Splenomegaly in New Guinea

by

P. D. Marsden
D. H. Connor
A. Voller
A. Kelly
F. D. Schofield
and
M. S. R. Hutt

1. Introduction

In a detailed clinical investigation of 64 adult Ugandans with marked splenomegaly, the cause of the splenomegaly remained obscure in 34 and, in 29 of these 34, the liver sinusoids were dilated and contained large numbers of lymphocytes (Marsden et al., 1965). This syndrome (marked splenomegaly and hepatic sinusoidal lymphocytosis) has been described in Algeria, Aden and India (Cattoir & Marell, 1950; Pawdry, 1955; Chaudhuri et al., 1956). More recently similar reports have appeared from the Sudan (Mustafa, 1965) and Ghana (Ratnesar et al., 1966). Most of these authors have speculated on the role of malaria in this syndrome.

1. Departments of Clinical Medicine and Parasitology, London School of Hygiene and Tropical Medicine.

2. Geographic Pathology Division, Armed Forces Institute of Pathology, Washington, D.C.


4. Department of Pathology, Makerere Medical School, Uganda.

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A number of special studies were performed on the 29 Uganda patients with splenomegaly and hepatic lymphocytosis. In a group of 15, the average serum gamma globulin was $2.74/\text{s100 ml}$, as compared with a normal figure for the area of $1.86/\text{s100 ml}$ (Leonard & Shaper, 1965; Marsden & Hutt, 1966), the fluorescent malaria antibody titres were higher than in patients with splenomegaly from known causes and higher than in patients without splenomegaly (Marsden et al., 1965; subsequently Gebbie et al., 1964) and finally, in a group of 20 of these patients there was a higher parasite rate in the peripheral blood than in a group of 25 patients without this syndrome. *Plasmodium malariae* was the species most frequently identified in this group.

Several lines of investigation arose from this study. One related to the portal haemodynamics in such patients. Leather (1961) first showed that some of these patients had portal hypertension without intra- or extrahepatic portal obstruction and he suggested that increased blood flow might be the cause. Recently, Williams et al. (1966) measured hepatic and splenic flow simultaneously and independently in 15 patients and showed this to be true in some Ugandans with idiopathic tropical splenomegaly.

Our purpose here was to determine whether or not New Guineans have hepatic lymphocytosis and if so whether it is associated with splenomegaly or other evidence of malaria. The Uganda studies held promise because they showed an association between elevated malaria antibody titres and *P. malariae* parasitaemia. Furthermore, we hoped that this peculiar liver change might indicate a specific entity or at least serve as a pathologic marker in the larger group of patients in the tropics who have splenomegaly of unknown cause. The summary in the initial paper stated that although malaria and more specifically quartan malaria was not proven as the cause for this syndrome, it should be further investigated (Marsden et al., 1965). Schofield et al. (1964) have described the effects of three years' malaria control on splenomegaly in adults in isolated villages in the Maprik area of New Guinea. They greatly reduced malaria transmission in villages (Wingei) by spraying the huts every six months and giving the inhabitants antimalarials. This decreased the percentage of adults with enlarged spleens; the average size of all palpable
spleens was no smaller than before malaria control. This was especially evident in women of the child-bearing age and Schofield (1962) has related this to the stresses of multiparity in marginally nourished females.

In view of the evidence from the initial study in Kampala it seemed possible that this residual splenomegaly in New Guinea after malaria control might be due to persistent occult malaria infection either quartan or vivax. In addition, since comparable villages where no antimalarial measures had been instituted were also under study, a unique opportunity presented itself to assess the frequency of changes in liver histology, malarial antibody titres and parasitaemia in these two populations in adults with and without splenomegaly. Accordingly, from January to March 1965 groups of villages from Wingei (the protected villages) and Wam (the malarious villages) were studied and material obtained to assess these parameters. Although thalassaemia gene abnormalities do occur in this area neither leishmaniasis, schistosomiasis nor brucellosis have been reported from New Guinea.

2. Materials and methods

The ecology of Wingei and Wam have been described elsewhere (Schofield & Parkinson, 1963). They are situated in the Maprik subdistrict where malaria is stable and transmission very intense if no control measures are taken (Peters, 1960; Peters & Standfast, 1960). For five years at Wingei every six months routine spray rounds with residual DDT have been performed. At the same time the whole village population received a chloroquine pyrimethamine mixture by mouth. In Wam malaria control did not commence until after this study was completed.

