Papers and Originals

Pathology of Malaria in West Africa*

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The pathology of malaria in West Africa is a subject in which I have been intensively interested for almost 30 years. In this lecture I intend to limit myself to those facets of the pathology which I have been most concerned with while working in Accra in Ghana, and later in Ibadan, Nigeria.

Pattern of Malaria in West Africa

The city of Ibadan, which has a population of over 500,000, is not really a city in the Western sense—rather is it a large village to and from which the Yorubas, the predominant tribe in the region, travel from their farms in the surrounding countryside. This fact is important in considering the pattern of malaria. Accra, on the other hand, is more urbanized.

Three species of malaria parasites occur in this area of West Africa—*Plasmodium falciparum*, *P. malariae*, and *P. ovale*, of which the first is by far the most important and ubiquitous. *P. ovale* is patchy in distribution and is not thought to be an important cause of ill-health and mortality in the region. A few years ago the same might have been said about *P. malariae*, but recent investigations in Ibadan have disclosed that the nephrotic syndrome is common in children and that *P. malariae* is closely associated with this (Gilles and Hendrickse, 1963). In addition, we have long recognized in Ibadan a syndrome of splenomegaly and anaemia in adults which responds to long-term suppressive antimalarial therapy (J. Watson Williams, personal communication, 1960), and workers in East Africa have suggested that *P. malariae* may be associated with a condition which may be similar and is termed "big spleen disease" or tropical splenomegaly (Gebbie *et al.*, 1964; Marsden, 1965).

The pattern of malaria in West Africa is holoendemic or stable, as defined by McDonald (1957). This implies that transmission occurs throughout the year and that the intensity of infection is fairly uniform. The pattern is repeated annually, with little variation over the years. There is marked resistance to infection in the community owing to this high degree of prevalence, and the main impact of the disease is seen in young children.

In a village survey I undertook in Ghana with Drs. Colbourne and Hughes in the late nineteen-forties (Colbourne *et al.*, 1950) malarial parasite, spleen, and liver rates were studied in different age groups, and the incidence of other diseases was also assessed. The percentage incidence of anaemia was found to be about 90, hookworm 52, ascariasis 76, schistosomiasis 9, streptocerciasis 21, yaws 75, and signs of malnutrition (mostly in children) 26. Hernias, scabies, trachoma, and upper respiratory infections were also found to be present. In other areas onchocerciasis and guinea-worm infection might have been an additional parasitic load. These figures are quoted to emphasize the multiple infestations and infections which may be found in areas of West

Africa, and they add to the difficulty of assessing the mortality and morbidity that is caused by malaria itself. The findings in our survey were closely similar to those found in a village in the Gambia shortly afterwards by McGregor and Smith (1952), and more recently by Giffles (1964) in a village near Ibadan.

The incidence of malaria, splenomegaly, and hepatomegaly in this village is shown in the Chart. The crude parasite rate shows the incidence of the parasites of *P. falciparum* malaria in the peripheral blood on one examination. The infection is absent in the neonate, gradually rises until the age of 2 to 3 years, and then gradually falls until adult life is reached—when about 10% of the population "normally" have scanty parasites in the blood. *P. malariae* follows a somewhat different epidemiological pattern. The peak parasite rate never rises much above 30% and falls more rapidly. It is rarely detected in the adult. Splenic and hepatic enlargement follows a somewhat similar pattern to the crude parasite rate, though the incidence rates are considerably lower.

Incidence of malaria, splenomegaly, and hepatomegaly in a random sample of the Akufo population 1959-63.

The neonate does not contract malaria, and congenital malaria does not occur. The mechanism of this transient resistance of the newborn to infection, which lasts for the first few months of life, has been the subject of much speculation. At one time it was suggested that the low level of para-aminobenzoic acid in maternal milk was inimical to the growth of the parasite. Then it was suggested that selective vector-biting might be responsible—the young infant being less likely to suffer from the bite of a female anopheline mosquito. Later, after the hypothesis that sickle-cell haemoglobin protected the bearer against *P. falciparum* malaria, it was suggested that perhaps foetal haemoglobin acted in a similar manner. However, since

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the work of Cohen *et al.* (1961) in the Gambia and of Edozien *et al.* (1962) on cord blood in Ibadan it is generally accepted that the relative immunity of the newborn is due to the trans-placental acquisition of humoral immunity from the mothers. The last-named workers showed that the 7 S (IgG) fraction of gammaglobulin extracted from the cord blood on neonates in Ibadan would clear parasites from the peripheral blood of children heavily infected with *P. falciparum* malaria. This protection gradually decreases over the first few months of life, and hence the first malarial infection in infants is therefore mild. Thus children are enabled gradually to build up their acquired immunity, until by the age of five years the clinical manifestations of malaria are mild and a considerable degree of immunity is established.

