

THE PATHOLOGIC ANATOMY OF MALARIA

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INTRODUCTION

Material. The autopsy material and records used in compiling the data presented in this section are largely from patients with malaria dying in the vicinity of the Panama Canal with some records from Tela, Honduras. We are grateful to the authorities of the Panama Canal and the United Fruit Company at Tela, Honduras, for their cooperation in making these records available. The majority of material is from the Board of Health Laboratory.

TABLE 140. RACIAL DISTRIBUTION OF AUTOPSY MATERIAL

	NUMBER MALA- RIA DEATHS	NUMBER BLACK- WATER DEATHS	TOTAL NUMBER DEATHS	PER- CENT- AGE BLACK- WATER DEATHS
Foreign whites*	117	50	167	29.9
Mestizos†	77	10	87	11.5
Negroes‡	211	22	233	9.4
Totals	405	82	487	16.8

* North Americans, Spanish, Italian.

† Natives of Central and South America showing varying mixtures of Spanish, Indian and Negro blood.

‡ Largely imported British West Indian laborers.

Ancon, Canal Zone. In the past, both authors have served as pathologist in this institution and in many instances the records are personal observations. They include 405 deaths directly attributable to uncomplicated *P. falciparum* infections and the racial distribution of these cases is presented in Table 140. From these cases our descriptions of the pathologic anatomy of this infection will be presented, along with a résumé of the available literature.

General Discussion. It is generally accepted

that pernicious malaria is the result of infection with *P. falciparum* (Marchiafava and Bignami, 1900; Marchiafava, 1931; Manson-Bahr, 1936; Strong, 1944; Ash and Spitz, 1945) although occasional fatalities have been reported as the direct result of *P. vivax* infections (Zwing, 1902; Deaderick, 1909; Billings and Post, 1915) and these latter infections have also been important complicating factors (Barker, 1895) in deaths from other causes. In our own experience and in the records studied, there are no fatalities from uncomplicated *vivax* or quartan infections. In a few instances the clinicians were convinced that *P. vivax* alone was the cause of death, but a careful search of tissue films at autopsy either revealed typical *P. falciparum* parasites, or other fatal conditions were found in the viscera. There are clinical reports of *vivax* infection causing cerebral malaria with fatalities, but autopsy studies were not performed and the possibility that an unrecognized mixed malaria or other type cerebral infection was present cannot be disregarded.

Human malaria infection is emphatically an infection of the erythrocytes, and the many forms and features that characterize the gross and microscopic changes in these cases must be considered in this light. With maturation of trophozoites, destruction of the infected erythrocyte is inevitable, and large numbers of infected erythrocytes are destroyed by phagocytosis before maturation of the parasite. Likewise, the extensive phagocytosis of nonparasitized erythrocytes in the internal organs is a contributing factor. In general, the severe infections show a rapid early loss of erythrocytes, which may amount to as much as 1,000,000 per cu. mm. for each paroxysm (Marchiafava and Bignami, 1900), and the drop

in erythrocytes is out of proportion to the number of red cells parasitized (Gambrell, 1941). This early loss tends to level off, although the paroxysms, infection, and clinical course may remain severe and even end fatally. Suggestions that parasitized erythrocytes may be incapable of carrying a normal oxygen complement have been made (Rigdon and Fletcher, 1945) but other research shows that definite variations in the blood oxygen content are present. Wong (1945), using an oximeter in studying patients with therapeutic *vivax* malaria, found that during a paroxysm the level of blood oxygen saturation rose slightly but after the paroxysm when the temperature rose the level of oxygen saturation fell. The fall in oxygen saturation depended upon the severity of the paroxysm and was seen to reach 70 per cent saturation. In these low saturation cases administration of pure oxygen did not produce recovery, and likewise transfusions of 300 to 600 cc. of red blood cells followed by oxygen administration did not return the blood oxygen to 100 per cent saturation, although 5 to 10 per cent increases were noted. Wong proposed the presence of some as yet unidentified factor which inhibited full oxygenation of the blood hemoglobin to explain this change. These findings in *vivax* malaria are especially valuable, in view of the recent interest in shock as the mechanism of death in *falciparum* infections. It has been suggested, but not verified, that with the rupture of merocytes, hemolysins and endotheliolysins are freed or produced, and also that malaria pigment acts as a hemolysin, and produces capillary hemorrhages (Strong, 1944). Recent studies of malaria pigment, now identified as ferrihemate, showed no hemolytic action upon erythrocytes, but did produce extensive vascular damage with thrombosis following intravenous injection of comparatively large amounts (Anderson and Morrison, 1942; Morrison and Anderson, 1942). In view of the insolubility of the malaria pigment as released from ruptured parasitized erythrocytes, Anderson and Morrison felt that this substance was not a factor in producing the lesions in experimental simian malaria. It has been proposed that free ferrihemic acid injected or liberated in the blood may combine with albumin forming methemalbumin which may be the agent responsible for the vascular damage. Studies (Rigdon, 1945) upon pigment present in the tissues in malaria have shown

that two types exist. One, giving a positive Berlin-blue reaction for iron, is presumably derived directly from red cell hemoglobin, while the other pigment (ferrihemate) which is formed in red cells by malaria parasites does not give the iron reaction. Rigdon feels that the malaria pigment may be slowly oxidized by the fixed phagocytic reticulo-endothelial cells (Kupffer cells) to an iron containing pigment which then gives a positive iron reaction. In chronic human malaria a varying degree of hypochromic anemia is usually described as being present; this, however, may be the result of factors other than malaria parasites, since the usual descriptions of bone marrow show active erythropoiesis.

Destruction of large numbers of erythrocytes releases parasites, pigment and erythrocytic debris, sometimes producing jaundice, but more important it throws a tremendous burden upon the phagocytic cells of the body. In these fixed and circulating phagocytic cells the characteristic histologic changes of malaria are found. In a critical survey of the literature and an experimental study of the cellular response to malaria infections (Tallaferrro and Mulligan, 1937) the comprehensive term LYMPHOID-MACROPHAGE SYSTEM is proposed to include the cells entering into the body defense against malaria and other infections. This LYMPHOID-MACROPHAGE SYSTEM includes the cells of the RETICULO-ENDOTHELIAL SYSTEM which are generally fixed macrophages, and the macrophages formed directly from the large TISSUE LYMPHOCYTES, from BLOOD LYMPHOCYTES, and by cellular division and proliferation of the fixed RETICULO-ENDOTHELIAL cells. Tallaferrro and Mulligan group the specialized cells lining the sinuses of reticular tissues, frequently called "littoral cells," under this system and mention that this cell is not ordinarily as phagocytic as the reticular cells of Billroth's cords, for example. As a result of the predominant localization of the cellular components of the LYMPHOID-MACROPHAGE SYSTEM, as we shall generally call it, the only specific tissues (not including the blood stream) which show a characteristic constant picture in acute malaria are those of the spleen, liver, and bone marrow. Considerable attention has therefore been accorded to these in considering the pathologic anatomy of malaria, and from their study much of our information has been derived.

The gross and microscopic findings at autop-

sy in cases of acute *P. falciparum* infection (and the other species) are influenced by several factors, and no one characteristic picture can be presented for guidance. The degree of parasitemia and the duration of the infection, for obvious reasons, play important parts in determining the extent of pigment (ferrihemate) deposits in the spleen, liver, bone marrow, and to a lesser extent other organs. However, considerable pigmentation may be present generally throughout the body in patients dying of short fulminating infections, if numbers of parasites show schizogony, with correspondingly large amounts of pigment both in erythrocytes and free in the capillaries. Another factor influencing the clinical symptoms and gross autopsy findings is an unexplained, seemingly selective localization of parasites in various organs or systems. With the exception noted in the brain, at autopsy the most dramatic localizations of parasites are usually present in the organs or systems to which the most prominent clinical features were referable.

Considerable evidence has accumulated that a limited immunity to malaria exists (Thomson, 1933) and may influence the clinical course and the gross autopsy findings of malaria infections, especially in the spleen (Boyd, 1930). Acquired immunity may result from single infections (Boyd et al., 1934; Boyd and Mathews, 1939; Coggeshall, 1940) or repeated infections in natives of malaria districts where surveys (Schilling, 1934) have shown that over 90 per cent of seemingly healthy young children have malaria parasites in their blood while in adults of the same village the rate of parasitization is much lower. Reference has also been made to the relative rarity of pernicious malaria in persons living in an endemic area who have had previous attacks, and likewise to the fact that individuals entering such endemic areas, not having previously been exposed to malaria, show a high degree of pernicious malaria (Marchiasava and Bignami, 1900). In a later report the following statement is made "... it is especially in organisms virgin to the malarial infection that the estivoautumnal parasites multiply more acutely, reaching at times enormous numbers; and, developing greater virulence, give rise to pernicious symptoms. And, on the other hand, with the persistence of infection in the organism, this acquires a relative and partial immunity, an immunity against the pernicious feature of the infection,

such as we see exemplified every day among the inhabitants of malarious communities" (Marchiasava, 1931).