Two workers (PDM and AK) lived in the villages during the period of investigation. Twenty-four Wingei villagers and 24 Wam villagers were persuaded to assist in the investigation. For both groups of patients data existed on their liver and spleen size, haemoglobin, and malarial parasitaemia taken every six months for the last five years. The goodwill built up among these people by this periodic field survey was valuable in enlisting their co-operation for the present investigation.
In both villages patients with palpable spleens (Hackett grade 2 or 3) were matched for age, sex and parity in the case of females against control patients without splenomegaly. A full physical examination was performed. Patients with detectable associated disease and pregnant women were excluded from the study.

Nine consecutive daily thick blood films were obtained from the Wingei villagers and 11 from the Wam villagers. Thin films were also taken. These were stained after 24 hours in a dessicator with freshly made up Giemsa solution buffered to pH 7.2. Approximately 5 mm$^3$ of finger prick blood sampled with a platinum loop was used for making the thick film, a method devised by Peters, 1957. In this way it was hoped to obtain data on the number of parasites per mm$^3$ but due to the scanty number of parasites seen and the fact that in some films partial "washing off" occurred during staining this had to be abandoned. Sera in sterile screw-capped containers containing penicillin and streptomycin were stored at 4°C within 72 hours of venepuncture in the field.

During the course of the above investigations, haemoglobin, bleeding and clotting time and estimation of number of platelets in the peripheral blood was done on each subject. Also in every case a course of 30 mg Vitamin K was administered orally one week before biopsy. Any patient with a severe anaemia or abnormality of blood coagulation assessed by the above investigations was not considered for liver biopsy.

Liver biopsies were done at the local health clinic. A modified Vim Silverman needle was used and specimens preserved in 10% buffered neutral formalin. After liver biopsy the patients remained supine for the rest of the day and their pulse and general condition checked frequently. Fresh group "O" negative blood was always available and facilities for transfusion. These were not used as there were no complications.

While in Lae it was possible to examine through the kindness of Professor W. R. Pitney and Dr D. Pryor and obtain liver biopsies and sera on 12 hospitalized patients with marked splenomegaly which were being used in a haematological investigation. Since all these patients were treated with chloroquine prior to admission, blood films for malaria parasites were not examined. These patients are discussed separately in the results section.
Later, in Washington, the liver biopsies were dehydrated, cleaned, embedded in wax and cut at 5 microns. Sections of each specimen were stained routinely with haematoxylin and eosin, Giemsa, for iron and reticulum. In selected cases the periodic acid Schiff, Comori methenamine silver, Ziehl-Neelsen, Brown and Brenn and 24-hour oil red "O" stains were used.

The sections were examined and graded by two of us independently (DHC and MSRH) without reference to the clinical key. Grading was determined by the concentration of lymphocytes in the sinusoids. Figs. 1-4 are examples of each grade and the captions explain the grading.

Malaria antibody titres were done on the sera by the indirect fluorescent antibody test as described by Voller (1964) using P. cynomolgi bastianellii as antigen. Unfortunately, the sera from Wingei had deteriorated, possibly from delay in refrigeration. These sera were bacteriologically sterile but malaria antibodies could not be demonstrated. To get some indication of the importance of thalassaemia in these villages haemoglobin solution from 10 Wingei patients and five Wam patients were collected for haemoglobin electrophoresis and foetal haemoglobin estimation. Five of the Wingei specimens were haemolysed and unsuitable for testing.

The whole area of the Giemsa stained thick films were examined under oil immersion by one investigator (FDM). Later the Wam films were examined again under oil immersion noting the parasites present in an area covered by 600 white cells (AV). Since frequently there were less than 10 parasites on the whole slide and rarely more than one per 10 oil immersion fields, the numbers of parasites were not counted.

In six patients with scanty parasites the species could not be identified and these are so recorded.

3. Results

In this section the liver biopsy appearance, malaria antibody titres and malaria parasites in the peripheral blood are considered in this order which is the order in which they were investigated in the initial study (Marsden et al., 1965). Together with splenomegaly all possible correlations for these factors have been analysed but where these have shown no detectable trend the table has not been included but just
the result mentioned. Unfortunately as the numbers are so small and the features so variable statistical correlations are not possible. The findings in the hospitalized Lae patients are considered separately as are other miscellaneous features noted in the material obtained from this study.

