académie des sciences* 262: 1113
- LANDAU, I. and KILICK-KENDRICK, R. (1966b). Rodent plasmodia of the République Centrafricaine: the sporogony and tissue stages of *Plasmodium chabaudi* and *P. berghei* yoelii. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 60: 633

- LANDAU, I., MICHEL, J. C. and ADAM, J. P. (1968). Cycle biologique au laboratoire de *Plasmodium berghei killicki* n. subsp. *Annales de parasitologie humaine et comparée* **43**: 545
- LANDAU, I., MICHEL, J. C., ADAM, J. P. and BOULARD, Y. (1970). The life cycle of *Plasmodium vinckei lentum* subsp. nov. in the laboratory; comments on the nomenclature of the murine malaria parasites. *Annals of Tropical Medicine and Parasitology* **64**: 315
- LIPS, M. (1960). *Anopheles* du Congo Belge. 2: Quelques espèces des galeries forestières. *Rivista di Parassitologia* **21**: 39
- MICHEL, G. (1963). Observations sur la gamétogèse du *Plasmodium berghei*. *Annales de la Société belge de médecine tropicale* **43**: 67
- MICKS, D. W. and FERGUSON, M. J. (1961). Microorganisms associated with mosquitoes. III. Effect of reduction in the microbial flora of *Culex fatigans* Windemann on the susceptibility to *Plasmodium relictum* Grassi and Feletti. *Journal of Insect Pathology* **3**: 244
- MOLINARI, V. (1961). The action of low temperatures on *Plasmodia*. *Journal of Tropical and Hygiene* **64**: 225
- MOREAU, R. E. (1964). The distribution of tropical African birds as an indicator of past climatic changes. In *African ecology and human evolution* Howell, F. C. and Boulière, F. (eds.) pp. 28–42 London: Methuen
- MORGAN, J. F., MORTON, H. J. and PARKER, R. C. (1950). Nutrition of animal cells in tissue culture. I. Initial studies on a synthetic medium. *Proceedings of the Society for Experimental Biology and Medicine* **16**: 118
- MOST, H., NUSSENZWEIG, R. S., VANDERBERG, J., HERMAN, R. and YOELI, M. (1966). Susceptibility of genetically standardized (JAX) mouse strains to sporozoite—and blood—induced *Plasmodium berghei* infections. *Military Medicine, Supplement* **131**: 915
- OTT, K. J., ASTIN, J. K. and STAUBER, L. A. (1967). *Eperythrozoon coccoides*: Influence on course of infection of *Plasmodium chabaudi* in mouse. *Science* **155**: 1546
- PEREZ-REYES, R. (1953). *Anopheles aztecus* (Hoffman, 1935) a new definitive host for the cyclical transmission of *Plasmodium berghei* Vincke and Lips, 1948. *Journal of Parasitology* **39**: 603
- PETERS, W. (1965). Competitive relationship between *Eperythrozoon coccoides* and *Plasmodium berghei* in the mouse. *Experimental Parasitology* **16**: 158
- PETERS, W. (1967). Chemotherapy of *Plasmodium chabaudi* infection in albino mice. *Annals of Tropical Medicine and Parasitology* **61**: 52
- PETERS, W., ROBINSON, B., RAMKARAN, A. and PORTUS, J. (1969). A virulent chloroquine-resistant strain of *Plasmodium berghei* transmitted through *Anopheles stephensi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**: 8
- RAMKARAN, A. E. and PETERS, W. (1969). Infectivity of chloroquine resistant *Plasmodium berghei* to *Anopheles stephensi* enhanced by chloroquine. *Nature, London* **223**: 635
- RODHAIN, J. (1952). *Plasmodium vinckei* n. sp. Un deuxième *Plasmodium* de rongeurs sauvages au Katanga. *Annales de la Société belge de médecine tropicale* **32**: 275
- RODHAIN, J. and VINCKE, I. H. (1953). Sur une deuxième souche de *Babesia rodhaini* du rongeur africain *Thamnomys surdaster*. *Acta tropica* **10**: 264
- ROSEVEAR, D. R. (1969). *Rodents of West Africa*. Publ. No. 677. London: British Museum (Natural History)
- RUTLEDGE, L. C., HAYES, D. E. and WARD, R. A. (1970). *Plasmodium cynomolgi*: sources of variation in susceptibility of *Anopheles quadrimaculatus*, *A. balabacensis*, and *A. stephensi*. *Experimental Parasitology* **27**: 53
- SERGEANT, E. (1963). Latent infection and premunition. Some definitions of microbiology and immunology. In *Immunity to Protozoa* Garnham, P. C. C., Pierce, A. E. and Roitt, I. (eds.) pp. 39–47 Oxford: Blackwell