Problem of Malaria in the Immune Adult

In Ibadan I have not attributed death to malaria in any child over the age of 4 years. In Accra, on the other hand, my impression was that deaths were beginning to occur in rather older children, because of the diminished transmission of the parasite owing to increasing urbanization. Death in Dakar in Senegal certainly occurs in older age groups (M. Payet, personal communication). In my experience there was only one exception to this observation in Nigeria. This was a Nigerian man suffering from chronic lymphatic leukaemia in whom splenectomy had been inadvertently performed. He died of cerebral malaria a few weeks after the operation. The immunological status of this patient had obviously been altered by disease of the reticuloendothelial system and a superimposed splenectomy. In this connexion it should be remembered that rupture of the spleen is not an uncommon complication of malaria, usually associated with mild trauma; and if splenectomy is performed in an area of stable malaria suppressive anti-malarial therapy must be considered. Overt symptoms of malaria in the immune adult are also said to be precipitated by surgical operations, anaesthesia, or any other form of stress. I doubt if there is convincing evidence to support this contention. The administration of corticosteroids in animal malaria results in a definite increase of parasitaemia, and this has been shown to occur in human malaria. Corticosteroid therapy is often used in Ibadan without antimalarial cover, and exacerbations of malaria in the immune have not been found to be a problem (T. Kinnear, personal communication). The development of miliary tuberculosis, however, has been found to be a real hazard.

It has been shown in Ibadan that pneumococcal meningitis occurs more often in pregnant women than in women who are not pregnant (Lucas, 1964), and my necropsy figures suggest also that fulminating amoebic dysentery is a greater hazard in the former group. Moreover, there is a lowering of resistance to malaria in pregnancy—spleen and parasite rates being higher in pregnant than in non-pregnant women (Bruce-Chwatt, 1952). Abortion and premature labour are also said to be complications of malaria, though from the examination of numerous uterine curettings I doubt whether in immune women malaria plays much part in miscarriage in the early months of pregnancy. Nevertheless, fairly severe attacks of malaria can occur in the later months, and birth weights have been shown to be less in mothers with infected placentas. Several placentas in Ibadan have shown fairly heavy malarial infection, with developing forms in the erythrocytes in the intervillous spaces and pigment in macrophages. An interesting finding has been the presence of pigment in the fibrin surrounding the chorionic villi. This has not stained with haemosiderin stains and has resembled malarial pigment. It is possible that the parasitized cells containing pigment tend to collect in the eddies of the intervillous spaces and cause premature deposition of fibrin around the villi, with consequent relative placental insufficiency. Non-iron-containing pigment has never been noted in the fibrin surrounding chorionic villi in non-infected placentas.

Basis of Immunity in Malaria

Immunity in malaria has been regarded as cellular or humoral—but it is doubtful if these factors can be separated, as they are probably interdependent. Erythrophagocytosis, which is such a feature of malaria, is probably dependent upon the presence of humoral factors in the plasma. Fluorescent-antibody techniques have been widely applied in malaria, and have been reviewed by Voller (1964).

Inherited and racial factors may play a part. In Ibadan we have observed a number of inherited factors in the blood: the sickle-cell trait is present in 24%, the Hb C trait in 6%, and the deficiency of the enzyme glucose-6-phosphate dehydrogenase in 22%, while there is a 0.1% incidence of the gene responsible for the persistence of foetal haemoglobin (Hb F) in West Africa. A fast-moving haemoglobin is present in 10% of neonates, while the gene for beta-thalassaemia is also present in the population. Allison and Beet independently many years ago suggested that the S gene, lethal in its homozygous expression, could be maintained in high incidence in a population only if the heterozygote was at an advantage when compared with persons carrying the genes for normal haemoglobin. It was suggested that the S heterozygote was protected from the lethal effects of *P. falciparum* malaria, the loss of normal genes from the effects of malaria more than balancing the loss of the S genes in children dying of sickle-cell anaemia.

