LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

MEMOIR 12

STUDIES ON THE EXO-ERYTHROCYTIC CYCLE IN THE GENUS *PLASMODIUM*

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by

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PREFACE

THERE exists today no adequate account of the exo-erythrocytic stages of the malaria parasites. These studies represent an attempt to set out the knowledge of the subject as it stands today together with some new information which might otherwise never reach the interested reader. This memoir cannot be treated other than as an interim report on a subject still swiftly growing after the thirty years in the wilderness (1903–1933). The discoveries of Raffaele, James, Huff, Kikuth, Mudrow, Shortt and Garnham bore abundant fruit and in an amazingly short time. 1954 is not an inappropriate year in which to look back on this twenty years of growth. The broad picture is apparent and the era of consolidation is now at hand.

The whole of these studies have been pursued in the Department of Parasitology of the London School of Hygiene and Tropical Medicine. I owe a debt of gratitude to the two Directors of this Department in my time which can be expressed simply by stating that these words and this work could not have been but for the constant presence of Professor H. E. Shortt and Professor P. C. C. Garnham at my elbow.

Many others have rendered me invaluable assistance and advice. Miss W. Wall and Mr. W. Cooper guided the stumbling steps of the tyro in the early stages. Dr. C. A. Hoare, Dr. J. Williamson, Dr. H. B. Fell and Dr. C. Wilcocks have all given unstintingly of their time and advice. I am greatly indebted to Mr. C. C. Barnard and his staff for bibliographical assistance, and to Miss B. J. Luckhurst for reading the proofs and helping with the compilation of the index.

Finally, for the typing of the manuscript and for her patient encouragement, I owe a deep debt of gratitude to my wife.

R. S. BRAY

December, 1954

NOTE

This work was completed in December 1953. The only information appearing in 1954, which has been included in this memoir, is that concerning *P. ovale*.

As burning fevers, agues pale and faint,
Life-poisoning pestilence and frenzies wood,*
The marrow-eating sickness, whose attaint
Disorder breeds by heating of the blood ; . . .

SHAKESPEARE, *Venus and Adonis*

* =mad, *O.E.D.*

CONTENTS

LIST OF FIGURES

All drawings of parasites made by using a *camera lucida*

STUDIES ON THE EXO-ERYTHROCYTIC CYCLE IN THE GENUS *PLASMODIUM*

CHAPTER I

DEFINITIONS, GENERAL CLASSIFICATION, TERMINOLOGY

DEFINITIONS

THE genus *Plasmodium* (Marchiafava and Celli, 1885) includes parasitic protozoa which reproduce sexually and by sporogony in an insect host and asexually by schizogony in two cycles in the vertebrate host, one in red blood cells producing pigment and the other in cells other than red blood cells.

The exo-erythrocytic cycle in this genus refers to that asexual schizogony which takes place in cells of the vertebrate host other than red blood cells. These cells are usually fixed tissue cells of one type or another, though there are several notable exceptions to this. In this cycle the parasite does not produce pigment. The term" exo-erythrocytic ", first coined by James and Tate (1937b), has gained general acceptance in reference to this cycle but it is not an ideal term as it defines what the cycle is not rather than what it is.

TERMINOLOGY

The terminology here elaborated and discussed refers only to the exo-erythrocytic cycle. Various terms are in use in the descriptions of this cycle and they will be listed roughly along national lines.