Liver biopsy. The liver specimens are graded as explained in Figs. 1, 2, 3 and 4. The histogram (Fig. 5) shows the number of villages with each grade for those with splenomegaly and those without splenomegaly in both villages (Wingei and Wam). The majority of biopsies showed minimal lymphocytic sinusoidal infiltration (Grade 1). However, three times as many biopsies showing grade 2 or 3 changes occurred in patients with splenomegaly than without and all five biopsies without lymphocytic infiltration came from villagers without splenomegaly. Nevertheless it is clear that this liver change occurs without clinical splenomegaly.

Fig. 6 compares the liver grades in the malarious and malaria controlled villages. Twelve out of 30 patients (40%) in Wam showed grade 2 to 3 livers. In Wingei, the malaria controlled village, seven out of 23 (30%) showed grade 2 or 3 changes. There were patients with no sinusoidal lymphocytes in both villages.

Of the 41 patients with splenomegaly in the study (and the Lae group is included here) it was impossible to relate spleen size to liver grade. However, patients with spleens extending into the right iliac fossa had maximal grades (Table 1). In the malarious village neither the presence of circulating parasites nor the malaria antibody titre could be correlated with the liver grade. For instance, both patients with grade 0 livers had circulating \textit{P. vivax} parasites (see Tables 2 and 3).

Malaria antibody titres. As mentioned, sera from the malaria controlled villages were non-reactive and thus there are no titres for this group. All patients in the malarious village had positive titres ranging from 1/160 to 1/5120. Higher malarial antibody titres were recorded in patients with splenomegaly in this village (Table 4). For example, six of the 16 patients without splenomegaly had titres of less than 1/640 whereas none of the 18 patients with splenomegaly had such a low titre. 14/18 of the splenomegalies had a titre about this level in contrast to
7/16 of the patients without splenomegaly. Table 5 also shows that the frequency with which circulating malaria parasites were detected could not be related to the height of the malarial antibody titre.

Malaria parasites in the peripheral blood. In the malarious village there were 61 positive slides for malaria parasites of the 176 examined from 16 patients without splenomegaly and 31 positive of the 198 slides from 18 patients with splenomegaly, approximately twice as many positive slides from patients without splenomegaly (see Table 6). All patients without splenomegaly showed parasites at some time or other during the 11-day period of observation. Of the 18 patients with splenomegaly, in five no parasites could be demonstrated and all these had antibody titres of 1/640 or above.

Table 6 also demonstrates that the malaria control measures were reasonably effective in the control village, since only seven patients showed parasites in the nine-day observation period out of the group of 24 (216 thick films examined).

In the malarious village P. vivax was the commonest parasite being encountered in 20 patients, P. falciparum in 12 and in six instances the scanty parasites could not be specifically identified and are so recorded. Mixed infections of P. vivax and P. falciparum occurred nine times in this village. Quartan malaria exists in the area but was not noted in these small groups of adult patients. Neither P. vivax nor P. falciparum infections appeared to be more frequently associated with an enlarged spleen.

Hospitalized patients at Lae. These patients had larger spleens than those seen in the field and clinically closely resembled those seen in Kampala as regards their hepatosplenomegaly, absence of clinically detectable ascites and degree of anaemia (average haemoglobin 9.5 g%). In one patient a faint systolic bruit could be heard over the spleen. Varying degrees of leucopenia and thrombocytopenia were present in some patients. There were six males and six females, the youngest 12 and the oldest 32 years of age. Four patients had spleens enlarged to Hackett grade 3, six were grade 4, and two were grade 5. Nine patients had hepatomegaly and in four it extended three fingers below the costal margin. In the liver biopsies there
were higher grades of sinusoidal infiltration than in patients with splenomegaly in the field study (six with grade 3, three with grade 2 and three with grade 1). The malarial antibody titres varied from 1/160 to 1/5120 but half of them were 1/2560 or above this dilution.

**Miscellaneous findings.** None of the liver specimens showed gross macroscopic changes. Although our primary interest was the number of sinusoidal lymphocytes, we recorded a great variety of microscopic changes in the whole group of 65 biopsies. The number of lymphocytes varied from none in the grade 0 biopsies to clusters and rows of four and five cells thick in the grade 3 biopsies. The individual biopsies varied greatly from one field to another; that is normal areas were interspersed with areas of intense sinusoidal infiltration. The area with the greatest concentration of sinusoidal lymphocytes determined the grade. Sinusoidal dilatation increased with the concentration of lymphocytes, and Kupffer cells tended to increase in size and number as the lymphocytes increased in number. The lymphocyte is the dominant cell and it is used as the sole criterion for grading. Although this simplified the grading, plasma cells, eosinophilic and neutrophilic leucocytes and monocytes formed a portion of the infiltrate in each case. In 37 specimens with leucocytes in the portal areas the infiltrate was mixed. Mast cells were seen in 40 cases.