- SHUTE, P. G. and MARYON, M. (1966). *Laboratory technique for the study of malaria*. 2nd Ed. London: Churchill
- SUNTHARASAMAI, P. and MARSDEN, P. D. (1969). Studies of splenomegaly in rodent malaria. I. The course of splenomegaly in mice infected with *Eperythrozoon coccoides*, *Plasmodium berghei yoelii* and the two infections combined. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**: 64
- TANDON, N. and BHATTACHARYA, N. C. (1970). Gametogenesis of *Plasmodium berghei* in corticosteroid treated albino mice. *Bulletin of the World Health Organization* **43**: 344
- TERZIAN, L. A., STAHLER, N. and MILLER, H. (1953). A study of the relation of antibiotics, vitamins and hormones to immunity to infections. *Journal of Immunology* **76**: 308
- TRAGER, W. (1959). A new virus of ducks interfering with the development of malaria parasites (*Plasmodium lophurae*). *Proceedings of the Society of Experimental Biology and Medicine* **101**: 579
- VANDERBERG, J. P., NUSSENSZWEIG, R. S. and MOST, H. (1968). Further studies on the *Plasmodium berghei*—*Anopheles stephensi*—rodent system of mammalian malaria. *Journal of Parasitology* **54**: 1009
- VANDERBERG, J. and YOELI, M. (1964). Some physiological and metabolic problems related to the *Plasmodium berghei* cycle in *Anopheles quadrimaculatus*. *Annales de la Société belge de médecine tropicale* **45**: 419
- VARMA, M. G. R. and PUDDNEY, M. (1969). The growth and serial passage of cell lines from *Aedes aegypti* (L.) larvae in different media. *Journal of Medical Entomology* **6**: 432
- VINCKE, I. H. (1950). Recherches sur le complexe *Anopheles duren*i, *Plasmodium berghei* et *Thamnomys surdaster* dans les galeries forestières du Katanga. *Compte rendus du Congrès scientifique d'Elisabethville* Communication 101
- VINCKE, I. H. (1954). Natural history of *Plasmodium berghei*. *Indian Journal of Malariology* **8**: 245
- VINCKE, I. H. (1967). The potential vectors of *P. chabaudi* and *P. b.yoelii* (Correspondence). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**: 154
- VINCKE, I. H. and BAFORT, J. (1968). Méthodes de standardisation de l'inoculum de sporozoïtes de *Plasmodium berghei*. *Annales de la Société belge de médecine tropicale* **48**: 181
- VINCKE, I. H., BAFORT, J. and SCHEEPERS-BIVA, M. (1966). Observations récentes sur la transmission cyclique du *Plasmodium berghei*. *Annales de la Société belge de médecine tropicale* **46**: 327
- VINCKE, I. H. and VAN DEN BULCKE, M. A. (1949). Observations sur l'entretien de la souche K173 du *Plasmodium berghei* Vincke et Lips, 1948. *Annales de la Société belge de médecine tropicale* **29**: 525
- VINCKE, I. H. and LIPS, M. (1948). Un nouveau *Plasmodium* d'un rongeur sauvage du Congo: *Plasmodium berghei* n.sp. *Annales de la Société belge de médecine tropicale* **28**: 97
- VINCKE, I. H. and LIPS, M. (1950). Note sur la transmission du *Plasmodium berghei*. *Annales de la Société belge de médecine tropicale* **30**: 1605
- VINCKE, I. H., SCHEEPERS-BIVA, M. and BAFORT, J. (1965). Conservation de gamétocytes de *P. berghei* viables et transmissibles à basse température. *Annales de la Société belge de médecine tropicale* **45**: 151
- VOLLER, A. and BIDWELL, D. E. (1968). The effect of *Eperythrozoon coccoides* infection in mice on superimposed *Plasmodium chabaudi* and Semliki Forest Virus infections. *Annals of Tropical Medicine and Parasitology* **62**: 342
- WEATHERSBY, A. B. (1963). Harvesting mosquito pupae with cold water. *Mosquito News* **23**: 249

- WELLDE, B. T., BRIGGS, N. T. and SADUN, E. H. (1966). Susceptibility to *Plasmodium berghei*: parasitological, biochemical and hematological studies in laboratory and wild mammals. *Military Medicine, Supplement* **131**: 859
- WERY, M. (1968). Studies on the sporogony of rodent malaria parasites. *Annales de la Société belge de médecine tropicale* **48**: 1
- WERY, 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. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**: 447
- YOELI, M. (1965). Studies on *Plasmodium berghei* in nature and under experimental conditions. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **59**: 255
- YOELI, M., ALGER, N. and MOST, H. (1963). Tree rat, *Thamnomys surdaster surdaster*, in laboratory research. *Science* **142**: 1585
- YOELI, M. and BONE, G. (1967). Studies on *Anopheles duren* Edwards, *Rivista di Malariaologia* **46**: 3
- YOELI, M. and MOST, H. (1960). The biology of a newly isolated strain of *Plasmodium berghei* in a rodent host and in experimental mosquito vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **54**: 549
- YOELI, M., MOST, H. and BONE, G. (1964). *Plasmodium berghei*: cyclical transmissions by experimentally infected *Anopheles quadrimaculatus*. *Science* **144**: 1580
- YOELI, M., MOST, H. and BONE, G. (1965). The natural history of *Plasmodium berghei* in the field and under experimental conditions. *Annales de la Société belge de médecine tropicale* **45**: 267
- YOELI, M., UPMANIS, R. S. and MOST, H. (1963). Gametogony and sporogony in a strain of *Plasmodium berghei* preserved at low temperature. *Journal of Parasitology* **49**: 926
- YOELI, M., UPMANIS, R. S., VANDERBERG, J. and MOST, H. (1966). Life cycle and patterns of development of *Plasmodium berghei* in normal and experimental hosts. *Military Medicine, Supplement* **131**: 900
- YOELI, M., VANDERBERG, J., NAWROT, R. and MOST, H. (1965). Studies on sporozite induced infections of rodent malaria. II. *Anopheles stephensi* as an experimental vector of *Plasmodium berghei*. *American Journal of Tropical Medicine and Hygiene* **14**: 927

THE ACTION OF 'TERRAMYCIN' ON THE PRIMARY EXOERYTHROCYTIC DEVELOPMENT OF *PLASMODIUM VIVAX* AND *PLASMODIUM* *CYNOMOLGI CEYLONENSIS* *

P. C. C. GARNHAM,† McWILSON WARREN ‡
and R. KILLICK-KENDRICK †

London School of Hygiene and Tropical Medicine

Cooper *et al.* (1949) and Imboden *et al.* (1950) showed that 'aureomycin' had some prophylactic action against the Chesson strain of *Plasmodium vivax* in volunteers bitten by infected mosquitoes. The prepatent period was delayed for two weeks beyond the normal after the daily administration of 4 gm of this antibiotic for seven days after infection, and up to three weeks after a dosage of 8 gm over the same treatment period. 'Chloromycetin' was found to have a slight action, while penicillin and streptomycin had none. More recently, Schmidt *et al.* (1970) described experiments on a big series of monkeys, dosed with 7-chlorolincomycin derivatives and infected with the sporozoites of *P. cynomolgi*. High doses were found to protect some of the animals completely from infection, and all showed a delay in the normal incubation period. Biopsies of the livers of the treated animals demonstrated the great scarcity of exoerythrocytic schizonts (only one abnormal form was seen), while normal schizonts (albeit in rather small numbers) were found in the control animals.

In a recent experiment, with a North Korean strain of *P. vivax* we had occasion to observe by chance the action of another antibiotic on sporozoite-induced infections. The paper is limited to a description of this incident and to a single attempt to repeat the work on a simpler model using monkeys and *P. cynomolgi*.

It is not intended of course to be a study in chemotherapy, and the observations are only reported to indicate the possible misinterpretations of results that may arise following the use of antibiotics in *Aotus* monkeys infected with human malaria parasites, or in other similar experiments.