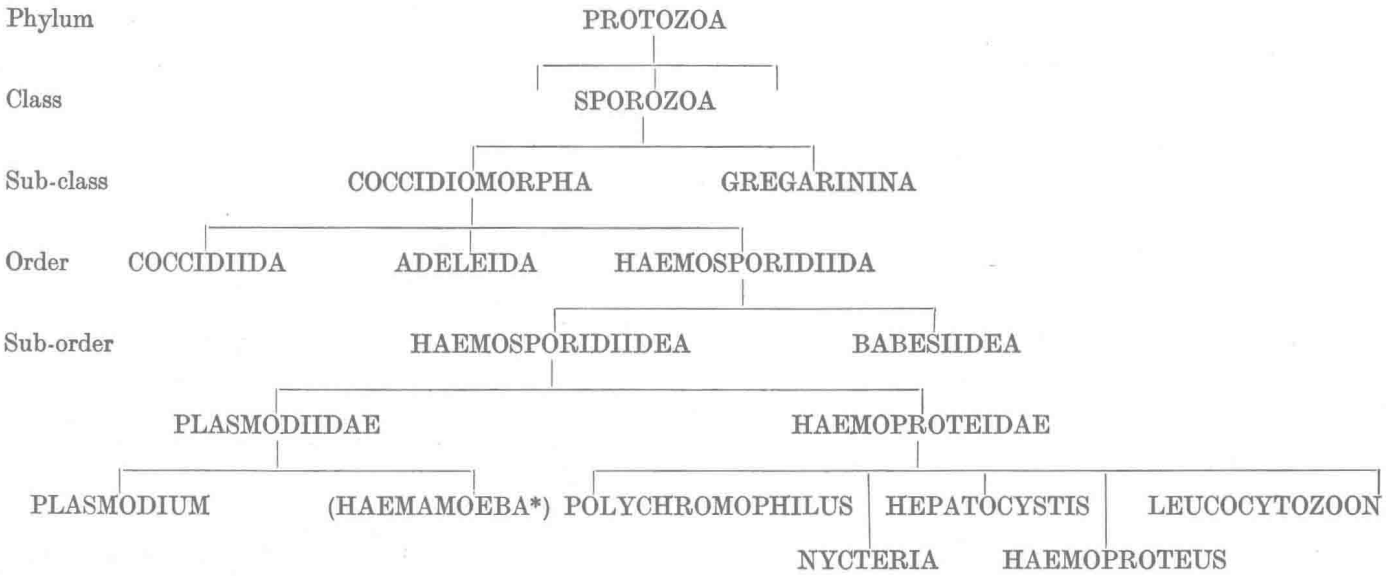
The English terminology derives from James and Tate (1937a) and Shortt and Garnham (1948a).

Exo-erythrocytic or e-e. (Henceforth this abbreviation " e-e " will be used in this work). This term was used by James and Tate to denote schizogony or stages of it in cells other than erythrocytes or reticulocytes. It was later modified by Shortt and Garnham to mean that schizogony in cells other than erythrocytes or reticulocytes occurring after parasitaemia was initiated or was theoretically possible. For the schizogony occurring before parasitaemia they used the term *pre-erythrocytic* or pre-e. (Henceforth this abbreviation " pre-e " will be used in this work.) Terms such as tissue phase or fixed tissue stages are also in use.

The Italian Terminology. Raffaele (1938a, 1938b) referred to the e-e schizogony as the primary monogonic cycle after Grassi. Corradetti (1941b, 1952), Corradetti and Gramiccia (1941b) used the terms :

(*a*) endo-haemoblastic for schizogony in red cell precursors ;
(*b*) endo-histiocytic for schizogony in reticulo- and vascular endothelial cells
(*c*) hepatic endo-epithelial for schizogony in liver parenchyma cells.

The taxonomic position of the genus with its closely allied genera is here accepted as follows :



This formulation combines features from Wenyon, Calkins, Hall and Garnham, and embodies the author's personal views on the phylogenetics of the genus *Plasmodium* (see pp. 153–6).

* The postulation of two genera in the family Plasmodiidae will be made in the last chapter and in the meantime the genus *Plasmodium* will be given its usual present-day scope.

These workers have made no differentiation between generations of schizogony. Their terms have not been generally accepted.

The French terminology. Ed. Sergent (1949b) rejected the term pre-erythrocytic in favour of pro-erythrocytic, as "erythrocytic" is a combination of two Greek words and " pre " is a Latin prefix. " Pro " represents the equivalent Greek prefix. He also appealed for a change from exo-erythrocytic to ex-erythrocytic for reasons not divulged, though perhaps to eliminate the consecutive occurrence of vowels. However, as " ex " is a Latin prefix whereas " exo " is the appropriate Greek prefix, this latter appeal would appear to nullify the former. This terminology has not been generally accepted. Various French authors have used the term histiocytic which is in common usage as a substitute for e-e in France.