Five specimens (7.8%) had haemosiderin in Kupffer cells and all of these had splenomegaly. One patient had a small amount of malaria pigment in the Kupffer cells and nine others had small amounts of malaria pigment in portal tracts. None of the Iae patients (who as a group had the biggest spleens and most prominent sinusoidal lymphocytes) had malaria pigment in their liver biopsies.

Thirty-one (48%) had slight to moderate fatty metamorphosis. The one with cirrhosis had some fat in parenchymal cells but there were no Mallory bodies nor was there satellitosis, or other evidence of parenchymal cell damage.

Five (8%) had megakaryocytes in the sinusoids but none of these five liver biopsies contained haemosiderin. In one of these five however there was Kupffer cell erythrophagocytosis.
Fifty-seven (87%) of the 65 biopsy specimens had lipofuscin in parenchymal cells. Forty-six of the 65 had some degree of portal fibrosis. In 61 of the 65 specimens there was at least one portal tract and of these 61, 46 (75%) had some portal fibrosis.

One patient (No. 442 Wingei) had cirrhosis (the hepatic parenchyma was reorganized into pseudo-lobules without central veins) but she did not have splenomegaly or other evidence of portal hypertension.

Sixty-one specimens (79%) had occasional eosinophilic leucocytes in either the sinusoids or the portal tracts and 39 (60%) had occasional neutrophils in the sinusoids. We found mixed lymphocyte-plasma cell infiltrates of the portal tracts in 51 (79%) cases.

Of the five haemoglobin samples from patients with splenomegaly in the malarious village all showed normal levels of haemoglobin A2 and haemoglobin F. Of the five haemoglobin samples from the controlled village, however, two showed haemoglobin A2 values above 4% and although the thin films were not very suggestive these patients could be carriers of the thalassaemia gene. Microfilaria of the Wuchereria bancrofti type were seen in 17 of 216 thick films examined from Wingei and 61 of the 374 films available from the Wam group. A microfilaria was seen in one of the liver biopsies. Since these films were taken during the day and the microfilaria exhibit nocturnal periodicity the incidence figures from these groups would be of little value.

4. Discussion

By examining the daily thick blood films for malaria parasites and estimating malaria antibody titres we hoped to correlate chronic malaria with splenomegaly and hepatic sinusoidal lymphocytosis. Also, in view of the evidence from Kampala, we believed that patients with residual splenomegaly in the protected villages might exhibit persistent vivax or quartan infections. As has been demonstrated in the previous section, our data fail to support these hypothesis. In spite of the protection afforded to the Wingei villagers seven out of 24 still had plasmodial infections and more of these 24 may have had undetected infections. It is unfortunate that the sera from this village were unsuitable for malarial antibody determinations.
It is known that only a proportion of malaria infections are detected on examination of a single blood film (Macdonald, 1931; Bruce-Chwatt, 1963). Even the methods employed here may be unsatisfactory for detecting latent malaria and a high proportion of parasites are lost on staining thick films (Dowling & Shute, 1966). The significance of a malaria antibody titre in these field conditions is difficult to define. We lack a practical field method for concentrating malaria parasites in the blood of patients with scanty parasitaemia.