RACIAL IMMUNITY to malaria apparently does not exist in the white race (Strong, 1944). The Negro race (Stephens and Christophers, 1908; Daniels, 1913; Clark, 1928) and Javanese (Oudendal, 1925) show smaller spleen weights than other races, indicating racial immunity, which according to some investigators (Giglio, 1932a), is quite definite and need not be activated by previous attacks of malaria (Strong, 1944). In Table 141 organ weights from the adult male uncomplicated cases of primary *P. falciparum* malaria in our series are presented. The difference between the Negro spleen, liver and brain weights and those present in the

TABLE 141. MEAN ORGAN WEIGHTS IN GRAMS IN UNCOMPLICATED ADULT MALE *P. falciparum* CASES

RACE	NO. CASES	BRAIN	LIVER	SPLEEN	KIDNEYS
Negro	101	1285	1687	292	304
	98				
	103				
	102				
Foreign white	35	1420	1913	440	355
	34				
	33				
	32				
Mestizo	13	1378	1927	473	271
	15				
	13				
	14				

foreign white and mestizo groups is striking and may represent the influence of racial immunity.

Autopsy Smear Preparations. In areas where malaria is endemic the routine preparation of thick and thin blood films from the right chamber of the heart, thin smears from the rib marrow, cut spleen surface and crushed thin smears from the brain cortex is advisable at the time of autopsy. These slides are stained as directed in the technical section and examined for the presence of malaria parasites or pigment. From this material it is possible to positively identify malaria parasites and in particular the species present (Figs. 254 and 255). Also an opinion can be formed regarding the degree of infection present and its role in causing death

in the particular case, since low-grade or chronic infections with *P. falciparum* or other species may be present in persons dying of other causes. In 405 proven *falciparum* malaria cases studied by us, the records of autopsy smear examinations are available in 381 instances, and revealed *P. falciparum* alone in 358 cases, *P. falciparum* and *P. vivax* mixed in twenty-one cases, and *P. falciparum* and *P. malariae* mixed in two cases. In most instances, therefore, it was possible to demonstrate some form of *P. falciparum* parasites in the autopsy smears,

parasite development, while the liver and brain smears show trophozoite and segmenting forms. A notable feature in all smears are the late forms of trophozoites which are rarely seen in the peripheral blood.

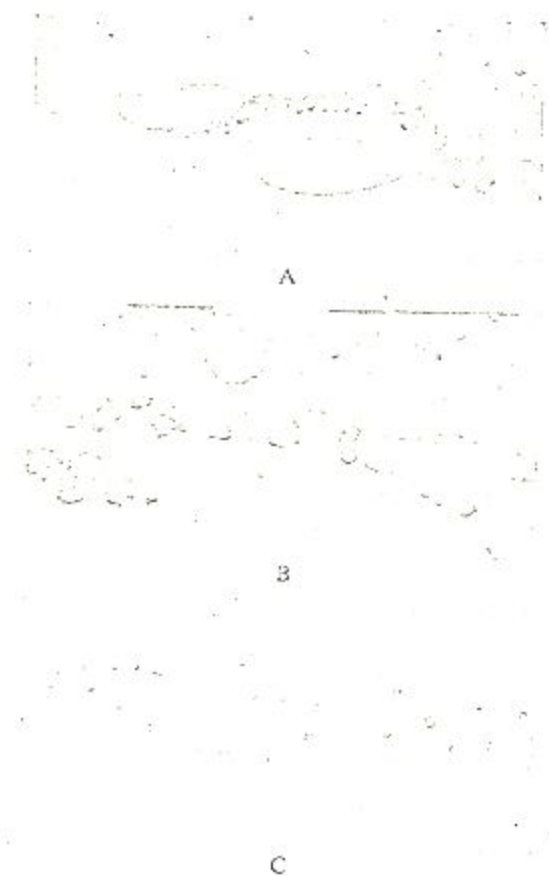


Fig. 254. A, Early *P. falciparum* trophozoites in brain smear (Giemsa, $\times 500$). B, Late *P. falciparum* trophozoites and early schizonts in brain smear (Giemsa, $\times 500$). C, Mature *P. falciparum* schizonts in brain smear (Giemsa, $\times 500$).



Fig. 255. A, *P. falciparum* trophozoites and schizonts in spleen smear (Giemsa, $\times 500$). B, *P. falciparum* trophozoites and gametocytes in bone-marrow smear (Giemsa, $\times 500$).

ACUTE *P. FALCIPARUM* INFECTIONS

Spleen. In acute infections with *P. falciparum* the spleen is described as being enlarged, soft, and ranging from a dark-red or chocolate color to a deep slate-gray or even black appearance. The malpighian corpuscles were usually described as inconspicuous as were the trabecular structures. Occasionally subcapsular hemorrhages and even infarctions are mentioned (Councilman and Abbott, 1885; Dock, 1894; Barker, 1895; Marchiafava and Bignami, 1900; Craig, 1909; Deaderick, 1909; Dudgeon and Clarke, 1917; Seyfarth, 1926; Marchiafava, 1931; Weil, 1934; Ash and Spitz, 1945).

In our foreign white and mestizo groups the spleen was always markedly enlarged (Table 141), soft and frequently showed subcapsular hemorrhages or areas of softening. The capsule

although in patients having received intensive therapy a long careful search of many smears was necessary. In these instances the presence of large pigment-laden macrophages in the spleen smears was also corroborative of a very recent malaria infection. In general, the spleen and bone marrow smears show all stages of

was thin and the pulp moved easily beneath the capsule resulting in a globular outline with loss of the convex phrenic surface and concave gastric and renal surfaces. The splenic edges were rounded and soft, and the cut surface bulged beyond the capsule and tended to flow out upon the sectioning board. Pulp color was influenced by the amount of malaria and other pigments present and depended upon the duration of the infection. In cases fatal in five to seven days, the pulp was intensely congested, and had a chocolate color, which deepened and became a violaceous slate-black in cases of longer duration. The trabeculae and malpighian follicles in these cases were usually very indistinct, owing to the extreme congestion and softening with occasional hemorrhages. Follow-

the severe muscle contractures in convulsions may produce rupture and in our autopsy series spontaneous rupture was present in six instances. At autopsy an impression smear, or better a thin, crushed tissue smear stained with any of the usual polychromatic blood stains, will reveal numbers of free and intracorporeal late trophozoites, schizonts and gametocytes of *P. falciparum* along with small delicate masses of free and phagocytized pigment (Fig. 255A).

In fatal cases of primary or recent *falciparum* infection, the histologic picture in the spleen is diagnostic only in that parasites and pigment are easily demonstrated. Without these the pattern is one of extreme congestion, hyperplasia even to exhaustion, and variable degrees of necrosis. The capsule is usually thin or of



Fig. 256. Spleen: Extreme congestion of Billroth's cords and venous sinuses in acute *falciparum* malaria. Note the swollen littoral cells lining the sinus. (Toluidine-blue phloxine stain, $\times 375$.)



Fig. 257. Spleen: Extreme hyperplasia of follicle with degeneration of central artery in late case of *falciparum* infection. The presence of parasites and pigment within follicle is not usual. (Depigmented, stained with toluidine-blue phloxine, $\times 250$.)

ing fixation and fresh sectioning, the malpighian follicles are usually easily visualized and likewise the slate-black color of malaria pigment becomes more apparent.

In the Negro race the gross splenic picture was similar but less extensive. Thus the organ was smaller (Table 141), and had less of a diffuent character although the cut surface was congested and pulp adhered to the knife. Malpighian corpuscles were frequently visualized and may be quite prominent. Variations in color are similar to those in the white group although hemorrhages were not usually as prominent in this group. Extreme care must be used in examining the spleen in all acute cases, because of the ease with which it may be ruptured. Severe episodes of coughing, vomiting or

average appearance, and occasionally shows small areas of hemorrhage. The trabeculae are usually thin, considerably distorted and show edema of the fibrous interstices. The congestion is primarily active in type and is characterized by erythrocytic engorgement of Billroth's cords with comparatively empty adjacent sinuses (Fig. 256); however, in many cases the infection has progressed to a point where this differentiation is not apparent and the hyperemia present is similar to that seen in passive or obstructive splenomegaly. Splenomegaly is in part produced by extreme hyperplasia of the malpighian follicles, the white pulp and the red pulp, and the similarity between hyperplasia in malaria and in other infections has been noted (Cannon, 1941).

The malpighian follicles vary, but in an average case will show marked hyperplasia of the reticular cells with numbers of lymphocytes among the reticular cells and at the transitional zones (Fig. 257). In severe cases there is a notable lack of these lymphocytes, while the elongated reticular cells are phagocytic for nuclear debris, and in instances show hyaline swelling and degeneration. In cases of this severity, phagocytized pigment may be patchily distributed in the reticular cells but it is very rare to find parasites. In the white pulp of the spleen (lymphatic sheaths of the small arteries) there is similar extreme hyperplasia of the lymphocytes with migration to the adjacent sinuses. The cells develop into the varied-size macrophages found in the spleen and are carried to other areas (Taliaferro and Mulligan, 1937). Here again the degree of phagocytosis present in the sheaths is usually minimal and in extreme cases the supporting framework of these sheaths, a loose reticular structure (Klemper, 1938) which is not phagocytic, is all that remains.