Materials and Methods

A young male chimpanzee, weighing 4 kg was splenectomized on May 6th, 1968, and treated

with standard doses of quinine for 10 days subsequently, in order to sterilize any malaria infection in the blood. On June 7th, 1968, 162,000 sporozoites of *P. vivax* were inoculated intravenously. The method of preparation of these suspensions and details of the strains used will be described in a subsequent paper. Three days later the animal became ill with broncho-pneumonia and diarrhoea, so 'terramycin' was given intramuscularly at a dosage of 35 mg/kg daily for two days and the animal recovered. On June 15th, a piece of liver was removed after a laparotomy; the material was fixed and stained by the Giemsa colophonium technique (Bray and Garnham, 1967). Sections of this biopsy material showed no exoerythrocytic schizonts, and parasitaemia did not begin until June 27th, *i.e.* after a delayed prepatent period of 20 days instead of the normal eight days.

This result suggested that 'terramycin' had a marked effect on the exoerythrocytic stage of *P. vivax* and the experiment was repeated on rhesus monkeys infected with sporozoites of the vivax-like parasite, *P. cynomolgi ceylonensis*; one animal was treated on the third and fourth days after inoculation with doses of 'terramycin' equivalent to those used for the chimpanzee, and another was kept as a control.

Biopsies of the liver of both monkeys were performed on the 8th, 11th and 21st days after the intravenous inoculation of 2,200,000 sporozoites into the experimental animal and 1,100,000 into the control. The sporozoites were obtained from the same batch of infected mosquitoes and were inoculated at the same

* Supported in part by a grant from WHO.

† Present address: Imperial College of Science, Field Station, Ascot, Berks, England.

‡ Present address: Central America Malaria Research Station, USAID, San Salvador, El Salvador.

Sections of liver of monkeys showing exoerythrocytic schizonts of *P. cynomolgi ceylonensis* and stained by Giemsa-colophonium method:
All at same magnification ($\times 1400$)

Fig. 1. Control; Figs. 2-5. Effect of treatment with 'Terramycin'.

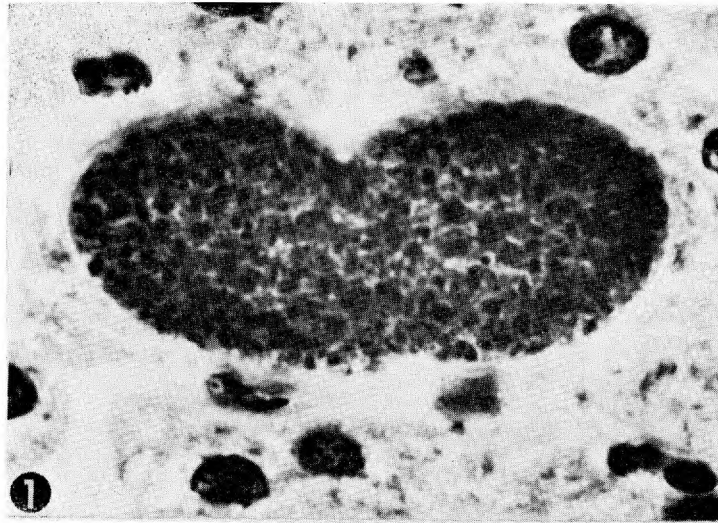


Fig. 1. Control. Biopsy taken eight days after inoculation of sporozoites. Normal EE schizont, nearing maturity.

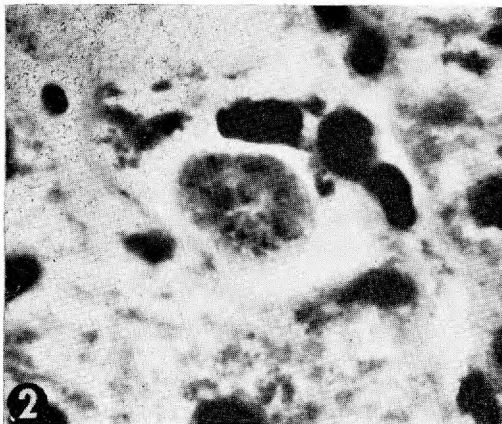


Fig. 2. 'Terramycin' treatment. Biopsy taken eight days after inoculation of sporozoites. EE schizont of much reduced size with highly abnormal nuclei.

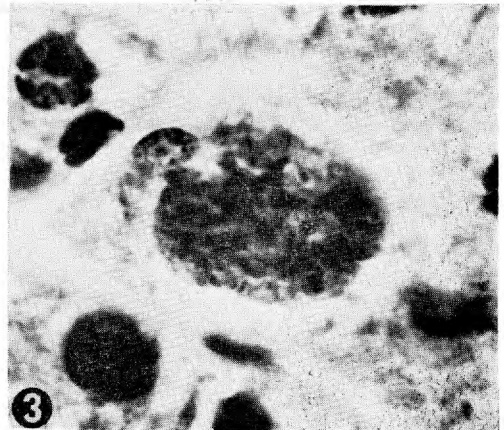


Fig. 3. 'Terramycin' treatment. Biopsy taken eight days after inoculation of sporozoites. EE schizont of reduced size with abnormal nuclei and large vacuole.

time. The material was treated as before, and the sections were compared. The experimental monkey weighed 2.6 kg and the control 2.3 kg. The former was given double the quantity of sporozoites, in order to increase the chances of seeing schizonts damaged by the drug.

Results

The monkey on the antibiotic showed scanty, small and otherwise abnormal exoerythrocytic schizonts on the eighth day and a delayed prepatent period of 12 days instead of a normal duration of eight days. The schizonts had a

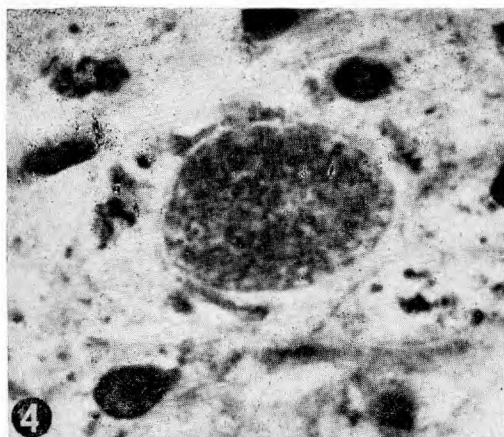


Fig. 4. 'Terramycin' treatment. Biopsy taken 11 days after inoculation of sporozoites. EE schizont still small and immature with peculiar large nuclei.

mean length of 21.6μ and a width of 13.9μ ; a single schizont was as large as $39 \times 14 \mu$. Apart from the reduction in size, the schizonts were much vacuolated on the periphery; the nuclei were often angular and with an indistinct outline; the cytoplasm was unusually dense (Figs. 2 and 3). The vacuoles were filled with an eosinophilic material and were about 2μ in diameter. The surface membrane of the parasite was inconspicuous and smooth. The parasitaemia, although late in appearing, otherwise followed a normal course.