Liver biopsy findings. Our findings show that hepatic sinusoidal lymphocytosis (HSL) previously reported in indigenous peoples of tropical Africa, Asia Minor and India, occurs also in natives of New Guinea. Saha & Chaudhuri (1957) first noted that patients with "tropical splenomegaly" had hepatic sinusoidal infiltrates. In the present study we found HSL in villagers with splenomegaly but also in some villagers without splenomegaly. We found also that the more severe degrees of HSL occurred in those with splenomegaly living in the unprotected village. Walters & Maogregor (1960) noted in Gambia that malarious children had more prominent sinusoidal changes than protected children. In another report Telcharov & Todarowa (1950) described, round-cell infiltrates, Kupffer cell "mobilization" fibroblastic activity and Hodgkins-like granulomas in liver biopsies from 45 patients with malaria, but they did not describe lymphocytes in the sinusoids. Corcoran et al. (1953) however in a study of liver biopsies from 20 Korean war veterans with vivax malaria found infiltration of the sinusoids and portal tracts with both chronic and sometimes acute inflammatory cells. More recently Blackburn et al. (1966) have described a high incidence of "pericholangitis" in liver biopsies from inhabitants of the eastern and western New Guinea highlands. Malaria parasites were seen in 3.5% of blood films examined. They relate their findings to those described in Uganda but they do not mention sinusoidal lymphocytosis, the hallmark of the Uganda biopsies and a prominent feature of the present New Guinea biopsies. It is clear from these reports that HSL is not a constant finding in people living in malarious areas, nor is it a constant finding in patients with malaria. However, all patients with HSL so far reported have lived in malarious areas and in the present study the unprotected villagers had more pronounced changes than the protected villagers.
Fifty of the 65 liver biopsies had lymphocytes in the portal tracts and these tended to vary in intensity with the sinusoidal lymphocytes. Lymphocytic infiltrates in the portal tracts are usually a manifestation of reticuloendothelial hyperplasia and the frequency with which portal infiltrates occur in association with HSL suggests to us that the latter is also a manifestation of reticuloendothelial hyperplasia. However, grade 3 HSL may be present without marked portal infiltrates.

Cells collect in hepatic sinusoids in a number of other conditions. Extra-medullary haematopoiesis, infectious mononucleosis and lymphocytic leukaemia, are three diagnoses which may come to the pathologist's mind when he first sees HSL in biopsies from patients in the tropics. Extra-medullary haematopoiesis can usually be excluded after studying thin Giemsa stained sections. Lymphocytes predominate and erythropoietic or granulopoietic cells are absent. Infectious mononucleosis is more difficult to exclude and in fact, it may be impossible to distinguish these two conditions from the biopsies alone. There are a number of features however which may help. In infectious mononucleosis parenchymal cell mitoses, acidophilic bodies, bile stasis and iron in Kupffer cells may all be present (Klatskin, 1963; Ishak & Renedo, 1966 (Personal communication)) and these are not features of HSL. In lymphocytic leukaemia large numbers of tumour cells may accumulate in the liver sinusoids and in the portal tracts, but the tumour cells are usually larger than the lymphocytes of HSL and they have more variation in nuclear size and staining than the sinusoidal cells in HSL. Furthermore, the intensity of the sinusoidal infiltrate in lymphocytic leukaemia tends to be more uniform than in HSL.

We found parenchymal cell lipofuscin in 57 biopsies. Lipofuscin accumulates in wasting diseases (especially chronic infections) in states of undernutrition and in old age. Chronic undernutrition and chronic parasitism may be factors causing the high incidence of lipofuscin in this series.

Malaria antibody titres. Higher antibody titres occur in those patients with splenomegaly in the malarious village but the height of the titre could not be correlated with malaria parasitaemia.
It can be seen from Table 4 that there was a difference in the distribution of antibody titres in the patients with splenomegaly and in those without splenomegaly. Both groups were from the malarious village. The titre is higher in the group with splenomegaly. Table 2 shows that in this admittedly rather small group there is no correlation between malarial antibody levels and the degree of sinusoidal infiltration. These findings are in contrast to the clear association of high malarial antibody with splenomegaly and hepatic sinusoidal lymphocytosis reported in Uganda by Gebbie et al. (1964) and Marsden et al. (1965) in hospitalized patients.

It must be emphasized however that the groups of villagers in this study had smaller spleens than the hospitalized patients in Kampala. The small Loe group was more comparable to the Kampala study and these patients showed higher antibody levels and more marked hepatic sinusoidal lymphocytosis similar to the Ugandan series.

In the present study there was no relationship between antibody levels and parasitaemia. However this is not surprising as the high incidence of positive blood films probably means that all of the subjects would be infected at some time, even over a short period.

Malaria parasites. Longitudinal studies of malaria parasitaemia have demonstrated that in many individuals in endemic areas blood parasitaemia can only be demonstrated intermittently (Bruce-Chwatt, 1963). It is likely that some chronic malaria infections were missed in these New Guinea patients since the observation period was short. Yet in this study neither parasitaemia nor the species of parasite could be correlated with clinical splenomegaly or sinusoidal infiltration on liver biopsy.