The German terminology. Kikuth, Mudrow and other German authors have at times used the words endothelial phase and E-stage, but generally they have conformed with the English terminology.

The American terminology. Huff, Coulston and Cantrell (1943) and Huff and Coulston (1944, 1946) have been responsible for the more precise American terms which at present apply only to the avian plasmodia. They use the terms *cryptozoite* and *metacryptozoite* to describe the first and second pre-e schizogonic generations respectively derived from sporozoites and occurring before parasitaemia. All other e-e generations are termed *phanerozoite.*

For the purpose of this work the English terminology will be used in general but the American terminology may be used when it is necessary to be precise in dealing with the avian malarias. The following definitions are therefore adopted.

Pre-erythrocytic cycle. The schizogony generations occurring in cells other than erythrocytes or reticulocytes and completing their schizogony prior to the demonstrable or theoretically possible invasion of erythrocytes or reticulocytes.

Exo-erythrocytic cycle. In general all generations of schizogony occurring in cells other than erythrocytes or reticulocytes. In particular when used in contradistinction to pre-erythrocytic, the term denotes all generations of schizogony occurring after parasitaemia on the above bases.

Cryptozoite generation. That pre-e generation deriving from sporozoites.

Metacryptozoite generation. That pre-e generation deriving from the cryptozoite generation.

Phanerozoite generations. All e-e generations occurring after demonstrable or theoretically possible parasitaemia. The first of these generations may derive from the cryptozoite or metacryptozoite generation.

These definitions represent a view which differs in some details from that of the Drafting Committee of WHO on Malaria Terminology (Covell, Russell and Swellengrebel, 1953). The Drafting Committee in one paragraph defined the metacryptozoite stage as being those generations arising from the cryptozoic stage and occurring before the appearance of parasitaemia. In another paragraph the Committee states : " The progeny of the second and of succeeding generations

after exo-erythrocytic schizogony are called metacryptozoites. Some of the metacryptozoites, especially after three or four generations appear to penetrate erythrocytes. . . ."

It has been felt that such a definition of metacryptozoites is too indefinite as to the actual duration of metacryptozoite schizogony. The actual appearance of parasitaemia may be after the first metacryptozoite generation or may be delayed months involving scores of generations of the e-e cycle. Therefore the definition has been adopted involving the use of the term " theoretically possible parasitaemia " which has the effect of reducing in fact the number of generations of metacryptozoite schizogony to one at the most in those species of plasmodia adequately studied. The admitted drawback of the definition is that it may be self-eliminatory in that parasitaemia may be theoretically possible after cryptozoite schizogony in all species.

CHAPTER 2

INTRODUCTION

INTRODUCTORY NOTES ON THE PLASMODIA

THE plasmodia are exclusively obligate parasites and cause the disease of vertebrates loosely known as malaria. The persistent geographical distribution of the genus in the vertebrate host is apparently confined only by the presence of a suitable insect or definitive host though temperature must play some part. In the vertebrate or intermediate host the biological distribution is widespread, appearing as it does in penguins and toucans, in lizards and in man. Fish are the only class among the vertebrates in which plasmodia have not been described.

Host specificities divide the various species of plasmodia into groups. On the one hand definitive host specificities divide the plasmodia into two groups. The one where the parasites complete their sexual cycle exclusively in anopheline mosquitoes, the other where the sexual cycle may take place in anopheline or culicine mosquitoes. The intermediate host specificities also divide the true plasmodia into two groups. The one group contains those parasitizing vertebrates having nucleated mature red blood cells—the avian and saurian plasmodia. The other group contains those parasitizing vertebrates having non-nucleated mature red blood cells—the human, simian, rodent and chiropteran plasmodia.

These groupings by host specificity coincide, so that we have two main groups.

1. The mammalian plasmodia which are cyclically transmitted exclusively by anopheline mosquitoes.
2. The avian and saurian plasmodia which may be cyclically transmitted by either anopheline or culicine mosquitoes. (This is not proven as yet in the case of saurian plasmodia but may be presumed.)