The red pulp, containing the finer ramifications of the vascular system in the sinuses and Billroth's cords (Klemper, 1938), presents a cellular matrix of cytoplasmic and fibrillar reticulum with free cells, and is probably the most important single defense area against malaria infections. The basic cellular pattern of the area responds to infections by active phagocytosis, cell division and the formation of more phagocytic cells. These cells are at first similar to lymphocytes and then mature into larger macrophages. Lymphocytes and macrophages produced in the malpighian follicles and white pulp of the spleen likewise migrate to the red pulp and reinforce the phagocytic activity. The formation of macrophages from lymphocytes has been the subject of a recent review with presentation of a mass of supporting evidence from experimental simian malaria (Taliaferro and Mulligan, 1937). Examination of large numbers of spleen sections from human malaria cases shows essentially the same picture of lymphoid hyperplasia with development of macrophages, and for this reason we have adopted their terminology "lymphoid-macrophage system" which is essentially composed of the reticulo-endothelial system (Aschoff) and the lymphoid cells which they follow through transitional polyblast stages into large typical macrophages. In the red pulp there are

also found large numbers of plasma cells, polymorphonuclear leukocytes (heterophils) and in some cases eosinophils. In our material there was no conclusive evidence that any of these cells materially contributed to the phagocytic defense against malaria. In rare instances, usually in young children, hemocytoblasts and normoblasts were identified.

The greatest phagocytic activity occurs in the red pulp cords (Billroth) where the blood flow is slowed and the most active phagocytic cells are concentrated. The cells are the large rounded reticulo-endothelial phagocytes of the pulp, and the numerous phagocytic cells intermediate between lymphocytes and the large macrophages called polyblasts by Maximow (1932). They appear in ordinary sections as pigment, para-



Fig. 258. Spleen: Heavy parasitemia and extreme hyperplasia of Billroth's cords. Large macrophage and numerous polyblasts containing parasites at arrows. (Depigmented, stained with toluidine-blue picrocarmalum, $\times 400$.)

site, and erythrocyte containing ameboid cells in which particulate debris and fat globules are also present (Fig. 258). In preparations that have been depigmented (see technical section), the nuclei of the largest macrophages are oval and vesicular with prominent nuclear membranes and delicate chromatin threads arranged in a lacelike pattern. The intermediate or developing macrophage (polyblast) has an irregular or frequently indented nucleus which is more dense and compact, and resembles the nuclear structure of the large lymphocytes formed in the spleen (Taliaferro and Mulligan, 1937; Taliaferro and Kläver, 1940). In large macrophages that have been depigmented we have found as many as six intact mature schizonts of *P. falciparum*, and it is very easy

to demonstrate three to five erythrocytes containing trophozoite forms in these cells.

Degenerative changes are present in the spleen in most cases. A frequently noted change (Barker, 1895; Dudgeon and Clarke, 1917; Gaskell and Millar, 1920) is degeneration of the malpighian follicles with varying degrees of lymphocytic destruction, while rarely one may

roth's cords appear as large engorged areas filled with masses of erythrocytes, parasites, pigment and macrophages (Figs. 259 and 260). The fibrillar reticulum shows edema, hyaline and fatty changes, and fragmentation resulting in the occasional formation of small areas of thrombosis. In this extreme picture the littoral cells lining the venous sinuses appear swollen, prominent, show degenerative changes and occasionally contain phagocytized pigment.

In the arteries and small arterioles throughout the spleen we fail to find evidence of capillary endothelial cell proliferation or phagocytosis. Degenerative changes of a fatty, vacuolated nature are present, and probably account for the more massive areas of infarction with gross rupture that are occasionally encountered (see



Fig. 259. Spleen: Fragmentation of stroma with degeneration of littoral cells and pulp cords. Large macrophage in venous sinus at arrow might be mistaken for phagocytic littoral cell. (Partly depigmented, stained with toluidine-blue phloxine, $\times 250$.)



Fig. 260. Spleen: Similar to Fig. 259, showing more marked littoral cell changes with polyblasts in venous sinus. (Partly depigmented, stained with toluidine-blue phloxine, $\times 250$.)

find small hemorrhages from the follicle capillaries. As a general rule the extreme hyperplasia of the lymphoid cells in the follicles and white pulp is arrested or exhausted in cases of appreciable duration, while the remaining cells show degenerative changes. In the red pulp there is a similar picture of exhaustion and depletion of the cytoplasmic reticular pulp cells, and Bill-

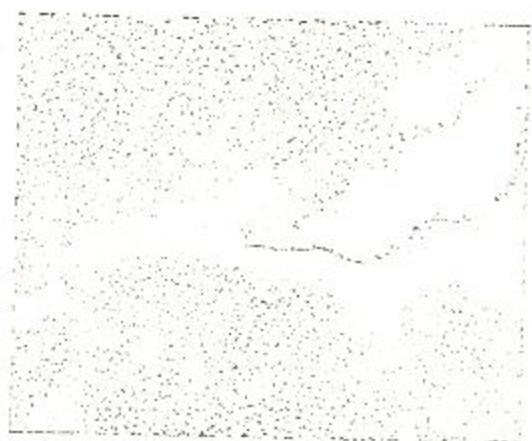


Fig. 261. Spleen: Cellular hyperplasia in trabecular vein wall with perforation of endothelium. Note the degeneration in the trabecular tissue. (Toluidine-blue phloxine, $\times 110$.)

Fig. 257). In the splenic vein we likewise have not found thrombosis or other changes. However, in the trabecular veins of many cases, scattered foci of cells are found frequently protruding through, or definitely displacing the endothelial layer (Fig. 261). These cells are characteristically cytoplasmic-reticular and lymphocytic in appearance and many become quite large. Phagocytosis is not an important feature in these areas and their presence is best explained on the basis of hyperplasia concomitant with the changes in the remainder of the spleen. In a few instances true partial thrombus formation by platelets, erythrocytes and fibrin was present in these areas. This change has been noted in experimental simian malaria (Taliaferro and Mulligan, 1937), and in a recent study (Rigdon, 1942) it is suggested that splenic infarction in

malaria may be the direct result of these hyperplastic nodules of reticular-like cells in the venous sinuses acting as *nidi* for thrombus formation in the sluggish circulation. In view of the doubt concerning parasitic thrombi in malaria this explanation seems more logical to explain the occurrence of small foci of hemorrhagic infarctions in the pulp. If these were located near the capsule they would result in the small hemorrhagic bleblike lesions seen grossly.

Liver. In the acute phases of the infection the liver is enlarged (Table 141), tense and has a basic chocolate-brown appearance. There may be considerable malaria pigment present producing a slate-gray cast, but it never reaches the intensity present in the spleen. The enlargement is a direct result of the extreme congestion present. Considerable loss of the normal lobular pattern on the cut surface may be present from the intense phagocytic reaction, sinusoidal hyperplasia and acute swelling of the parenchymal cells *per se*. These changes may be so pronounced that gross softening and extreme flabbiness may be present. Rarely hemorrhagic areas are present. No cases of acute degeneration were encountered in our series. The description above, from our cases, does not materially differ from that of other observers (Marchiafava and Celli, 1887; Councilman and Abbott, 1885; Dock, 1894; Barker, 1895; Marchiafava and Bignami, 1900; Craig, 1909; Deaderick, 1909; Dudgeon and Clarke, 1917; Seyfarth, 1926).

Jaundice of appreciable degree is frequently present, and Marchiafava and Bignami (1900) report a case showing intense jaundice with parenchymatous degeneration. Most frequently the jaundice results from excessive destruction of erythrocytes and the inability of the congested engorged liver to handle the excess hemoglobin released in this manner. The gallbladder and biliary ducts are usually filled with an extremely viscid green-black bile. No gross changes were recognized in the gallbladder walls.

Microscopically the liver is basically the site of an extreme congestion of the entire vascular tree. Free phagocytic cells containing pigment, parasitized and nonparasitized erythrocytes and other debris are found throughout the vascular system. It must be emphasized that many of these phagocytic cells are morphologically identical with the polyblasts and macro-

phages found within vascular channels in other viscera, and are undoubtedly transported by the blood flow to the liver (Barker, 1895; Thayer, 1897), where the numerous sinusoids act as stagnant pools to facilitate phagocytic activity. There is no actual production of phagocytic cells in the arteries, veins or capillaries of the liver although this has been reported (Marchiafava and Bignami, 1900; Dudgeon and Clarke, 1917). It is probable that reference was made to the liver sinusoids with their Kupffer cells which show true phagocytosis. These sinusoids are enclosed by a membrane not typically endothelial and are regarded by many as a syncytium in which two kinds of cells are identified (Mann, 1928). One, closely resembling an endothelial cell, has an oval, compact, small nucleus with a granular cyto-



Fig. 262. Liver: Showing degenerative changes in parenchyma with extreme congestion, attached and free phagocytic Kupfer cells with prominence of sinusoidal endothelial cells. (Toluidine-blue ploxine, $\times 275$.)

plasm parallel with the liver sinusoid. The other cell (Kupfer) is larger, has a stellate nucleus and long cytoplasmic process which appears to extend between the parenchymal cells and along the sinusoid channels. These cells are relatively few in ordinary histologic preparations but in instances where phagocytosis is prominent they are numerous. Thus in malaria they are found with ease and show shortening of the cytoplasmic processes with resultant increased width and contain amazing amounts of phagocytized material (Fig. 262). It is possible for them to contain six to eight mature schizonts, numerous trophozoite forms both in erythrocytes and free, along with huge quantities of malaria pigment. The diffuse slate-gray color of the liver results from these cells and the vascular content of macrophages and

parasites. The Kupffer cells constitute an efficient cleansing agent for the blood which is physiologically slowed in passing through the sinusoids. In some instances they are visualized detaching from the sinusoidal lining and becoming free macrophages. The basic sinusoidal lining cells are regarded as undifferentiated endothelial-like cells (Taliaferro and Mulligan, 1937) and retain developmental potentialities to form Kupffer cells but per se do not ordinarily exhibit phagocytosis, and when they show phagocytosis should be considered as Kupffer cells. The hepatic parenchymal cells contain hemosiderin, and bile pigment is usually present in the small canaliculi, but phagocytosis of malaria pigment or parasites by these cells, which has been reported (Ewing, 1902; Dud-

not found evidence of thrombosis, while the explanation that large fixed and free phagocytes slow the circulatory rate hardly seems tenable in view of the dilatation universally present in the sinusoids which would obviate mechanical obstruction if sufficient hydrostatic pressure was present.