Discrepancies between the spleen index and blood parasite index have been frequently noted (Spicer, 1945; Boyd, 1949) and the many palpable spleens without positive blood films suggests that splenomegaly persists longer than detectable parasitaemia (Darling, 1934). The spleens in the survey were chosen for their large size and it has been observed that the larger the spleen gets the fewer parasites are detected (Covell, 1927). Hackett (1944) records a poor correlation between splenomegaly and parasitaemia.
Boyd (1949) has discussed the spleen reaction to different species of parasites and *P. falciparum*, *P. vivax* and *P. malariae* have all been favoured as parasites causing greater degrees of splenomegaly in different parts of the world. The inconsistency of these reports may not only depend on variations in species prevalence and transmission rates in these different geographical locations but also as the cross-sectional nature of most of these surveys. Longitudinal studies give a clearer picture of the dynamics of the host parasite relationship.

Apart from looking for the parasites in the peripheral blood other measures can be attempted to infer an association with malaria. Attempts to induce vivax infections in Indian patients with idiopathic tropical splenomegaly were not successful (Konar & Choudhury, 1955) suggesting that such patients were immune. Xenodiagnoses using laboratory-bred mosquito vectors is a possible way of demonstrating latent infection in patients with low undetected parasitaemia (Covell, 1960). Of an association between malaria and all degrees of splenomegaly there can be no question (Boyd, 1949) and some aspects of the pathology have been documented (Maegraith, 1948).

Bruce-Chwatt (1956) has recorded a large series of spleen weights from African children and some adults obtained at autopsy in Lagos, Nigeria. These African spleens were generally larger than their Caucasian counterparts. The histogram of the frequency distribution of spleen weights in African children showed a few individuals with spleen weights up to three times the mean value.

Even in an area of low malaria endemicity higher spleen rates are recorded than in a non-malarious area (Smith, 1945). In adults infected with *P. vivax* or *P. falciparum* treatment results in resolution of the splenomegaly although the "ague cake" spleen of chronic malaria resolves more slowly (Sinton, 1927; Russell, 1935).

In view of the difficulties of even demonstrating an association between idiopathic tropical splenomegaly and malaria at the present time it seems reasonable to consider the possible mechanisms of etiology with regard to plasmodial infection. First tropical splenomegaly may be caused by malaria infection alone, reflecting an unusual host response, or it may be a response dependant on the combination of plasmodial species or the frequency of sporozoite challenge. There is little
information available in this respect on the effect of comparable infections in a population of individuals as regards spleen size. Certainly the marked variations in malarial antibody titre suggest these adults vary in the amount of antibody they produce in a stable malarious environment. The spleen is an important organ monitoring both cellular and humoral immunity in malaria, and its size may also vary (Stratman-Thomas, 1935; Boyd, 1940).

Patients in New Guinea and Kampala with marked splenomegaly admit that the tumour has been present for some years, and in the majority of the patients in this study it has been present throughout the five years' observation period though fluctuating in size. In induced P. vivax infections Stratman-Thomas (1935) observed that if a marked splenomegaly persisted such relapse was more likely. McKendrick (1915) concluded that there is a direct relationship between the frequency of attack and the size of the spleen. Hackett (1944) suggests that where frequent and repeated inoculations result in mixed and overlapping infections, each infection could act almost independently producing a splenic reaction and giving rise to a protracted and genuine chronic enlargement. Christophers (1929) felt that host response was constant in children and that a certain unit enlargement of the spleen (a splen) could be conceived in response to a single infection. The effect of the superimposed infection was then additive in terms of splenic enlargement.

Experimental primate infections have yielded interesting information. Coggshall (1937) using an oncometer observed that in Rhesus monkeys P. inui infections produced larger spleens for a longer period than the more pathogenic P. knowlesi. During the latent phase of P. inui infections the spleen tended to change in consistency becoming hard and firm. It has been suggested that the firmer enlarged spleen of continued malaria results from cellular changes arising from the development of acquired immunity (Taliaferro & Mulligan, 1937).

A second tenable hypothesis is that the sporadic occurrence of marked splenomegaly in adults in a malarious community may be attributed to the parasitic infection plus some other factor or combination of factors.

A racial difference between normal spleen weights (Bean & Baker, 1919) and also spleen weights in malarious areas has been noted (Clark, 1928). Malnutrition, it has been suggested, may be implicated in the etiology of Bengal splenomegaly
(De & Tribedi, 1939). Other infectious agents are a possibility. It has been noted that an outbreak of measles can raise the spleen rate in children (Spicer, 1945). Hookworm has been implicated in the etiology of the Columbian spleen (Reeder, 1911). Thalassaemia must be excluded in New Guinea as a contributory cause for persistent splenomegaly particularly in the malarial controlled village.