The control monkey showed numerous exoerythrocytic schizonts of normal morphology and size on the eighth day when the blood was invaded by malaria parasites in the usual way for this sub-species (Dissanaike *et al.*, 1965). The schizonts measured $47.5 \times 24.5 \mu$ (mean) and were slightly immature (Fig. 1); at maturity the dimensions are slightly higher ($48 \times 30 \mu$). The nuclei were round or bar-shaped and cytoplasmic clumps were present; vacuoles were not seen.

Further biopsies on both monkeys were made on the 11th day; in the treated animal the schizonts, though larger, were still highly abnormal. Their size had now increased to a mean of $29 \times 18 \mu$. Large vacuoles (about 6μ in size) were present in many. The cytoplasm was often dense and contracted from the cytoplasm of the host cell (Fig. 5). A definite membrane enclosed the parasite (Fig. 4) and some vacuolated schizonts had a cystic appearance. The nuclei were again abnormal and sometimes were in the form of large masses 1.8μ in diameter.

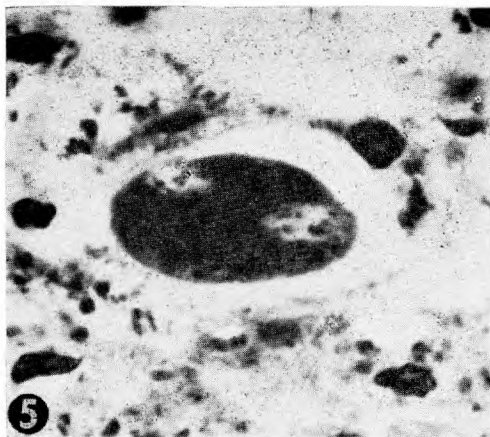
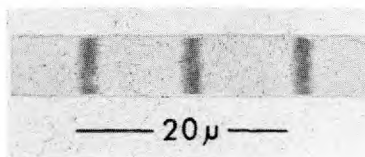


Fig. 5. 'Terramycin' treatment. Biopsy taken 11 days after inoculation of sporozoites. EE schizont of reduced size, vacuolated with excessively dense cytoplasm.



A third biopsy was made of the liver of the drug-treated monkey on the 21st day after infection, but no parasites were seen in the sections.

No exoerythrocytic schizonts were found in the liver of the control monkey on the 11th day, as all had presumably ruptured at the normal time, two or three days earlier.

The number of schizonts per section was approximately of the following order:

Drug-treated monkey

Biopsy on 8th day 1 per section

Biopsy on 11th day 5 per section

Control Monkey

Biopsy on 8th day 10 per section

Biopsy on 11th day 0 per section

Although the drug-treated animal received twice the dosage of sporozoites, the number of schizonts in its liver was much reduced as compared with the control.

Discussion

These observations were made in the course of research in relapses in malaria, in which sporozoites of certain parasites of primate malaria were subjected to various adverse influences, *e.g.* exposure to radiation, in attempts to modify the course of the infection. We particularly wanted to see if the delayed incuba-

tion period and long term relapses, characteristic of certain strains (e.g. the North Korean *P. vivax*, Serguiev and Tiburskaya, 1967) could be induced experimentally.

The apparent effect of 'terramycin' on the sporozoites or early exoerythrocytic schizonts of two species (*P. vivax* and *P. cynomolgi*) was demonstrated; the prepatent period was lengthened by 12 and four days respectively, but no retardation comparable to the 6-15 months' delay (of North Korean and other strains) occurred. It is clear from the morphological evidence of the *P. cynomolgi* infection, that the antibiotic had caused gross damage to the developing schizonts. Probably a very small number of schizonts escaped and gave rise to merozoites on the eighth day, but parasitaemia did not build up to a sufficient height to become patent until several days later. It is possible however that the infection in the blood, delayed to the 20th and 14th days (in the chimpanzee and rhesus respectively), was the result of the maturation and discharge of merozoites from damaged schizonts, although once the parasites had entered the erythrocytes, no abnormalities were detectable. Schmidt *et al.* (1970) favour the second hypothesis *i.e.* the "recovery from injury" concept, as an explanation of the greatly delayed incubation periods after 'lincomycin' administration.

Summary

1. 'Terramycin' at a dosage of 33 mg per kg was given to a splenectomized chimpanzee three and four days after the intravenous inoculation of sporozoites of *Plasmodium vivax*. The antibiotic destroyed most of the exoerythrocytic

stages of the parasite in the liver and delayed the prepatent period by 12 days.

2. The experiment was repeated on a rhesus monkey infected with the sporozoites of *Plasmodium cynomolgi ceylonensis*, and a second untreated monkey was used as a control. Exoerythrocytic schizonts in the liver of the treated monkey were found to be grossly damaged on the eighth and 11th days after infection and the prepatent period was delayed for four days. The infection in the liver and blood of the control monkey was normal.

References

- Bray, R. S. and Garnham, P. C. C. (1962). The Giemsa-colophonium technique for staining protozoa in tissue sections. *Indian J. Malariol.*, v16, 153-155.
- Cooper, W. C., Coatney, G. R., Imboden, C. A. and Jeffery, G. M. (1949). Aureomycin in experimental Chesson strain *vivax* malaria. *Proc. Soc. exp. Biol. Med.*, v72, 587-588.
- Dissanaike, A. S., Nelson, P. and Garnham, P. C. C. (1965). Two new species of malaria parasites, *Plasmodium cynomolgi ceylonensis* subsp. nov. and *Plasmodium fragile* sp. nov. from monkeys in Ceylon. *Ceylon J. Med. Sci.*, (D), v14, 1.
- Imboden, C. A., Cooper, W. C., Coatney, G. R. and Jeffery, G. M. (1950). Studies in human malaria. XXIX Trials of aureomycin, chloramphenicol, penicillin, and dihydrostreptomycin against the Chesson strain of *Plasmodium vivax*. *J. Nat. Malaria Soc.*, v9, 377.
- Schmidt, L. H., Harrison, J., Ellison, R. and Worcester, P. (1970). The activities of chlorinated lincomycin derivatives against infections with *Plasmodium cynomolgi* in *Macaca mulatta*. *Am. J. trop. Med. Hyg.*, v19, 1.
- Serguiev, P. G. and Tiburskaya, N. A. (1967). A propos de l'évolution du *Plasmodium vivax*. *Path. Exp. Microbiol.*, v27, 473-484.