There were few instances of appreciable mantling of the periportal areas by lymphocytes and the relationship between this change and acute malaria is doubtful, while they may represent sources of phagocytic cells in chronic malaria or a response to other conditions.

In general, our material agrees with that of other descriptions but regenerative processes of appreciable degree (Marchiafava and Bignami, 1900; Deaderick, 1909) were not present, possibly because our cases did not present diffuse or marked necrotic changes which they and others (Dudgeon and Clarke, 1917; Gaskell and Millar, 1920; Klotz, 1929) have emphasized.

Brain. At the time of autopsy the brain affords the best material for preparation of a thin, clean tissue film by crushing a small fragment of cortex between two glass slides and then separating them lengthwise while compressed in a jerky uneven spiral fashion. Such films fixed and stained as directed in the technical section reveal thick and thin areas of tissue on the slide. Examination of the thin areas will reveal numerous stretched capillaries which frequently contain parasitized erythrocytes and pigment, and the type parasite or the presence of a mixed infection can be determined (Fig. 254 A, B, and C). In our experience and in others (Marchiafava and Bignami, 1894) fatalities were due to *P. falciparum* alone or mixed with other types, but no fatalities from uncomplicated *P. malariae* or *P. vivax* were found.

Considerable variation exists in the gross appearance of the brain in fatal *falciparum* malaria. In patients having presented cerebral symptoms the brain is usually heavy and edematous, with flattened convolutions and partial obliteration of the sulci, accompanied by congestion of the meningeal and cortical vessels, but none of these changes are diagnostic. In cases showing marked parasitemia there is extreme congestion of the pial and cortical vessels with rare small petechia, especially over the cerebellum (Fig. 265), producing an appearance similar to that seen in acute meningitis before a purulent exudate is grossly visible. This



Fig. 263. Depigmented liver showing degenerative parenchymal changes with better visualization of Kupffer cells (arrows) containing parasites. (Toluidine-blue phtoxine, $\times 250$.)

geon and Clarke, 1917), must be considered an erroneous interpretation or artefact.

In general, the parenchymal degenerative changes in the liver follow the severity of the clinical picture (Fig. 263). Fatty infiltration is frequently present but the pathologic significance of this is difficult to evaluate. In the majority of our clinically severe cases early fatty degeneration was present as a fine foamy vacuolization of the cytoplasm with rare degenerative changes in the nuclei. However, in a few cases there were widely separated areas of central necrosis, and also jaundice at autopsy. The mechanism of the fatty degeneration and patchy necrosis is difficult to interpret. It has been attributed to toxicity, and to interference, even thrombosis in the circulation. Another possibility is anoxia from shock which has recently been emphasized in malaria. We have

appearance is locally called "pink brain" and is very striking (Fig. 264). Depending upon the pigment content of the parasites in the vessels, varying shades of a faint to an intense ashen-gray hue may be present and this coloration is diagnostic of a severe malaria infection. Sectioning such a brain reveals that the extreme "pink" appearance, the pigmentation, and



Fig. 264. Typical "pink brain" of *falciparum* malaria showing extreme congestion, edema and petechial hemorrhages with faint pigmentation.

rarely the petechial hemorrhages are localized in the cortical substance. This apparent pigment localization results from the marked capillary vascularity present in the cortical structures as compared with the white medullary structures. In very severe cases, however, the pigment may be apparent in the medullary portion. The majority of the hemorrhages, when found, are present just beneath the cortical layer in the medullary substance but occasionally may be

so extensive as to produce a mottled turkey-egg appearance throughout cross sections of the brain. Gross thrombosis of vessels or areas of degeneration were not present in our series of cases.

In the routine performance of autopsies in cases of malaria one soon realizes that there is no true correlation between the clinical picture of cerebral malaria and the gross changes present in the brain at the time of autopsy. Some patients presenting marked cerebral symptoms up to the time of death may show only congestion, slight pigmentation and no hemorrhages, while others not presenting cerebral symptoms may show extreme congestion, pigmentation, and occasional medullary hemor-



Fig. 265. Cerebrum: Showing subpial edema, cortical hemorrhages with dissection to surface, degenerative changes in Purkinje cells and dispersion of the molecular layer in *falciparum* malaria. (Toluidine-blue phloxine, X50.)

rhages. In persons showing the extreme gross changes the cerebral symptoms were always quite prominent. This apparent lack of gross correlation was noted by Frericks (quoted by Marchiafava and Bignami, 1900) and emphasized by Kean and Smith (1944). In a histologic study of cerebral malaria cases, however, the correlation becomes evident, and it is possible that intensive therapy may change the gross appearance.

The histologic changes in the brain in severe fatal *falciparum* infections showing cerebral symptoms while the patient was alive have been described in many studies (Marchiafava and Bignami, 1894, 1900; Dock, 1894; Mannaberg, 1894, 1905; Craig, 1909; Dudgeon and Clarke,

1917, 1919; Gaskell and Millar, 1920; Dürck, 1923, 1925; Seyfarth, 1926; Alvarez, 1938; Rigdon, 1942, 1944; Rigdon and Fletcher, 1945; Ash and Spitz, 1945). Our findings in this series of cases are essentially in agreement with these descriptions, and a composite description would be as follows: There is intense congestion of the vessels throughout the brain and spinal cord with special prominence in the cortical layer of both the cerebrum and cerebellum. These capillaries contain both parasitized and non-parasitized erythrocytes (Fig. 266), depending upon the terminal degree of parasitemia and localization. Frequently one area of the cortex will show scattered parasites while other areas will reveal almost complete parasitization of the erythrocytes, and no satisfactory explanation for this phenomena is available. The

cell body. Extensive studies of the functions of the endothelium of common blood vessels (Maximow, 1932) have shown that these cells are unable to transform into wandering phagocytic polyblastic cells, and it is extremely doubtful in view of this finding, and our critical survey of autopsy material, if the endothelial cells of the brain blood channels are factors in the phagocytic response to malaria infections. Other recent reports (Rigdon, 1942, 1944; Rigdon and Fletcher, 1945) fail to note endothelial phagocytosis of malaria parasites or pigment in severe human and experimental animal infections showing extreme gross and microscopic cerebral changes.

In general, the endothelial cells throughout the brain capillaries in cases of severe malaria appear swollen, faintly granular and may con-



Fig. 266. Brain: Medullary portion of brain showing marked capillary parasitemia, endothelial cell swelling and degeneration of nerve substance. (Toluidine-blue phloxine, $\times 350$.)



Fig. 267. Brain: Cerebral arteriole showing *falciparum* parasites, endothelial-cell swelling, perivascular edema, and degenerative changes in nerve cells. (Toluidine-blue phloxine, $\times 350$.)

capillaries contain circulating pigment-laden macrophages resembling those seen in peripheral smears. Although phagocytosis of pigment and parasites by the fixed endothelial cells of these capillaries has been described (Barker, 1895; Marchiasava and Bignami, 1900; Craig, 1909; Gaskell and Millar, 1920; Seyfarth, 1926; Thomson and Annecke, 1926; Fitz-Hugh, Pepper and Hopkins, 1944) or by macrophages formed from the endothelial cells in these capillaries (Thayer, 1897; Dudgeon and Clarke, 1917; Gaskell and Millar, 1920; Seyfarth, 1926; and in *vivax* infections by Bructsch, 1932a, 1932b) we have not found convincing evidence of this change. Adherence of pigment granules to swollen degenerated endothelial cells was occasionally present (Jaffé, 1927) but the pigment was definitely not contained within the

tain a few yellow-brown granules of lipochrome material totally distinct from malaria pigment (Fig. 267). Fatty deposits are frequently present in these cells and there is marked enlargement of the pericapillary spaces. Also occasional pericapillary areas of lymphoid infiltration may be present (Gaskell and Millar, 1920; Marinesco, 1921; Dürck, 1923, 1925; Thomson and Annecke, 1926) and these are usually associated with areas of necrosis. These degenerative changes in the cerebral capillaries have been ascribed to poisonous decomposition products of malaria parasites and to malaria toxins (Mannaberg, 1894; Lafora, 1912; Gaskell and Millar, 1920; Dürck, 1923, 1925; Seyfarth, 1926; Thomson and Annecke, 1926; de Vries, 1927) but it generally is questioned whether any specific toxins are liberated by

malaria parasites. Rigdon (1942, 1944, 1945) feels that the pericapillary enlargement, endothelial changes and others mentioned later are indicative of severe shock, cerebral anoxia and that the areas of necrosis throughout the medullary structures are the result of anoxia. Similar, though less extensive, degenerative changes are frequently present in the smaller arterioles and venules of the brain in severe cases, and the perivascular spaces are quite prominent in these areas.