A third possibility is that these giant spleens of the tropics are not associated with malaria at all, but no feasible alternative etiology has been suggested to date, such splenomegaly persisting after malaria control has been noted before in New Guinea (Metselaar & van Thiel, 1959). The findings of Schofield et al. (1964) on the persistence of splenomegaly in Wingei requires explanation. This study suggests that malaria infections were still present in the village and more frequent in Wingei patients with splenomegaly (Table 6), although the transmission rate is probably greatly reduced.

While it is not easy to even establish an association between idiopathic tropical splenomegaly and malaria, all the areas from which cases have been reported have in fact been malarious. In India, Chaudhuri (1962) is convinced that chronic massive splenomegaly (Bengal splenomegaly) is associated with malaria and notes that following malaria control the prevalence of this condition has been on a steady decline. It is not clear at the present time why relatively few individuals in such an area develop marked splenomegaly.

5. Conclusion

Clinical splenomegaly tends to be associated with greater hepatic sinusoidal infiltrations in New Guinea villagers exposed to hyperendemic malaria. Higher malaria antibody titres were more common in patients with splenomegaly in this village. In adult villagers protected for five years the incidence of malaria parasitaemia was much lower than the malarious village and the sinusoidal infiltrates were also diminished.

No associations between parasitaemia, malaria antibody titre, and hepatic sinusoidal infiltration could be demonstrated in the malarious village. The implications of these findings are discussed. The question of malaria being responsible for the marked splenomegaly encountered in tropical practice is evaluated.
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RESUME

Les auteurs ont cherché à établir si chez les habitants de la Nouvelle-Guinée la lymphocytose sinusoïdale du foie associée à la splénomégalie était en rapport avec le paludisme. Ils ont travaillé sur deux groupes, dont l'un (24 sujets) habitait un village protégé depuis cinq ans par des cycles semestriels de pulvérisations d'insecticides à effet rémanant associées à une chimiothérapie de masse, tandis que l'autre (34 personnes) habitait un village non traité. Dans les deux cas, des enquêtes semestrielles avaient permis de réunir pour les cinq dernières années des données sur les dimensions du foie et de la rate, sur le taux d'hémoglobine et sur la parasitémie. On a également pratiqué des observations sur les modifications histologiques du foie, sur les titres d'anticorps du paludisme et sur la parasitémie dans les deux groupes en comparant les sujets atteints et les sujets exempts de splénomégalie.

Les auteurs ont constaté que la splénomégalie clinique tend à être associée à des infiltrations sinusoïdales du foie plus importantes chez les villageois exposés au paludisme hyperendémique et que les titres élevés d'anticorps sont plus fréquents chez les malades atteints de splénomégalie dans le village non protégé. Dans le village protégé depuis cinq ans, au contraire, la fréquence de la parasitémie est beaucoup plus faible chez les adultes, et les infiltrats sinusoïdaux sont moindres.

Les auteurs n'ont pu mettre en évidence aucune association entre la parasitémie, le titre d'anticorps et les infiltrations sinusoïdales du foie dans le village impaludé. Ils examinent la portée de ces résultats et s'efforcent de déterminer dans quelle mesure la splénomégalie marquée observée par les médecins dans les régions tropicales est imputable au paludisme.
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### Table 1. To show the relationship between degree of sinusoidal infiltration and spleen size in 41 patients

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<tr>
<th>Spleen size (Hackett grading)</th>
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<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2. To show relationship between malaria antibody titre and degree of sinusoidal infiltration in 30 patients from the malarious village

<table>
<thead>
<tr>
<th>Malaria fluorescent antibody titre(^a)</th>
<th>Degree of sinusoidal infiltration on liver biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>&gt;5 120</td>
<td>0</td>
</tr>
<tr>
<td>5 120</td>
<td>0</td>
</tr>
<tr>
<td>2 560</td>
<td>0</td>
</tr>
<tr>
<td>1 280</td>
<td>1</td>
</tr>
<tr>
<td>640</td>
<td>1</td>
</tr>
<tr>
<td>320</td>
<td>0</td>
</tr>
<tr>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\) Using *Plasmodium cynomolgi* bastianelli as antigen.
<table>
<thead>
<tr>
<th>Liver grade on degree of sinusoidal infiltration</th>
<th>Number of patients with liver biopsy grade</th>
<th>Without malaria parasites</th>
<th>P. vivax</th>
<th>P. falciparum</th>
<th>Mixed P. vivax and falciparum</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Grade I</td>
<td>16</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Grade II</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Grade III</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