THE DEVELOPMENT OF TRYPANOSOMES, LEISHMANIAE AND ASCITIC TUMOUR CELLS IN THE TESTICLES OF LABORATORY ANIMALS

R. B. HEISCH,* R. KILLICK-KENDRICK,** M. W. GUY*** AND J. DORRELL
London School of Hygiene and Tropical Medicine,

It was first pointed out by UHLENHUTH and EMMERICH (1913) that *Trypanosoma evansi equiperdum* and *T. brucei gambiense* have a special predilection for the testicles of rabbits, and a technique related to this was used with success for the diagnosis of *T. e. equiperdum* in horses by a number of workers (CIUCA, 1933; DOMILESCU, 1938; SOLDINI, 1938, 1939; HAIG and LUND, 1948). CORDIER and MENAGER (1934) used the technique with guinea-pigs for the detection of *T. e. equiperdum* in donkeys.

Recently it has been shown that other trypanosomes of the subgenus *Trypanozoon*, and *T. cruzi*, grow well in the testicles of laboratory animals (HEISCH, KILLICK-KENDRICK, DORRELL and MARSDEN, 1968). The object of the present paper is to give these findings in greater detail and to include observations on the behaviour of other trypanosomes, leishmaniae and ascitic tumour cells in the testicles of laboratory animals.

Material and methods

Infective material was inoculated directly into the testicle of a laboratory animal, usually a rabbit, guinea-pig, hamster or rat, with strict aseptic precautions. The scrotal skin over the testicle to be inoculated was cleaned very carefully with spirit. 0.5-1.5 ml. of the inoculum, usually blood, was sufficient, depending on the size of the testicle to be inoculated. Better results were obtained if antibiotics were not added; the inoculum therefore had to be absolutely sterile.

When obtaining testicular material for biopsy a sterile 5 or 10 ml. syringe with the plunger drawn back was attached to a hypodermic needle of rather large bore, which was pushed into the body of the testicle; the needle was then withdrawn and the contents squirted on to a slide for examination. This method was usually sufficient but sometimes a small vessel in the skin over the testicle was punctured and only blood was obtained. To overcome this the finger and thumb of the left hand were used to squeeze a portion of the testicle, the pressure being kept up for 2 to 3 minutes to expel the blood. The hypodermic needle was then driven between the finger and thumb and when withdrawn usually contained a small plug of testicular tissue. This was either smeared on a slide and stained with Giemsa's stain, or mixed with a drop of citrate or saline, covered with a coverslip and viewed by phase contrast illumination.

*Deceased.

**Present address: Dept. of Zoology and Applied Entomology, Imperial College Field Station, Ashurst Lodge, Ascot, Berks.

***Present address: Nuffield Institute of Comparative Medicine, Zoological Society of London, Regents Park, N.W.1.

During this work the senior author was supported by the Ministry of Overseas Development. The Ministry also provided funds for a visit to Nigeria. We wish to thank Mr. T. M. Leach for kindly providing facilities for work at N.I.T.R., Kaduna, Nigeria, and Mr. N. Mitchell for technical advice and assistance.

Results

As reported elsewhere (HEISCH et al., 1968), *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* grow readily when inoculated into the testicles of rabbits. Even when the blood of rats and mice showing 2 to 3 trypanosomes per field ($\times 100$ obj.) was inoculated intratesticularly, after 2-3 weeks many trypanosomes (50 to 300 per field when viewed by phase contrast with $\times 40$ objective) appeared in biopsy material. The infection continued 3-4 months, the number of trypanosomes gradually being reduced. The inoculated testicle slowly diminished in size and became hard and fibrotic. *T. b. rhodesiense* usually caused more acute infections in rabbits, the testicle and overlying skin becoming gangrenous. The three trypanosomes usually developed more slowly in the testicles of guinea-pigs, and only became numerous 3-4 months after inoculation. In rats trypanosomes were often scanty in the testicles, but quite numerous in the epididymis. Although *T. b. gambiense* developed more slowly in the testicles of rabbits than the other two trypanosomes, the test was fairly sensitive. Thus when 1.5 ml. of apparently negative blood from a chronically infected monkey was inoculated into the testicle of a rabbit, trypanosomes appeared after a few months and eventually became quite numerous (20-40 per field under phase contrast and a $\times 40$ objective).

About a week after infections had become established in the testicles of rabbits and guinea-pigs, trypanosomes began to appear in the testicle on the other side. This was useful because if the inoculated testicle became infected with secondary bacteria, the testicle on the other side remained sterile and the trypanosome was not lost.

A curious observation was that although spermatogenesis was reduced or abolished in the inoculated testicle this did not occur when trypanosomes spread to the other side. This was not due to trauma caused by the inoculation, for when uninfected blood was inoculated into the testicles of rabbits and guinea-pigs spermatogenesis was unaffected.

Portions of rabbit and guinea-pig testicles infected with *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* were fixed in Carnoy, sectioned and stained with Giemsa's stain using the colophonium method. Trypanosomes, which could usually be seen quite easily, were confined to the interstitial tissue and were extracellular. The tubules were often atrophied, with a complete absence of spermatocytes. There was intense and often focal round cell infiltration of the interstitial tissue. In more advanced infections of the testicles there was some interstitial fibrosis, but the tubules though atrophied were never completely obliterated.

Other trypanosomes inoculated into the testicles of laboratory animals were *T. cruzi*, *T. vivax* and *T. lewisi*.

T. cruzi did not develop well in the testicles of rabbits, but in the testicles of hamsters appeared as amastigotes in the Sertoli cells of the tubules, giving a beautiful appearance in sections stained with Giemsa's stain. *T. cruzi* was never seen in the interstitial tissue, and never spread to the contralateral testicle.

T. vivax did not develop in the testicles of rabbits but multiplied rapidly in the testicle of the kid, numerous and very active intermediate forms appearing after 4-5 days; the infection was brief and died out after about a fortnight. Sections of the infected kid's testicles were not examined.