The question of cerebral capillary "thrombosis, plugging or blocking" as mentioned in the numerous older descriptions (quoted by Marchiafava and Bignami, 1894; Cropper, 1908; Dudgeon and Clarke, 1917) and recent publications (Hudson, 1943; Fitz-Hugh, Pepper and Hopkins, 1944) is discussed in other reviews (Gaskell and Millar, 1920; de Vries, 1927; Kean and Smith, 1944; Rigdon, 1944, 1945). Conclusive evidence of intravascular thrombosis, erythrocytic, parasitic or pigment agglutination was not found, and in our cases satisfactory evidences of these phenomena were lacking at the time of autopsy. The 100 fatal *falciparum* malaria cases studied by Kean and Smith (1944) are from the autopsy records at the Board of Health Laboratory, Ancon, C. Z., and are therefore included in our material. In vivo studies (Krisely, Stratman-Thomas and Elliot, 1941; Lack, 1942) of simian and avian malaria are reported as showing deposits of fibrin or fibrin-like substances upon infected erythrocytes with resultant adherence and clumping of these infected erythrocytes. Such clumps are at first susceptible to phagocytosis by "hepatic and other phagocytes" but with increased plasma viscosity and reduction of capillary flow rates there is slowing of phagocytosis and a marked increase in the tendency of ordinary leukocytes to coat the now "sticky" ordinary endothelium. The "terminal symptoms are manifestations of damage resulting from greatly slowed capillary circulation rates," and Rigdon interprets these changes as being the result of shock. In view of the lack of supporting evidence for true embolic phenomena in human malaria cases it is doubtful in our opinion if they can be a prominent factor in producing cerebral or other lesions.

It is highly probable that shock as earlier mentioned (Marchiafava and Bignami, 1894, 1900) and recently emphasized (Cannon, 1941; Rigdon, 1942, 1944, 1945; Kean and Smith,

1944) is responsible for the microscopic appearance of the capillaries containing large numbers of packed parasitized and nonparasitized erythrocytes, and results in anoxia of the brain cells producing the lesions which will be described. Wong's (1945) studies showing appreciable decreases of blood oxygen saturation in *vivax* malaria indicate a definite decrease in the corpuscular oxyhemoglobin content and such



Fig. 268. Brain: Dilated medullary capillary showing endothelial degeneration with escape of parasitized erythrocytes (arrows) into adjacent edematous nerve tissue. (Toluidine-blue phloxine, $\times 350$.)



Fig. 269. Brain: More extensive hemorrhage from medullary capillary entering at right and ruptured with escape of erythrocytes, parasitized erythrocytes and phagocytes into adjacent nerve tissue. (Depigmented, stained with toluidine-blue phloxine, $\times 350$.)

cells stagnated in the cerebral and other capillaries would produce rapid tissue anoxia. Changes similar to those in malaria brains, but not showing parasites or pigment, have been emphasized in sickle-cell anemia, where the oxygen carrying capacity of erythrocytes is diminished (Tomlinson, 1945) and in severe shock from other causes (Hartman, 1938; Steiner, 1941). Small hemorrhages from the damaged capillary walls occur (Figs. 268 and 269), and

Rigdon believes the hemorrhagic foci are the result of erythrocytes escaping through the damaged endothelial cells into the already damaged adjacent tissue. It is probable that this does occur but also thrombosis in situ from stagnation is a factor in producing the diffuse terminal hemorrhages. The severe convulsive attacks these patients exhibit may be a prominent factor in rupturing the damaged capillary walls.

In the cortex there is evidence of pericellular edema and a general uneven staining reaction of the cortical cells. This results in a spongy appearance with loss of Nissl's substance and frequently shrunken cytoplasmic processes. The nuclei show pyknotic changes with central clumping of chromatin and intensification of the nuclear margins (Fig. 270). Neuronophagia



Fig. 270. Cerebral cortex in *falciparum* malaria showing prominent degenerative changes with neuronophagia. Parasites at arrow. (Toluidine-blue ploxine, $\times 475$.)

is relatively common in some areas while evidence of depletion of the cortical nerve cells is apparent in all cases that exhibited cerebral symptoms. Occasional hemorrhages in the sub-cortical area tend to dissect through the cortex and present beneath the pia mater, especially in the cerebellum (see Fig. 265). In the medullary substance the lesions in typical cases are very striking. There is a marked accentuation of the vacuolated appearance resulting from myelin degeneration in these areas, and a general decrease in glial cells is apparent. Scattered throughout the section are roughly oval areas of necrosis having deep-staining centers composed of cellular debris, malaria pigment, proliferating glial cells and mononuclear phagocytic cells (Figs. 268 and 269 and 271). Usually the centers of these areas show

small capillaries, or capillary remnants can be visualized. Some of these areas show a central zone of hemorrhage which is not accompanied by a cellular response and in these, parasitized erythrocytes may be identified. In other areas the center is an acellular granular mass with a surrounding ring of nonparasitized erythrocytes and in this type lesion the swollen or necrotic vessel wall is usually surrounding the acellular debris. In lesions of obvious longer duration there is a definite granulomatous appearance (Dürek, 1923, 1925) from palisading of the mononuclear phagocytic cells and neuroglial proliferation. The end stage of such a lesion is represented by a small focus of glial elements, pigment, scattered phagocytic cells and a marked porosity of the adjacent area. In the ventricle linings of the brain and in the



Fig. 271. Brain: Medullary area in *falciparum* malaria showing edema, rarefaction, degeneration and granuloma formations. (Toluidine-blue ploxine, $\times 75$.)

choroid plexuses no changes were demonstrable beyond capillary congestion and corresponding degrees of parasitemia.

In cases of fatal *falciparum* malaria not presenting cerebral symptoms there is no characteristic histologic pattern. There is usually congestion, although this may be very minimal and the capillary endothelium may show minor changes. An occasional parasitized erythrocyte may be identified or these parasitized cells may be quite numerous depending upon the terminal parasitemia. It is usually possible to demonstrate minor foci of nerve-cell degenerations especially in the cortical areas, but hemorrhages are not usually present. In our material we did not find marked histologic changes in the brains of persons showing no cerebral symptomatology.

Eye. No gross external changes in the eyes of

our series are recorded as the result of acute malaria, and no eyes were removed for study of the internal structures. Punctiform hemorrhages of the retina have been described (Marchiafava and Bignami, 1900) and the same authors report the results of a study by Guarnieri as follows: The capillaries were injected with parasitized erythrocytes which were "deformed and took eosin poorly." The veins were also dilated, showed edema of the lymphatic sheath, and contained parasitized erythrocytes and phagocytized pigment sometimes to the point of obstructing the vessel lumina. The hemorrhages primarily involved the external plexiform layer of the retina. Phagocytosis by the vessel endothelium was not present. "In the nervous apparatus of the retina we find changes secondary to the hemorrhages, as well as to the accompanying stasis and oedema. Up to the present time, these changes have appeared to consist in a confused arrangement of the cells of the internal granular layer." In several of our cases sections of the optic nerves were available for study. The capillaries and other small vessels seen in cross section contained scattered parasitized erythrocytes and a few pigment-laden circulating macrophages but no evidence of degeneration was present in the capillaries or in the neural tissue.

Bone Marrow. The bone marrow in the acute fulminating infections is soft, diffuent, dark red-brown and shows little pigment deposit (Marchiafava and Bignami, 1900; Seyfarth, 1926). In our experience, infections lasting more than four to five days reveal a more firm, granular marrow which, with the pigment deposits in the basic dull-brown appearance, has led to the use of the descriptive term "apple-butter marrow" in this area. Pigmentation in the bone marrow never becomes as pronounced as that present in the spleen or liver, and tends to be removed more rapidly than that present in the other organs (Craig, 1909).

Active hyperplasia of the white and red cell series in the bone marrow, accompanied by phagocytosis of malaria pigment, parasites, degenerating erythrocytes and cellular debris, is the basic change in the bone marrow of the acute infections (Councilman and Abbott, 1885; Dock, 1894; Marchiafava and Bignami, 1900; Thayer, 1897; Craig, 1909; Seyfarth, 1926). The phagocytic activity has been ascribed to large mononuclear marrow cells, leukocytes, macrophages and pigmented phagocytes. Os-

good (1935) describes heterophils (polymorphonuclear leukocytes) and heterophil metamyelocytes as phagocytic cells from aspiration studies on sternal marrow. The cells, in our experience, that show the most active phagocytic activity are those of the lymphoid-macrophage system and are most numerous in the polyblast 2 and 3 stages. The bone marrow is a favorite site for the development of parasites, especially gametocytes (Fig. 272) as shown by the large numbers found in smears and sections of the marrow when the infection has been present for approximately seven days. Degeneration, thrombosis or hemorrhage was not found in the material available for study.



Fig. 272. Bone marrow showing hyperplasia with *falciparum* gametocytes at arrow and other forms throughout the section. (Toluidine-blue stain, X500.) (Originally published in *Am. J. Clin. Path.* 14:322, 1944.)

Heart. As a general rule, there are no gross changes present in the heart which are diagnostic of malaria. The earlier descriptions mention occasional cases of "sub-pericardial hemorrhages," flaccidity and dilatation of the right ventricle (Barker, 1895; Marchiafava and Bignami, 1900). Later studies emphasize the extreme dilatation, flaccidity and tawny appearance of the heart especially in those cases classified clinically as "cardiac" or "septicemic" (Gaskell and Millar, 1920; Michelotti, 1929; Rigdon, 1942). In our uncomplicated adult cases (103) the heart weights were within the normal range (290 to 339 gm.). Punctiform hemorrhages of the epicardium and endocardium were present in three cases, and pigmentation of the pericardium and epicardium was mentioned once. Mention of flaccidity and

right-sided dilatation is found in eighteen of these cases.