The phylogenetics of the genus are somewhat obscure. Huff (1938) argues that it may once have been confined to the present insect hosts. There is more convincing evidence, on the other hand, that it represents the furthest remove in a gradual drift away from the coccidia to which it is undoubtedly related.

The disease which these parasites cause has gone under many names, but malaria is now the accepted term. In man it is a disease of the first magnitude, but it is not intended to discuss either the epidemiology or clinical effects of the disease here.

The disease in animals varies widely in intensity in unnatural hosts, but of all natural hosts man is the only one to be constantly and seriously disturbed by the presence of the parasites. There have been isolated reports of heavy infections in some wild birds, and death attributed to the plasmodial invasion, but these are

exceptions (e.g. in blackbirds, Jacobs and Shortt, 1951). On the other hand, there may well be a high death rate among nestlings with plasmodia as a contributory cause. In unnatural hosts, however, some plasmodia can achieve an astonishing virulence.

Domestic animals are remarkably free from plasmodia. The only known outbreak of any importance being that of *Plasmodium durae* in turkeys (Purchase, 1942) though *P. juxtanucleare* can occur in small foci in chickens (Paraense, 1947). Thus the economic importance of the parasites is confined to their effect upon man, in which field they amply compensate for their unimportance elsewhere.

The normal life cycle of a *Plasmodium*, as far as it is known in nature, is briefly and broadly as follows :

A mosquito having in its salivary glands the sporozoites of the *Plasmodium* bites a vertebrate, and the sporozoites are injected intradermally with the mosquito's saliva. The sporozoites are taken up into the general blood circulation and are demonstrable there for 30–50 minutes. They then find their way into fixed tissue cells or cells of the haemopoietic system and reproduce there asexually without the production of pigment. The type of cell parasitized depends on the species of *Plasmodium* involved. This is a schizogonic cycle producing merozoites and takes place once or twice prior to the invasion of erythrocytes. This e-e cycle continues to reproduce itself independently of the erythrocytic cycle as merozoites continue to reinvade the same type of non-erythrocytic cells.

Many of the merozoites from the first or second pre-e generations invade erythrocytes or reticulocytes. In these cells the parasites reproduce asexually by schizogony, producing merozoites which reinvade red blood cells and in the species of group 2 (above) also invade non-erythrocytic cells, thus reinforcing the e-e cycle. This cycle in red blood cells produces pigment.

Some of the plasmodia in the red blood cells differentiate into male and female gametocytes. These sexual forms can fertilize each other and develop fully only in the gut of a mosquito, When a suitable species of mosquito bites the vertebrate having gametocytes in the blood they are taken up into the mid-gut of the insect. Here the male gametocyte produces the gametes which penetrate and fertilize the female gametes forming zygotes. The zygote elongates to form an oökinete which makes its way to the outside of the mosquito's gut wall to come to rest between the gut wall and the membrane surrounding the gut. Here the oökinete reproduces by sporogony to become an oöcyst which eventually contains some thousands of spores or sporozoites. These then break out and the majority come to rest in the salivary glands of the insect. The insect is now infective.

This, as can be seen, is no simple life cycle and only the intense rate of reproduction can explain the remarkably successful survival of the genus.

CHAPTER 3

HISTORICAL

(*a*) THE GENUS AND THE DISEASE

THE disease caused by the genus in man has been known from time immemorial. It is referred to in the Orphic poems (*c.* 1,000 B.C.) and in the philosophizings of an early Chinese emperor. It was first described and defined as a disease entity by Hippocrates (*c.* 400 B.C.). The fevers were described in some detail by the Roman physicians Celsus and Galen.

In the middle ages the quotidian, tertian and quartan agues were well-recognized fevers and were often referred to in contemporary chronicles. Many great men are recorded as having suffered from the disease, especially in the lands around the Mediterranean.

The disease of itself is one which has truly shaped the courses of history, playing as it did an enormous part in any military or social endeavour in the Middle and Far East, in Africa and in the Balkans. The victorious Alexander was turned back from virtual mastery of the land from Hellas to India by the disease. Such a conquest, if consolidated, would have resulted perhaps in an entirely different culture in the Mediterranean basin.