T. lewisi was tested in the testes of rabbits and guinea-pigs, but no multiplication occurred.

Two leishmaniae were tested—*Leishmania enriettii* and *L. donovani*. *L. enriettii* grew rapidly in the testicles of hamsters, amastigotes appearing in large numbers in cells of the interstitial tissue about 10 days after inoculation. A few cells of the lamina propria

were infected to a lesser extent. *L. donovani* developed more slowly, amastigotes appearing in cells in the interstitial tissue about 4-5 weeks after inoculation; a few cells of the lamina propria also contained parasites.

Ascitic fluid from the peritoneal cavity of a mouse infected with a sarcoma of the Downe's type, with about 20-30 cells per field (phase contrast and a $\times 40$ objective), was inoculated into the testes of a rabbit, hamster and a white rat. There was no growth in the rabbit's testicle but columns of tumour cells were seen in the interstitial tissue of the other two animals. Growth continued for about a fortnight and then the tumour cells disappeared.

Discussion

The observations recorded in this paper show that the "testicular technique" may help to distinguish various protozoa. Thus the development of *T. cruzi* as amastigotes in the Sertoli cells of the tubules was strikingly different from that of trypanosomes of the subgenus *Trypanozoon* (*T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense*) in the interstitial tissue. Development of the last three trypanosomes was much the same except that *T. b. rhodesiense* tended to cause gangrene of the testicle in rabbits, and *T. b. gambiense* was the slowest to develop. *T. vivax* could be distinguished from the other trypanosomes because it did not develop in the testes of laboratory animals but caused heavy infections in the testicles of the kid. The two leishmaniae tested in hamsters could usually be differentiated because although both developed in the interstitial tissue, *L. enriettii* developed more rapidly and profusely than *L. donovani*.

The "testicular technique" was also found of value when trying to isolate trypanosomes from domestic animals in Nigeria. *T. b. brucei* was isolated on several occasions and one strain behaved like *T. b. gambiense* when inoculated into white rats, with a very light and transitory infection developing on the 18th day.

Intratesticular inoculation of rabbits may have a special use in the diagnosis of Gambian sleeping sickness. Strains of *T. b. gambiense*, particularly those from West Africa, usually fail to infect laboratory rodents when inoculated intraperitoneally, and the attempts to demonstrate the parasite in this way seldom succeed. The method may also help to recognize *T. b. gambiense* in an as yet undiscovered animal reservoir.

The inhibition of spermatogenesis caused by trypanosomes of the subgenus *Trypanozoon* was difficult to understand, particularly as it did not occur if the infection spread to the contralateral testicle. *T. cruzi* also inhibited spermatogenesis, but never spread to the testicle on the other side.

The growth of sarcoma cells in the interstitial tissue of the testes of hamsters and white rats was of especial interest, because after about a fortnight the tumour cells disappeared.

Summary

T. b. brucei, *T. b. rhodesiense* and *T. b. gambiense* multiply in the interstitial tissue when inoculated into the testicles of rabbits and guinea-pigs; *T. b. gambiense* grows more slowly than the other two. *T. b. rhodesiense* sometimes causes gangrene of the testicle and overlying skin of the rabbit. All three spread to the uninoculated testicle of rabbits and guinea-pigs after 1-3 weeks.

T. cruzi developed readily in the testicle of a hamster, numerous amastigotes appearing in the Sertoli cells of the tubules. No parasites were seen in the interstitial tissue.

T. vivax did not develop in the testicles of rabbits, but multiplied readily in the testicle of a kid.

T. lewisi failed to multiply in the testicles of rabbits and guinea-pigs.

Leishmania enriettii grew vigorously and rapidly in the testicles of hamsters, numerous amastigotes appearing in cells in the interstitial tissue, and occasionally in cells of the lamina propria.

L. donovani developed similarly but more slowly.

In testicles inoculated with *T. b. brucei*, *T. b. rhodesiense*, *T. b. gambiense* and *T. cruzi* spermatogenesis was interfered with. With the first three trypanosomes inoculated testicles became smaller and harder, and sections showed interstitial fibrosis, particularly in rabbits.

Ascitic tumours of the Downe's type grew in columns in the interstitial tissue of the testicle of a hamster and a white rat. The tumour cells disappeared after about a fortnight.

REFERENCES

- CIUCA, A. (1933). *Bull. Off. int. Epizoot.*, **7**, 168.
 CORDIER, G. & MENAGER, J. (1934). *Revue vét. Toulouse*, **86**, 185.
 DOMILESCU, G. C. (1938). Thesis, Bucarest. (Abst. in *Vet. Bull. Weybridge*, **11**, 159 (1941)).
 HAIG, D. A. & LUND, A. S. (1948). *Onderstepoort J. vet. Sci. Anim. Ind.*, **23**, 59.
 HEISCH, R. B., KILLICK-KENDRICK, R., DORRELL, J. & MARSDEN, P. D. (1968). *Trans. R. Soc. trop. Med. Hyg.*, **62**, 5.
 SOLDINI, M. (1938). *Bull. Acad. vét. Fr.*, **11**, 557.
 ——— (1939). *Bull. Soc. Path. exot.*, **32**, 334.
 UHLENHUTH, P. & EMMERICH, E. (1913). *Dt. med. Wschr.*, **39**, 642.

Blood parasites of scaly-tailed flying squirrels in the Ivory Coast

R. KILLICK-KENDRICK AND L. BELLIER

*Department of Zoology, Imperial College of Science and Technology and O.R.S.T.O.M.,
Centre d'Adiopodoumé, Abidjan, Ivory Coast*

During a search for malaria parasites of African rodents, blood films were prepared from 37 specimens of flying squirrels of 3 different species in the Lokota district of the Ivory Coast. The following parasites were seen:

1. *Plasmodium*. Malaria parasites were found in heart blood from *Anomalurus peli* and *A. derbianus* (= *A. fraseri*; see ROSEVEAR, 1969). Parasitaemias were light in 6/15 *A. peli* and 2/6 *A. derbianus*, but the pigmented appearance of the spleens of the other animals, suggests that all specimens of these flying squirrels were probably infected. We think that *A. peli* in the Ivory Coast is possibly a host of 2 species of *Plasmodium* both differing from *P. anomaluri* PRINGLE, 1960, described from a single specimen of *A. derbianus* in Tanzania. One of the parasites from the Ivory Coast causes an intense reddening of infected erythrocytes. The other, which does not alter the host cell in this way, is unusual in that erythrocytic schizogony seems to be restricted mainly to the blood capillaries of the lungs.