In severe infections showing marked parasitemia there are numerous parasitized erythrocytes present in the capillaries of the heart, and circulating phagocytic cells likewise contain malaria pigment. In some cases these parasitized erythrocytes simulate a blockage of the capillaries, but thrombosis was not present. Unfortunately sections were not available in the three cases showing epicardial hemorrhages in our series. We have not found malaria parasites or pigment outside of the regular vascular channels, although parasites have been described in myocardial fibers (Gaskell and Millar, 1920) but this is probably an erroneous interpretation, artefact, or another condition unrecognized. Considerable edema is present in the interstitial tissue and cloudy swelling with fragmentation may be prominent in the

marked weight increases were present (Table 142). Grossly the lungs were described as congested and edematous in 105 instances by pathologists not concerned with the possible role that pulmonary edema and shock may play in malaria. The presence of pulmonary edema has been previously mentioned (Marchiafava and Bignami, 1900; Rigdon, 1942; Keen and Smith, 1944). Patchiform visceral and parietal pleural hemorrhages were present in six instances, terminal bronchopneumonia was present in twenty-four cases and lobar pneumonia was present in one case.

Considerable controversy exists regarding the relationship between pneumonia, bronchopneumonia, pneumonitis and malaria. Marchiafava and Bignami (1900) conclude that bronchopneumonia or lobar pneumonia may develop in cases of long duration and that this represents a complicating infection not specifically related to malaria. In a recent review of pneumonitis (pneumonia) in association with malaria infections (Applebaum and Shrager, 1944) a report of 125 consecutive patients with pneumonia and malaria admitted to Gorgas Hospital, Ancon, Canal Zone, is presented. Malaria parasites were evenly divided between *P. falciparum* and *P. vivax* and the degrees of parasitemia were comparable. The patients were admitted during the "rainy" season which corresponded to the peak incidence of malaria infections and also of pneumonia. The condition was clinically benign (one death from cerebral malaria showing associated noncontributory bronchopneumonia) and responded in most instances to adequate malaria therapy and sulfonamide compounds. Applebaum and Shrager conclude that the cases may be classified as follows: (1) atypical or virus pneumonia running a self-limited course and not responding to therapy, (2) bacterial pneumonitis with adequate response to sulfonamide compounds, and (3) malaria pneumonitis, with a favorable response to malaria therapy.

Sections of lung from the average case of fatal malaria will show scattered parasitized erythrocytes with a few phagocytic cells containing malaria pigment within the capillaries but none in the alveolar walls, spaces, or supporting stroma. In cases of extreme parasitemia these findings are more prominent, but in our material the lung did not appear to be important in phagocytizing parasites or pigment, or in the production of macrophages for this

TABLE 142. ADULT LUNG WEIGHTS IN *P. falciparum* CASES

	NUMBER CASES	RIGHT	LEFT
Negro.....	121*	463*	389
Foreign white.....	37	712	627
Mestizo.....	37	523	456

* Grams.

myocardial fibers. Frequently there is an appreciable increase in plasma cells and myocytes throughout these areas. Deposits of lipochrome pigment are usually present at the polar ends of the nuclei and may be quite extensive. Patchy fat deposits are present in the myocardial fibers, but areas of necrosis as reported (Micheletti, 1929) were not found in our cases. The degenerative and other changes conform to other reports with the exceptions noted (Dudgeon and Clarke, 1917; Gaskell and Millar, 1920; Wenyon, 1922; Micheletti, 1929; Rigdon, 1942; Ash and Spitz, 1945).

Lungs. In general there were no gross changes diagnostic of acute malaria present in the lungs at autopsy in our series. In severe cases (eight) showing extensive pigmentation of other viscera, a slate-gray cast was apparent in the lungs and this has been noted (Rigdon, 1942). In 190 adult cases that showed no complicating pneumonia, tuberculosis or other lesions

purpose (Marchiafava and Bignami, 1900). Cases showing pneumonic processes grossly revealed no microscopic characteristics, except parasitized erythrocytes, which distinguished these processes from those of pneumonia not associated with malaria.

Kidneys. In our material no diagnostic gross changes were present. As a rule the kidneys were slightly enlarged (Table 1-4) and occasionally showed cloudy swelling and rarely mucosal pelvic petechiae. In very severe cases a faint slate color could be seen but there was also a severe general melanosis. The kidneys have been described as showing enlargement, cloudy swelling, pelvic hemorrhages and congestion (Marchiafava and Bignami, 1900; Dudgeon and Clarke, 1917; Pringault, 1921). Allen (1926) studying twenty-six fatal cases of malaria in Jamaica found a definite change in the kidneys of twelve cases and he attributed death to these changes in four instances. The kidneys were nearly twice normal size and had reddish-gray smooth capsules. They were firm and the cortical markings were indistinct with greasy, blurred medullary portions. Rempicci's work upon the relationship between malaria and nephritis is discussed by Marchiafava and Bignami (1900). In a study of 350 cases of malaria infection (type not specified) Rempicci found eighty instances in which nephritis developed while under strict observation, and he felt that there was a definite relationship between malaria and nephritis which could become severe, and end in uremia or be less fulminating and develop into chronic nephritis. He was convinced that the damage resulted from toxic changes and not parasitic localizations in the kidneys.

Microscopically in our cases there were no degenerative or toxic lesions present that could be attributed to the malaria infection. In a few instances pigment-containing macrophages were seen in the capillaries of the interstitial tissue and in cases showing a heavy general parasitemia parasites were present in these capillaries and rarely in the glomeruli. Allen (1926) described marked degeneration of the tubule cells with areas of granular degeneration and infiltrations of large pigment containing mononuclear cells in the interstitial tissue. The glomeruli showed loss of the lining cells of Bowman's capsule and malaria pigment was likewise present in the glomerular tufts. He felt the changes were recent, not inflammatory and

not due to quinine, but malaria parasites were not present in the kidneys. Similar extensive changes have been described by Dudgeon and Clarke (1917).

Gastro-intestinal Tract. In our records there were no notable variations from the reported descriptions. The gastro-intestinal tract may appear grossly normal, may be moderately congested and may be slightly pigmented. In cases showing algid or choleraic manifestations there are usually marked alterations present. The walls are edematous, markedly congested and may show petechial serosal hemorrhages. The mucosal surfaces may show only intense dark red-brown congestion or may also be pigmented, depending upon the stage of parasite development. Ulcerations of the congested surface are frequent and petechial hemorrhages are occasionally present in the submucosal area. The intestinal contents are usually watery, dark-brown with flecks of mucus present. The watery content has a tendency to segmental localization resembling paralytic ileus in this regard. The lymphoid deposits, especially Peyer's patches, are swollen, gray and prominent on the opened mucosal surface.

The cases with no appreciable gross changes show minor edema and occasional parasites within the mucosal capillaries. In the algid or choleraic types there is a marked dilatation of the mucosal villi capillaries and these are frequently filled with parasitized erythrocytes and pigment containing phagocytes. The intense edema present in these areas, with the interference in circulation, apparently results in small areas of necrosis in the epithelium. Some of these areas progress to ulcers extending to the submucosa and are obviously secondarily infected from intestinal bacteria. In marked contrast to the capillary picture in the mucosa is the relative absence of parasitized erythrocytes in the capillaries of the serosa and muscularis. In our experience the lymphoid deposits in the intestines are not primarily involved in the production of phagocytic cells but seem to be reacting to the ulcerations present, and malaria pigment and parasites were not prominent.

Pancreas. The pancreas usually shows congestion and firmness. In cases of some duration, where pigment deposits are easily seen in the spleen and liver, the pancreas shows a striking slate-gray hue over the normal faint-yellow glandular background.

The microscopic picture is one of intense congestion with many of the erythrocytes, especially in the small capillaries, showing parasites in various stages of development. Large macrophages containing pigment are present around these dilated capillaries. Their origin is uncertain since no endothelial hyperplasia is present. They may develop from stromal histiocytes but probably have migrated from the blood into the pericapillary areas. No degenerative changes attributable to malaria were seen in the glandular structures.

Lymph Nodes. The lymph nodes, especially in the upper abdomen around the liver and spleen, are enlarged, edematous and show pigment deposits corresponding to the general findings in other organs. The cut surface is frequently markedly congested and we have seen instances of hemorrhage in the medullary portions of the nodes. No record of hemorrhages in the lymph nodes is available although our cases correspond otherwise with previous reports (Doek, 1894; Barker, 1895; Marchiafava and Nigamti, 1900; Mannaberg, 1905; Seyfarth, 1926).

The histologic reaction resembles that present in the spleen, consisting of marked phagocytic cell production from hyperplasia of the reticular cells in the medullary cords and from the lymphoid cells in the transitional zone of the cortical follicles. Frequently there is extreme derangement of the follicle architecture. Parasitized erythrocytes are found in the dilated vascular channels; rarely phagocytized parasites are recognized in large macrophages within the subcapsular lymph sinuses or in the medullary sinuses. Pigment containing macrophages, however, may be relatively numerous in these areas. The cases of gross hemorrhage showed extreme hyperplasia of the fixed reticular cells with weakening of the stroma and hemorrhage apparently from lack of support around the extremely dilated damaged capillaries. No evidence of hyperplasia or malaria pigment was seen in the endothelial cells lining these capillaries. There are few microscopic descriptions of the lymph nodes in this infection. Some observers (Tulliaferro and Mulligan, 1937) feel that the reaction is nonspecific, at least in their simian research material, but we feel that the lymph nodes contribute some macrophages in the infection.