**TABLE 4. DISTRIBUTION OF MALARIAL ANTIBODY TITRES IN THE PATIENTS WITH AND WITHOUT SPLENOMEGALY IN THE MALARIOUS VILLAGES**

<table>
<thead>
<tr>
<th>Malaria antibody titre</th>
<th>With splenomegaly</th>
<th>Without splenomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/160</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1/320</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1/640</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>1/1 280</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1/2 560</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1/1 520</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1/1 520</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>16</td>
</tr>
</tbody>
</table>
TABLE 5. FREQUENCY WITH WHICH CIRCULATING MALARIA PARASITES WERE FOUND ON CONSECUTIVE EXAMINATIONS IN PATIENTS IN THE MALARIOUS VILLAGE RELATED TO THE MALARIA ANTIBODY TITRE

<table>
<thead>
<tr>
<th>Malaria antibody titre dilution</th>
<th>P. vivax</th>
<th>P. falciparum</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/160</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1/320</td>
<td>17</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>1/640</td>
<td>17</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1/1280</td>
<td>7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1/2560</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>1/5120</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1/5120</td>
<td>9</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>57</strong></td>
<td><strong>32</strong></td>
<td><strong>6</strong></td>
</tr>
</tbody>
</table>

TABLE 6. FREQUENCY WITH WHICH PARASITES WERE FOUND IN PATIENTS WITH AND WITHOUT SPLENOMEGALY IN THE TWO VILLAGES

<table>
<thead>
<tr>
<th></th>
<th>Protected village</th>
<th>Malarious village</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parasites found</td>
<td>Parasites not found</td>
<td>Parasites found</td>
<td>Parasites not found</td>
</tr>
<tr>
<td>With splenomegaly</td>
<td>5</td>
<td>8</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Without splenomegaly</td>
<td>2</td>
<td>9</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7</strong></td>
<td><strong>17</strong></td>
<td><strong>29</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>
Fig. 1

Grade 0 - no increase in sinusoidal lymphocytes. The sinusoids are narrow and sinusoidal lymphocytes are inconspicuous. Kupffer cells (arrows a) may be slightly more prominent than normal and specimens containing occasional sinusoidal lymphocytes (arrow b) are included in this group. The architecture and parenchymal cells are normal. (Case 1040437, H&E, X 265, AFIP Neg. No. 66-2349)

Fig. 2

Grade 1 - increase in lymphocytes, up to and including single rows within the sinusoids. Many sinusoids are slightly dilated and contain scattered lymphocytes (arrow a) which in some areas, are more numerous, forming single rows (arrow b). Kupffer cells are slightly increased in size and number. The basic architecture is preserved. Some of the parenchymal cells contain finely granular lipofuscin (arrow c) but they are otherwise not remarkable. There are 3 lymphocytes in the central vein on the left. (Case 1040435, H&E, X 265, AFIP Neg. No. 66-2350)
Fig. 3

Grade II - increase in lymphocytes up to and including double rows in the sinusoids. Specimens in this group also have single lymphocytes, single rows, and double rows of lymphocytes within the sinusoids. (Case 1040472, H&E, X 265, AFIP Neg. No. 66-2351)

Fig. 4

Grade III - increase in lymphocytes beyond double rows. Here the sinusoids are focally dilated ("sinusoidal lakes"). The cells are mostly lymphocytes but there are occasional plasma cells, monocytes and eosinophilic and neutrophilic leukocytes as well. The basic architecture is preserved and the parenchymal cells are normal. (Case 1040470, H&E, X 265, AFIP Neg. No. 66-2352)
FIG. 5

LIVER BIOPSY GRADE IN PATIENTS WITH AND WITHOUT SPLENOMEGALY (BOTH VILLAGES)

No. OF PATIENTS

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

LIVER BIOPSY GRADE

NO SPLENOMEGALY
24 PATIENTS

29 PATIENTS

WHO 70297
FIG. 6

LIVER BIOPSY GRADE IN PATIENTS FROM BOTH CONTROLLED AND MALARIOUS VILLAGES

No OF PATIENTS

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

LIVER BIOPSY GRADE

0  I  II  III  0  I  II  III

CONTROLLED VILLAGE
23 PATIENTS

MALARIous VILLAGE
30 PATIENTS
The purpose of the WHO/Mal series of documents is threefold:

(a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;

(b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;

(c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

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