2. *Trypanosoma*. Trypanosomes were found in 10/15 *A. peli* and 5/6 *A. derbianus*. There were two species; one resembles *T. denysi* RODHAIN, PONS, VANDENBRANDEN and BEQUAERT, 1913 (= *T. anomaluri* SCHWETZ, 1933; see HOARE, in press), but the other is previously undescribed. The new parasite is a large (34-48 μ) species of the subgenus *Herpetosoma*.

3. *Hepatozoon*. Two new haemogregarines were seen in the leucocytes of the pygmy flying squirrel, *Idiurus macrotis*. The commoner of the two was in blood films from 4/16 animals; its gametocytes were similar in appearance to those of common species of *Hepatozoon* of other rodents such as *H. muris*, *H. microti* or *H. griseisciuri*. (One of us (R. K-K.) later found this same parasite in 10/16 *Idiurus macrotis* collected in West Cameroons). In the blood of one infected *Idiurus*, a second haemogregarine of curious morphology was found. The gametocytes of this parasite were unusually long and slender (27.5-38.5 μ $\times$ 1.5 μ) and rapidly left the host cell if films were not dried quickly. Because of their shape, the gametocytes of this second parasite were at first mistaken for microfilariae.

4. *Microfilariae*. In the heart blood of 1 *A. peli* microfilariae morphologically similar to those of *Onchocercella katangensis* of the giant elephant shrew *Petrodromus tetradactylus* in Katanga were seen. No adult worms were found in the thoracic and abdominal cavities, the mesentery, heart, lungs, liver and spleen.

Many of the flying squirrels were examined for ectoparasites shortly after death, but no fleas, lice, mites or ticks were found.

We thank Professor P. C. C. Garnham, Dr. C. A. Hoare and Dr. R. Muller for advice on the identity of the parasites. The senior author thanks the Medical Research Council and the World Health Organization for financial support.

REFERENCES

- HOARE, C. A. (in press). *The trypanosomes of mammals*. Oxford: Blackwell.
PRINGLE, G. (1960). *Trans. R. Soc. trop. Med. Hyg.*, **54**, 411.
RODHAIN, J., PONS, C., VANDENBRANDEN, F. & BEQUAERT, J. (1913). *Rapport sur les travaux de la mission scientifique du Katanga Octobre, 1910, à Septembre, 1912*. Brussels.
ROSEVEAR, D. R. (1969). *Rodents of West Africa*. London: British Museum (Natural History).
SCHWETZ, J. (1933). *Annls. Parasit. hum. comp.*, **11**, 287.

EPIDEMIOLOGICAL SIGNIFICANCE OF *STRONGYLOIDES* IN SPUTUM

SIR,—Recently we received a sample of sputum collected from a patient in Bristol (U.K.) on June 25th, 1971, with a request to identify some nematodes found in the specimen. It had been delayed in the post and was not delivered to us until June 30th, 5 days after collection. On first examination we found rhabditiform bacteriophagous larvae of *Strongyloides* sp.; a few larvae, however, were filariform infective stages. The specimen was left on the bench and when examined again next day, almost all the larvae were found to have developed into filariform stages. SEABURY et al. (1971) have similarly reported the culture of *S. stercoralis* in sputum.

These observations raise the question of the possible spread of infection by promiscuous expectoration. Sputum, especially if associated with lung conditions accompanied by the copious production of mucus, provides a moist viscous medium for a rich bacterial culture with ideal foraging conditions for rhabditiform larvae and their subsequent growth to infective forms.

Although this appears to be the first record of *Strongyloides* in sputum in the U.K., there are scattered reports of the parasite in human sputum, pleural exudate and lungs from other parts of the world (GAGE, 1911; FROES, 1930; YOSHINO, 1932; LAPTEV, 1945; CAMAIN et al., 1955; BROWN & PERNA, 1958; SEABURY et al., 1971). These infections appear to have been noticed only in patients with broncho-pneumonic symptoms. Premature development of *Strongyloides* in the lungs, which would be accompanied by the presence of larvae in sputum, is known to occur (MACKIE, 1948), but to provide evidence to support our suggestion that expectoration is of greater epidemiological importance in strongyloidiasis than usually realized, we need information on the incidence of larvae in the sputum of uncomplicated cases.

There is no reason to suppose that the case in Bristol was an autochthonous infection. Although the patient had not been abroad since 1945, we assume this was a long-standing, low-grade infection. It has been suggested that chronic infections sometimes erupt "in patients who are weakened, ill or have some deficiency." (GALLIARD, 1967). In the present case the patient suffered terminal carcinoma and died on the day we received the sputum.

We thank Dr. Francis T. Page, Dr. D. S. Reeves and Mr. Martin Bywater of Southmead Hospital, Bristol, for bringing this case to our notice, and Mr. P. S. Gooch of the Commonwealth Institute of Helminthology for helping us to trace reports of *Strongyloides* in sputum.

We are, etc.,

NEIL A. CROLL,
R. KILLICK-KENDRICK,
Department of Zoology and Applied
Entomology, Imperial College,
London University.

17 August, 1971

REFERENCES

- BROWN, H. W. & PERNA, V. P. (1958). *J. Am. med. Ass.*, **168**, 1648.
CAMAIN, R., DESCHIENS, R. & SENECAI, J. (1955). *Bull. Soc. Path. exot.*, **48**, 51.
FROES, H. P. (1930). *Annls Parasit. hum. comp.*, **8**, 171.
GAGE, J. G. (1911). *Arch. intern. Med.*, **7**, 561.
GALLIARD, H. (1967). *Helm. Abs.*, **36**, 247.
LAPTEV, A. A. (1945). *Klinich. Med.*, **23**, 75.
MACKIE, T. T. (1948). *Dis. Chest*, **14**, 894.
SEABURY, J. H., ABADIE, S. & SAVOY, F. (1971). *Am. J. trop. Med. Hyg.*, **20**, 209.
YOSHINO, K. (1932). *Taiwan Igakkai Zasshi*, **31**, 99.

THE LOW PATHOGENICITY OF *TRYPANOSOMA BRUCEI* TO CATTLE

SIR,—In the discussion following Dr. Goodwin's paper on the pathology of African trypanosomiasis, Dr. Hoare lays the ghost of the pathogenicity of *Trypanosoma brucei* to cattle. Early accounts of *T. brucei* killing cattle (e.g. BRUCE, 1896; FOY, 1911) were almost certainly based, as Dr. Hoare suggests, on infections mixed with *T. congolense* (or *T. vivax*). But WENYON'S (1926, p. 542) view that cattle infected with this trypanosome rarely recovered seems to have been the result of typographical error.