For many years now I have had haemoglobin electrophoresis routinely performed on all subjects coming to necropsy, and in almost 50 children dying of cerebral malaria sickle-cell haemoglobin has not been detected. We now fully accept Allison and Beet's hypothesis. Unfortunately, confirming previous work (Edington and Laing, 1957), we have examples of children with the Hb C trait dying of cerebral malaria, so we cannot accept at present that C haemoglobin confers a partial protection against *P. falciparum* malaria. The person with a C gene, however, is only at a slight disadvantage, and detectable differences are likely to be small. Hence we are continuing to collect information on this question, though it will take several years to obtain statistically valid figures.

Preliminary studies in Ibadan did not suggest that G-6-PD deficiency gave any protection against malaria, but more recent studies are perhaps showing evidence of this (Gilles *et al.*, 1966).

Gorman (1964) has put forward a most interesting hypothesis about the possible selection against the Rh-negative gene by malaria. The incidence of the Rh gene is generally low in areas in which malaria is or was endemic. It was suggested that a population subject to a heavily malarious environment might be superior antibody producers owing to selection by the elimination of poor antibody producers. If this were so, erythroblastosis foetalis should be more intense in malarious areas, and Rh-negative genes should be selectively eliminated if the frequency of the gene is or was below 0.50 (Wiener, 1942). Hence Rh-negative mothers in a malarious area should show a higher incidence of sensitization to an Rh-positive foetus than their counterparts in northern areas. We have recently studied over 400 Rh-negative pregnant multiparae in Ibadan, and, apart from some who had had previous transfusions of Rh-positive blood, the incidence of those sensitized by pregnancy was only 2.5%—a lower incidence than the figures recorded from Europe and elsewhere (L. Luzzatto, personal communication). We could not therefore substantiate this hypothesis. Barr and McGregor (1962) have also shown in the Gambia that the antibody response to tetanus toxoid was lower in malarious children when compared with those protected against the infection—an interesting finding when one considers the morbidity caused by malaria.

The last factor to be considered in immunity is the racial—a feature which is difficult to divorce from the environmental. Nevertheless, the absence of *P. vivax* infection in West Africa and the known resistance of the U.S. negro to this infection are factors in favour of the existence of racial immunity.

Pathology of Malaria in the Susceptible Population

It is obvious that the most severe effects of malarial infection in stable areas are seen in children aged 6 months to 5 years. Though *P. falciparum* in the nonimmune may present in many pernicious forms—algid, dysenteric, choleraic, etc.—in Ibadan it presents in its severe form from the pathologist's point of view as either cerebral malaria or malarial anaemia. Though blackwater fever occurs in children (R. G. Hendrickse, personal communication), I have not recorded it as a cause of death in Ibadan. Moreover, before blackwater fever can be diagnosed a haemolytic anaemia due to drug sensitivity in a G-6-PD-deficient patient must be considered as well (Gilles and Ikeme, 1960).

Malarial Anaemia

Most of the factors responsible for malarial anaemia are ill understood. Nevertheless, the following points are established: (1) parasitized and unparasitized cells are phagocytosed and destroyed; (2) anaemia is not necessarily related to the degree of parasitaemia; (3) transfused cells in a malarial patient may be destroyed more rapidly than in a normal recipient; (4) there is a fall in complement during the acute attack; (5) in animals malarial antigens alone, in the absence of infection, may adversely affect the red cells; (6) fluorescent antibody techniques will differentially stain malarial parasites, Schüffner's dots, and Maurer's clefts (Tobie and Coatney, 1961; Voller and Bray, 1962); and (7) corticosteroid therapy may prove of value in blackwater fever.

Though unequivocal proof is lacking, increasingly auto-immune processes are being considered to explain the excessive anaemia seen in malaria (Zuckerman, 1966). Dixon (1966) has suggested that the erythrocyte is affected by circulating malarial antigen-antibody complexes, which may be absorbed on to the red cell. With complement fixation, opsonization and phagocytosis—or even outright lysis—may occur. Irrespective of the process, malarial anaemia is not uncommon in Ibadan (Hendrickse and King, 1958), sometimes complicated by folic-acid deficiency, and deaths do occur.

Cerebral Malaria

Children, however, dying of malaria in Ibadan more usually have the cerebral form, and it is usually the well-nourished, chubby child who dies of this condition. In my experience it is most unusual for the marasmic or kwashiorkor child to be found at necropsy to have had severe malaria. Since it has been said that malaria is more virulent in malnourished populations, it is difficult to explain this observation—unless the deficiency of amino-acids is so great that it prevents excessive multiplication of the parasite.