Ductless Glands. In our material we were unable to find gross changes in the ductless

glands (thyroid, parathyroid, hypophysis and adrenals) that were the result of malaria infection. In cases of severe terminal parasitemia the usual intracapillary parasites were present in these organs but no instances of appreciable phagocyte formation was present.

Formation of phagocytic histiocytes from the endothelial cells lining the hypophyseal sinusoids has been described in therapeutic vivax malaria by Brutsch (1932a) although this author notes that the change was rather minimal.

In the adrenal glands microscopic examination reveals some definite changes. In the acute fulminating cases a rather constant finding is depletion of cortical lipid material with no evidence of inflammation. Some cases show marked congestion of the capillaries by parasitized erythrocytes. Phagocytized malaria pigment is present in large macrophages within the capillaries and in the supporting stroma of the glands. Changes similar to these have been described accompanying acute fulminating infections, which frequently show profound prostration (Paisseau and Lemaire, 1916, quoted by Dudgeon and Clarke, 1917; Weryon, 1922).

Paisseau and Lemaire, and Weryon also describe hemorrhages, vessel thrombosis and necrotic foci throughout many of the glands, changes which we did not find in our cases. Dudgeon and Clarke likewise refer to the presence of malaria pigment in the adrenal cortical cells but phagocytosis by these cells is very difficult to understand while brown pigmentation from degenerative changes is occasionally encountered. The change is present in the adrenals we believe to reflect the high fever and general toxemia (nonspecific) present in severe malaria, and similar changes are present in many other conditions.

Skin. In most cases the skin was considered more pallid than usual, and in eighty-nine instances jaundice was mentioned. In a very few cases the skin was described as gray or ashy, but this probably was an agonal or post-mortem change, since malaria pigment was not recorded in the skin sections studied and these cases did not show an extreme parasitemia or degree of pigmentation throughout the general viscera. No specific or unusual histologic changes were recognized in the sections of skin studied. An occasional parasitized erythrocyte could be found in the small capillaries.

Abdominal Fat. Dock (1894), Marchiafava (1931) and Marchiafava and Bignami (1900) describe marked localizations of parasitized erythrocytes in the mesenteric and intra-abdominal fat. In several severe cases studied by us there were numerous parasites demonstrable in the capillaries of the fat and in some areas there were small hemorrhages. These changes, in our opinion, are present in severe overwhelming infections, and the same heavy degree of parasitemia is present in most of the capillaries regardless of their location. Thus in a series of skeletal muscle sections studied we found similar degrees of capillary parasitic content in some instances.

Pregnancy and Placenta. In our autopsy series there were forty adult women of whom seventeen were pregnant, and they all miscarried at intervals of from five months to almost term gestation. These figures are not a reliable index of the incidence of fetal and maternal deaths generally in malaria, since the mothers had severe and ultimately fatal courses. Other reports, however, emphasize the seriousness of malaria in pregnancy; thus, Gorn (1881) reported that nineteen of forty-six cases ended in premature labor, Blacklock and Gordon (1925), reported 36 per cent fetal mortality, Torpin (1941), 4 per cent maternal and 60 per cent fetal mortality, and Villar (1942) stated that malaria was very dangerous to the fetus.

As a result of the large vascular spaces and the slow circulation rate, the placenta, after the third month, is an important site for the maturation of parasites and therefore infection of other erythrocytes. The placentas were edematous, markedly congested and showed very pronounced pigmentation in most instances. Smears from the raw surface of the placenta are an exceptionally good source to study the development of trophozoites, schizonts and gametocytes. Examination of numerous sections from this material has not revealed malaria parasites in the fetal circulation. Large numbers of parasitized erythrocytes in all stages of maturation are present in the intervillous channels. There is an accompanying phagocytosis by macrophages identical with those present in the venous channels of other organs (Fig. 273). No evidence of blocking or interference with the maternal circulation by reticulo-endothelial cells (Graham) was present in our autopsy material or in infected placentas received from deliveries in the hospital.

From other data made available* some very interesting facts are obtained. In examinations of placental smears performed for a forty-two-month period on consecutive routine deliveries, 1603 smears were examined with parasites (*P. falciparum* 95 per cent) present in 199 instances or 12.4 per cent, and pigment alone present in nineteen instances. Heavy infections were recorded in eighty-eight cases and slight or minimal infections in 111 cases. The heavy placental infections always showed parasites in the peripheral blood smears while approximately 35 per cent of the slight or minimal positive cases showed no peripheral parasites in the admission blood smears or in blood smears taken at the time of delivery. In considering fetal mortality in this series it was the



Fig. 273. Placenta in *falciparum* malaria showing heavy localization of parasites and circulating phagocytic cells. (Partly depigmented, stained with toluidine blue phloxine, X475.)

impression of the workers (Drs. Walker and Wright) that children born alive from heavily infected placentas had as equal a survival chance as those from noninfected placentas, and that impression is verified by our observations in the hospitals of the Canal Zone and the Republic of Panama.

In the period covered in making the placental smear study, and in other records available from there (Blacklock and Gordon, 1925), in an extensive study in Kenya (Garnham, 1938), in Torpin's (1941) experience, and in the records of the Canal Zone, no instances of uncomplicated transplacental malaria infections are

* Personal communication from Dr. A. J. Walker making available the data collected by him and Dr. E. J. Wright at Freetown, Sierra Leone, West Africa, covering the period July 1, 1937 to December 31, 1940.

found. Transplacental malaria infection has been reported (Laffont, 1910; Tissier and Brumpt, 1913; Lavier, 1925; Wickramasuriya, 1935; Da Leas, 1936; Schwartz, 1934; Garcia, 1938; Gammie, 1944; and others quoted by Strong, 1944). The senior author reported a case showing a potential communication between the maternal and fetal circulation. In our opinion, and others (Ash and Spitz, 1945), transplacental transmission of malaria cannot occur through an otherwise normal placenta.

ACUTE *P. VIVAX* INFECTIONS

General. The amount of material available for the study of uncomplicated infections is obviously limited. Barker (1895) reported the autopsy findings in a patient with severe *vivax* infection dying from streptococcal septicemia. Ewing (1902) reported one case of presumably pure *vivax* infection, and refers to a case showing both *vivax* and *falciparum* parasites. Another reported fatality from *vivax* infection (Billings and Post, 1915) might well be considered an example of hemoglobinuria the result of, or accompanying, a heavy *vivax* infection terminally. Extensive studies on the histopathology of therapeutic *vivax* malaria in paresis (Brutsch, 1932a, 1932b) are available. In our local records there are four patients dying of other causes showing mild degrees of *vivax* parasitemia in routine autopsy smears, and through other sources we have been able to gather records and material from eleven patients with paresis dying during or immediately after the interruption of *vivax* therapy. From the material studied personally and the cases reported the following descriptions are obtained. No chronic *vivax* reports are available and the question of chronic malaria splenomegaly and hepatomegaly will be discussed later.

Spleen. Frequently large numbers of *P. vivax* parasites are present in the blood of these patients after death. Melanosis is relatively slight in the general viscera although present in the liver and spleen. The spleen usually is enlarged (400 to 850 gm.) and has a tense, smooth capsule. The consistency is firm with some hyperemia but rarely hemorrhages. The pulp is dark red-brown and even black depending upon the severity and duration of the infection. The malpighian corpuscles are small and usually indistinct while trabecular changes are not present. One fatality was the direct result

of a spontaneous splenic rupture in a patient with paresis. Cases of abscess formation in the spleen have been reported and were reviewed by Buhr and Brutsch (1928). Some cases were considered to follow trauma while others were considered primary infarctions from malaria becoming secondarily infected. The possibility of primary septic infarction, as occurs in non-malaria cases, cannot be excluded. Microscopically the spleen in this infection does not show the extreme hypereemic and degenerative changes present in *P. falciparum* cases. The hyperplastic response is typically the LYMPHOID-MACROPHAGE system with the formation of large numbers of lymphocytes, polyblasts and macrophages. The malpighian corpuscles are active, especially the secondary nodules, and many lymphocytes and macrophages are present in the transitional zones and pulp cords. Active phagocytosis of parasites and pigment is most intense in the pulp cords with but minor activity in the transitional zones while polyblasts and large macrophages are usually present in the sinuses. Areas of exhaustion of the malpighian follicles or depletion of the pulp cords similar to the changes in *falciparum* infections were not present. Another notable difference is the mild hyperplasia but never degeneration that is present in the littoral cells of the venous sinuses. Rare changes of a proliferative nature are seen in the walls of the trabecular veins. In the patient dying from hemorrhage following spontaneous rupture of the spleen the congestion was intense but thrombotic processes were not recognizable. These may have been destroyed at the time of rupture, but certainly, if present, they were not general.