The story is told in a little-known publication by HORNBY (1930). He says of *T. brucei*: "There is still a good deal of misapprehension concerning the action of this trypanosome on domestic animals, for a little of which I was unconsciously responsible. In my article on "The Trypanosomes found in domestic animals in South-Central Africa" (1919), I used a clumsy sentence to the effect that in a certain experiment 83% of cattle infected with *T. brucei* did recover. Unfortunately the compositor in England turned "did" into "died" so that the printed sentence ended "of which 83% died." This article was seen by Wenyon and probably influenced him in stating that cattle rarely recover. It was therefore in the manner of announcing a discovery that as late as 1928, DUKE, in the Final Report of the League of Nations International Commission, wrote (p. 80): "The experience of workers in Uganda, I think, goes to show that an uncomplicated infection of *T. brucei* in cattle does not often, and certainly does not always, prove fatal." The experience of veterinarians in Uganda and elsewhere showed this quite clearly ten years previously. In the Laboratory one can easily set up an infection of bovines with *T. brucei* which is usually transient, though occasionally fatal, but in sixteen years' experience of nagana I have not met half-a-dozen cases of naturally contracted severe disease of cattle due to *T. brucei*."

T. brucei is common in cattle in many parts of Africa, but infections are usually sub-patent (e.g. GODFREY and KILLICK-KENDRICK, 1961) and there appear to be no field reports of deaths due to this trypanosome alone. It is probably true to say, however, that some strains of *T. brucei* give rise to chronic long-standing infections in cattle which occasionally lead to death. If 83% of Hornby's cattle recovered, presumably 17% did not. Furthermore GRAY (LEACH, 1964, p. 28) experimentally infected two cattle with different cyclically transmitted strains of *T. brucei* and found that while one recovered completely, the other died a year later, apparently of trypanosomiasis.

Should the term nagana be used only for the acute disease caused by *T. brucei* in horses? When applied to the same infection in cattle, perhaps it perpetuates the mistaken view that *T. brucei* is highly pathogenic to these animals.

I am, etc.,

R. KILLICK-KENDRICK,

Department of Zoology and Applied Entomology,
Imperial College Field Station,
Ashurst Lodge, Ascot, Berkshire.

16 December, 1970

REFERENCES

- BRUCE, D. (1896). *Further Report on the Tsetse Fly Disease or Nagana in Zululand*. London: Harrison and Sons.
FOY, H. A. (1911). *J. trop. Med. Hyg.*, **14**, 301.
GODFREY, D. G. & KILLICK-KENDRICK, R. (1961). *Ann. trop. Med. Parasit.*, **55**, 287.
HORNBY, H. E. (1919). *Vet. J.*, **75**, 128.
——— (1930). Control of Animal Trypanosomiasis. In *Proc. 11th Int. Vet. Congr.*, London: Bale, Sons and Danielson.
LEACH, T. M. (1964). *Ann. Rep. nig. Inst. Tryp. Res.*
WENYON, C. M. (1926). *Protozoology*. London: Baillière, Tindall & Cox.

Abstract of paper
presented at the
2nd International
Congress of Para-
sitology, Washington,
1970.

89. The life-cycles of two murine malaria parasites from Nigeria.

R. KILLICK-KENDRICK, Department of Zoology and Applied Entomology, Imperial College of Science and Technology, Ascot, Berks, England.

The life-cycles of Nigerian strains of *Plasmodium berghei* ssp. indet. and *P. vinckei* ssp. indet., isolated

in 1967 from wild-caught *Thamnomys rutilans* (1), were studied in laboratory-bred rodents and mosquitoes.

In white mice, the blood stages of the Nigerian *P. berghei* (strain N.67) were indistinguishable from those of strains and subspecies from other localities.

Cyclical transmission was easily obtained using *Anopheles stephensi*. The size of the mature oocysts, the time taken for completion of sporogony and the yield of sporozoites were affected by the temperature at which the mosquitoes were incubated. At 21 C, oocysts grew larger (70μ) than at 24 or 28 C ($50-62 \mu$). The sporogonic cycle was shorter at 28 C (6 days) than at 21 or 24 C (9-11 days). The yield of sporozoites from mosquitoes incubated at 28 C was much less than at the lower temperatures, but the sporozoites were, nevertheless, infective to white mice. As few as 80 sporozoites from the salivary glands of mosquitoes incubated at 24 C always produced infections in white mice when inoculated intravenously.

The sporozoites were remarkably long. The mean length of 50 in a dried stained smear was 16.72μ (S.E. 0.22μ) with a range of $10.0-19.5 \mu$; statistically there was found to be a highly significant difference between this length and the length of sporozoites of two strains of *P. b. berghei* (ANKA and RLL) from Katanga.

Tissue schizonts of the Nigerian strain were similar to those of *P. b. yoelii* from the Central African Republic (2) and *P. b. killicki* from Brazzaville (3). Like the latter subspecies, hypertrophy of the nuclei of infected parenchymal cells was often seen.

Observations were made on two strains (N48 and N54) of *P. vinckei* from Nigeria. In white mice, the morphology of blood stages was very similar to that seen in a naturally infected *Thamnomys*. There were no important differences seen in the blood forms of these new strains and those of a strain isolated in 1953 from a *Praomys* collected in the same locality (4).

Sporogony was obtained in *A. stephensi*, *A. quadrimaculatus*, and *A. labranchiae atroparvus* incubated at $25 \text{ C} \pm 1 \text{ C}$; *A. gambiae* (B) was found to be insusceptible. The highest numbers of oocysts and sporozoites were seen in *A. stephensi*, but as in all 3 species of susceptible mosquitoes, the yield of sporozoites was always low, and sporozoites were never infective to white mice or laboratory-bred *Grammomys* or *Praomys*.

The normal size of mature oocysts was $50-60 \mu$ in diameter, but forms measuring $40-70 \mu$ were encountered. The mean length of 50 sporozoites in dried stained smears of infected salivary glands was 14.7μ (S.E. 0.5μ) with a range of $10-24 \mu$. This wide range suggests a higher than usual proportion of malformed sporozoites.

A third strain of Nigeria *P. vinckei* is presently being studied in an attempt to obtain infective sporozoites and to demonstrate primary exoerythrocytic schizonts.