In cerebral malaria the brain is leaden in colour or deeply congested. The smaller vessels in the grey matter are packed with parasitized erythrocytes containing pigment. Ring haemorrhages occur in the white matter. Histologically these consist of a central "blocked" arteriole or capillary, containing an agglutinated mass of parasitized pigmented erythrocytes surrounded by brain tissue and then a ring of extravasated red blood cells, some of which may be parasitized. In older haemorrhages necrosis of the midzonal brain tissue and a glial reaction occur—the so-called granuloma. Eventual scarring is said to occur, but whether malaria in children leads to residual brain damage has still to be decided. These changes are due to sludging of blood, with stasis and local anoxaemia affecting the endothelial cells, and eventually the vessel wall (Maegraith, 1948). They are initiated by a reduction in the surface electrical charge of the erythrocytes, which become "stickier" and tend to adhere to leucocytes and to each other. A fine fibrin deposit has been described around them.

Recently a reduction of coagulation factors in the blood in animal malaria and in United States military personnel suffering from chloroquine-resistant malaria has been described, the findings being suggestive of increased coagulation, and it has been postulated that this may be precipitated by phospholipids released from the stroma of destroyed erythrocytes (Dennis *et al.*, 1966). An increase of circulating fibrinolysis in African populations has been suggested as a possible explanation of the very low incidence of coronary thrombosis in this part of the world. Could this be initiated by possible increased fibrin deposition in parasitic infections which are intense in childhood? I have purposely referred to the intravascular lesion in cerebral malaria as a mass of agglutinated erythrocytes, as I do not think that true thrombosis is a feature, and this is borne out by the usual prompt and rapid recovery which ensues with efficient therapy. I have seen death occurring with few parasites in the peripheral blood; parasites were, however, present in the cerebral lesions, and a history of treatment before death was usually obtained (Edington, 1954).

Necropsy Findings

The liver at necropsy in children dying of cerebral malaria is enlarged, tense, and brownish red or even grey in colour. The sinusoids are congested with parasitized cells and the Kupffer cells are swollen, hypertrophied, and packed with malarial pigment (haemozoin), occasional erythrocytes, and cellular debris. Haemozoin gives a negative Prussian-blue reaction, and I know of no specific histochemical test which will identify it. Haemosiderin derived from the breakdown of the host's erythrocytes is also usually described as being present in the reticuloendothelial system in malaria. It has not often been seen in my material, probably because of its rapid utilization in erythropoiesis and because of the substantial amount of iron incorporated in the haemozoin. We have shown in laboratory animals that in malaria the amount of histochemically demonstrable iron pigment varies inversely with the amount of haemozoin (Keeler *et al.*, 1960). Though centrilobular necrosis due to vascular stasis is described, it is unusual in my experience.

In children in whom immunity is developing the liver at necropsy tends to be congested and the greyish-black portal tracts stand out prominently. Histologically there is now little pigment in the Kupffer cells, apart from the periportal areas, and it is concentrated in histiocytes or lying free in the portal tracts, in contrast with the diffuse distribution seen in the lobule in the acute phase. Such a picture is constantly seen in young children, and the presence of other conditions (either acute, chronic, or associated parasitic) makes it extremely difficult for the pathologist to assess the eventual cause of death and the importance of malaria as a factor. It is generally thought that acute malaria accounts for 5 to 15% of deaths in children. It is impossible to assess the effect it has on morbidity, or even mortality, in the semi-immune.

With increasing immunity the amount of haemozoin, first in the Kupffer cells and later in the portal tracts, gradually decreases until with established immunity none may be detectable. The hepatomegaly (see Chart) is thought to be due, at least in part, to the sinusoidal congestion and hypertrophy of the reticulo-endothelial cells which I have described. Though the portal tracts may show a mild lymphocytic infiltration and occasionally stellate fibrosis, I have been unable to trace in over 700 liver sections a portal fibrosis in childhood progressing to cirrhosis, and I discount malaria as an aetiological agent in the cirrhosis which occurs in Ibadan.

Nephrotic Syndrome

I do not think that *P. falciparum* malaria is a common cause of renal lesions in my material, but it is doubtful if the same can be said of *P. malariae*. It has been shown that in Ibadan

over 90% of children presenting with the nephrotic syndrome have this parasite in the peripheral blood, the incidence in non-nephrotic ill children and healthy children being about 30% (Gilles and Hendrickse, 1963), so that an association between the two conditions is proved. In over 100 renal biopsies in children suffering from renal disease seen in Professor R. G. Hendrickse's department the pathological changes can best be described as a localized, focal, proliferative, and membranous glomerulonephritis. By this I imply a condition in which only a number of the glomeruli are affected (localized and not diffuse) and that the lesions in the affected glomeruli may affect only a portion of the tuft (focal and not generalized). In the affected glomeruli there is patchy endothelial cell proliferation and patchy basement-membrane thickening.