Shakespeare provided us with a less gloomy view of the disease by his use of the fevers as a parallel for that other common disease of man—love: " . . . for he seems to have the quotidian of love upon him." *As You Like It*, Act III, Scene 2.

In the Americas one wonders whether malaria played its part in " selecting " the Spanish *conquistadores* as the conquerors of the middle and southern portions of those continents. It is known, for instance, that a quartan ague terminated the clerkly career of Hernán Cortéz and sent him in search of more lucrative adventures. At all events, the Spanish conquest of Peru brought about the first relief for the civilized world from the chills and fevers of malaria. In the forests of Peru grew the cinchona tree, whose bark was an efficient febrifuge. About 1630 the bark is said to have cured the wife of the Governor of Peru, one Count of Chinchon, of an intermittent fever. This pleasant tale is probably apocryphal. About 1640 it is believed that a Jesuit father, truly *ad majorem Dei gloriam*, brought the bark to Europe and thus quinine came into general use in the treatment of malaria. In 1820 Pelletier and Caventou isolated the alkaloid in the pure state from the bark and the process was quickly commercialized and equally quickly abused.

In the latter part of the nineteenth century the classic researches of Pasteur and Koch on the aetiology of disease stimulated further studies in malaria with the intention of finding the aetiological agent. This was encompassed by Laveran in

1880, who described amoeba-like bodies in the red cells of patients suffering from malaria. He noted particularly the pigment and the gamete production of the male gametocytes.

Laveran's announcement was not immediately accepted by scientists then obsessed with bacteria identifiable by the carefully laid-down rules of Koch. However, Danilewsky observed similar parasites in the red blood cells of birds in 1885. In the late 1880s and early 1890s Laveran's discovery was confirmed and brilliantly extended by the work of the great Italian school of malariologists including Marchiafava, Bignami, Golgi, Celli and Bastianelli. Where Laveran had been somewhat vague and indecisive, these workers were precise and dogmatic and, all honour to them, remarkably correct. In only one major detail—the fate of the gametocytes—were they in any manner wrong.

The advent of the Romanowsky stains in 1890 greatly assisted the study of the parasites and, by the late 1890s, all three major species infecting man had been described by Golgi and others. Another plasmodium, *P. relictum*, of birds, had also been described by Grassi and Feletti.

In 1897–1898 an important observation was made by MacCallum, working with *P. falciparum*, when he recognized the true significance of the crescents and the " exflagellation " of the male gametocyte. He followed and described the full fertilization process. In 1898 Ross worked out the full sporogonous cycle of *P. relictum* in culicine mosquitoes and gave the world the surprising observation that mosquitoes carried malaria. This observation was confirmed in human malaria by Bignami, Bastianelli and Grassi also in 1898. They noted that it was the *Anopheles* which transmitted the human disease, which was confirmed by Grassi's work at Albanella and Manson's experiments in London and the Roman Campagna.

In the early 1920s malaria therapy of general paralysis of the insane was introduced and the study of the human plasmodia under controlled laboratory conditions was greatly facilitated thereby.

In the realm of antimalarials, 1926 saw the discovery of plasmoquine (pamaquine) and, in 1932, atebrin (mepacrine) was introduced. The discovery of these drugs was facilitated by the use of avian plasmodia, a number of which had been discovered by this time. Techniques for their use in drug assays had been worked out by Roehl, Schulemann and Kikuth in Germany.

In 1933 Sinton and Mulligan in India attempted to rationalize the muddle into which the contemporary knowledge of the simian malaria parasites had fallen.

In the next year Raffaele discovered the existence of the e-e cycle of avian malaria parasites and in 1937 James and Tate established the cycle as a definite and essential part of the life history of the plasmodia. In 1938 Kikuth and Mudrow gave the first description of the pre-e forms of avian plasmodia and, in 1943, Reichenow and Mudrow gave the first full description of the pre-e generations of avian plasmodia.