Results of electron microscopy studies have to date been reported in only one case, and I am grateful to Dr. P. B. Herdson, of Northwestern University, for his report. He found that the occasional glomerulus was essentially normal, and that in others there was patchy fusion of the epithelial foot processes and patchy deposition of basement-membrane-like material on the luminal surface of the basement membrane and lying between increased numbers of intracapillary cells. Additional lesions seen by light microscopy are adhesions and focal obliteration of the capillaries of the tuft by P.A.S.-positive material which gives a negative reaction for amyloid and fibrin and does not stain as fibrous tissue with van Gieson. Several glomeruli may be completely destroyed, but usually Bowman's space is patent; pseudo-tubule formation is not unusual; haemazoin has not been seen. The impression given is that of a relentless process progressively and gradually destroying individual glomeruli. The final picture is that of a chronic glomerulonephritis. These conclusions are perhaps borne out by the finding that 30% of subjects dying of chronic glomerulonephritis are under the age of 15 years, and that many others are young adults. My impression is that in many of these children with contracted kidneys the number of relatively unaffected glomeruli and the number of those showing hyalinization of the tuft with a patent glomerular space lined by prominent epithelial cells is greater than would be expected in Europe in chronic glomerulonephritis following the acute diffuse type. Comparative studies are, however, required.

The role that *P. malariae* may have in the pathology of the nephrotic syndrome in Ibadan is not as yet clear. It has, however, been shown by fluorescent-antibody techniques that in this condition there are heavy deposits of hosts' gamma and beta IC globulins along the capillary basement membranes (Dixon, 1966). This phenomenon has also been noted in simian malaria (Ward and Conran, 1966).

Other Complications

I have already mentioned the dangers of splenectomy in an area of stable malaria and also tropical splenomegaly. In the last-named syndrome East African workers have reported lymphocytic infiltration in the sinusoids of the liver in many of their patients, together with anaemia and thrombocytopenia. *P. malariae* and raised serum malarial-antibody titres have also been noted, and in some patients portal hypertension occurs. The aetiology is still obscure (Gebbie *et al.*, 1964; Marsden *et al.*, 1965; Hamilton *et al.*, 1966). I do not wish to discuss the problems of hypersplenism or the possibility of a blood-bone-marrow barrier in malaria except to state that a number of years ago we saw improvement in the hypersplenism syndrome after splenectomy (Dodu and Edington, 1956). On the basis of the observations of Watson Williams already mentioned, however, this procedure is now unjustifiable without prior trial therapy unless there are unequivocal clinical indications.

Bronchitis in children is common clinically, and the significance of "chronic" malaria in children dying of bronchopneumonia is difficult to assess. Gastroenteritis is another

common complication. Gross congestion is usual in the adrenals, but degenerate and necrotic lesions have been described. There is no evidence that malarial heart disease is an entity, and even in children dying of malarial anaemia there are few changes apart from oedema, as the illness is acute and of relatively short duration. Malaria would not appear to be a great problem in the blood-transfusion service (Edington, 1956), though malaria parasites are present in at least 10% of blood donors and appropriate antimalarial cover must be given to non-immune persons receiving blood and to others in whom a lowered resistance to infection is suspected; or it should be used routinely if post-transfusional supervision is inadequate.

Conclusion

I have tried briefly to outline some of the problems malaria presents to the pathologist in West Africa. In parts of the area probably 15% of children die of the infection, and the nephrotic syndrome associated with *P. malariae* infection is a potent cause of chronic renal disease. Though W.H.O.-assisted eradication schemes have probably reduced the number of annual deaths due to malaria in the world by about 1,000,000, there has been little improvement in the situation in parts of West Africa, and here Dr. Sadun's remark that D.D.T. has probably gone further to eradicate malariologists than mosquitoes is pertinent. It has been said that the full picture of malaria is rarely seen in stable, partially immune populations, but I hope I have said enough to convince you that we would not like to see the picture enlarged. Malaria is still a problem of major medical importance in West Africa, and there are still large gaps in our knowledge worthy of further and intensive fundamental research.

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