Liver. The liver is usually enlarged (1600 to 2000 gm.) and the edges are rounded. Moderate congestion is present and there is a diffuse slate-gray pigmentation. Degeneration and necrosis was not noted grossly in any instance. In general the cellular reaction to this infection resembles that of *falciparum* infections with the notable exception that our cases did not show appreciable parenchymal degenerative changes, although Billings and Post (1915) reported their presence. There are large phagocytic cells containing parasites and pigment free in the liver veins and dilated sinusoids. The Kupffer cells are rounded up, protrude into the sinusoids and contain fine globular masses of pigment and parasites. Phagocytosis was not demonstrable by the fixed lining endothelial cells. Minor

deposits of hemosiderin are present in the parenchymal cells along with some fat deposits but malaria pigment was not identified. The periportal areas showed minor lymphocytic hyperplasia but no phagocytic reaction or erythropoiesis. Recent studies of liver function in therapeutic *vivax* malaria cases have shown a definite transitory impairment (Cook and Hoffbauer, 1946) which is probably physiologic in most instances.

Brain. In our material and that reviewed in the literature gross changes due to *vivax* malarial infection are not recorded, and in the microscopic material available for our study there were no recognizable changes attributable to the malaria infection. (Considerable care must be exercised in studying the paresis cases or erroneous interpretations can be made.) In one case (Billings and Post, 1915) intracapillary masses (thrombi) of swollen, infected erythrocytes, free parasites and pigment are described, and one area of an older parasite thrombus is mentioned. Tigrolysis of the Purkinje cells and early degenerative changes in the cortical cells are likewise mentioned. Caution must be used in evaluating the nerve degenerative changes in this case since they are relatively minor and autopsy was not performed until ten hours after death.

Braetsch (1932a, 1932b) in studying therapeutic *vivax* malaria in paresis agrees that there is formation of macrophages from the specific endothelium (reticular-endothelial cells) but feels that in many instances additional sources of macrophages are available and utilized. One source he considers to be certain capillary endothelial cells (brain), which are midway in development between the histiocytic (reticulo-endothelial) lining cells and the common endothelial cells of larger vessels. These transitional forms of capillary endothelium in the brain, spleen, mesentery and other organs are considered by him to show stimulative changes of basophilism, vacuolization and detachment from the lining at which time they become phagocytic and are indistinguishable from other macrophages derived from specific endothelium. The other source of macrophages he describes as resulting from migration of the connective tissue histiocytes and stimulation of the undifferentiated mesenchymal cells giving rise to small basophilic round cells of hemocytoblastic properties. This rather extensive derivation of macrophages is not in accordance

with general impressions concerning fixed endothelial-cell phagocytic properties or with the studies of Maximow (1932) and has been seriously doubted by others (Taliaferro and Mulligan, 1937). In similar therapeutic *vivax* infections, with which Braetsch was working, and in the more highly activated *falciparum* infections, we failed to find evidence of capillary endothelial phagocytic activity or of stimulative changes in the common endothelium suggestive of phagocytic cell production while phagocyte formation from tissue histiocytes was certainly not a prominent feature.

Bone Marrow. The bone marrow grossly is soft, red-brown and slightly pigmented. Smears and sections reveal a general hyperplasia with myeloblastic activity being predominant. Throughout the marrow there are numerous mononucleated phagocytic cells resembling large lymphocytes and polyblasts. They contain malaria pigment with a rare parasite and occasional degenerating erythrocytes.

Kidneys. The kidneys in our autopsied cases showed no changes attributable to the malaria infection although the possibility of degenerative changes occurring in these cases is postulated on clinical evidence (Giglioli, 1930). In two other cases (Ewing, 1902; Billings and Post, 1915), severe degenerative changes of hemorrhagic nephritis and extensive renal tubule damage are respectively described. In the latter instance there is presumptive evidence from the general autopsy report that hemoglobinuria might have been the underlying condition.

Adrenals. In the material studied, gross changes attributable to the malaria infection were not present. Microscopically Barker (1895) found extensive phagocytic activity in the adrenals of his case but our material showed no similar changes. The discrepancy may be the result of the streptococcal septicemia present in his case. There is a variable degree of congestion present in the adrenal glands and parasitized erythrocytes are present in the vessel lumina depending upon the degree of infection. Active phagocytosis of parasites by the endothelial cells of the adrenals was not seen. No notable changes were present in the cortical or medullary layers.

Lymph Nodes. Grossly only minimal changes of enlargement, edema, softening and pigmentation were present. Microscopically the lymph nodes show edema and hyperplasia of the corti-

cal follicles. The same progression from large lymphocytes to macrophages seen in the spleen was evident. The medullary cords were likewise hyperplastic, and many pigment-containing macrophages were present along with some phagocytized parasites. There was no evidence of degeneration or necrosis. This picture in our opinion is the direct result of malaria infection and is less extensive than that seen in acute *falciparum* infections.

P. MALARIAE INFECTIONS

No information is available regarding the changes in acute infections of this type, and the material available for study in chronic infections is limited and open to the same criticism mentioned later in presenting chronic infections, since other species of parasites are usually endemic and frequently predominate in most malarial regions. In our material there were no recognizable quartan infections, although we obtained some slide preparations from supposed chronic quartan cases for study. Giglioli (1932a) reported the autopsy findings in five cases of chronic quartan nephritis in British Guiana and from this and other reports (Neron and Annamalai, 1933; Ash and Spitz, 1945) the following description is obtained.

The spleen is grossly enlarged, firm and has a thickened capsule. Splenic adhesions were not mentioned. The cut surface was firm, dry, dark-red and showed malaria pigmentation. Microscopically three of the five cases showed extensive fibrosis of the malpighian follicles and atrophy of Billroth's cords with the formation of an angiomatous appearance similar to that found in chronic splenic infections of undetermined types. Pigment as small, dense agglomerations was present around the margins of the follicles and to a lesser extent in Billroth's cords. The material we had the opportunity of studying personally could not be distinguished from other cases of chronic malarial splenomegaly.

In the liver, enlargement was constant though not extreme. The parenchyma was firm, showed congestion and usually a diffuse pigmentation. This pigment was found in the increased periblobular connective tissue and Kupffer cells in the sections and one case showed lymphocytic mantles in the connective tissue. Lymph node enlargement with edema and malaria pigmentation was constant and especially prominent in

those found at the hilum of the spleen and liver. Microscopically pigment is described around the cortical lymphoid follicle margins and in the subcapsular region.

The kidneys (it must be remembered that Giglioli was studying nephritis in this group) showed varying stages of glomerulonephritis, acute, subacute, and chronic or contracted with the gross picture conforming to such stages in cases from other etiologic agents. The histologic changes varied with the gross stage present and consisted of simple degenerative, inflammatory, and proliferative intra- and extracapillary glomerular lesions. The interstitial tissue showed lymphocytic infiltration with extensive fibrosis and dilatation of the tubules. There was fatty degeneration present in some glomeruli while this was a constant finding in the tubular epithelium.

CHRONIC MALARIA INFECTIONS OF UNDETERMINED OR MIXED SPECIES ORIGIN

General. In our experience it is not unusual to encounter enlarged spleens and livers that are pigmented and are apparently the result of chronic malaria infections. We have not been able to determine that the enlargement was due to chronic infection or repeated infections with one species of parasites. Indeed, the results of many years of surveying the native villages (Clark, 1928) reveal that the same individual will, over a period of time, show infections from *P. falciparum*, *P. vivax* and less frequently *P. malariae* during that time. It is our opinion that the demonstration of one type parasite in the blood or tissue smears at these autopsies does not warrant ascribing the changes present to chronic or repeated infections by that type parasite, and we will describe chronic malaria infections as a general group.

Chronic Malaria Splenomegaly. It is generally agreed that these infections produce a tremendous enlargement (Marchiafava and Bignami, 1900; Thayer, 1897; Deunderick, 1909; Christophers, 1927; Photakis, 1929; Strong, 1944; Ash and Spitz, 1945) which may reach several thousand grams, although one report (Lambert and De Oliveira, 1929) states that approximately 13 per cent of a small unselected series of malaria pigmented spleens from routine autopsies were definitely not enlarged. One

spleen we had an opportunity to study was surgically removed from an eighteen-year-old Nicaraguan having a known *vivax* infection for three years. It weighed 2750 gm. and was 34 cm. in length, 19 cm. wide and 18 cm. thick. Spleens of this type are firm, even brittle to touch, and have thickened gray-white capsules showing marked fibrous adhesions to the adjacent viscera. They lose the normal splenic contour, becoming more like a bread loaf and are known in many malaria regions as "ague cakes" while in Panama they are referred to as "Colombian spleens" from the frequency with which they were encountered in citizens of that country in the early construction days. In our autopsy cases of acute *falciparum* malaria, spleens of this type were not present and this is confirmatory evidence of an acquired immunity.

A few cases of recognizable malaria pigmentation of the spleen were obtained at autopsy, and these were supplemented by instances of surgically removed chronically diseased spleens in which accidental rupture had occurred. In some instances prolonged search of many smears from these would reveal rare parasites, most frequently *falciparum* in type. The cut surface of the chronic malaria spleen shows a varying degree of pigment depending upon the interval since the last infection. Congestion was never as marked as in the acute cases although the basic appearance was quite red, disregarding pigment deposits. Malpighian corpuscles are usually easily visualized and there is a definite increase in the trabecular tissue.

Microscopic examination of these specimens reveals extensive thickening and even calcification in the capsule, with a general increase in the fibrous tissue throughout the trabeculae. The malpighian follicles are frequently enlarged but the cellular content did not show appreciable mitotic activity as has been described (Deckerick, 1909), while some malpighian follicles are hyalinized, apparently from previous acute episodes. There is frequently a marked change in the appearance of the red pulp consisting in the formation of an angiomatous-like structure. This appearance results from condensation, contraction and fibrosis of Billroth's pulp cords and a resultant increased diameter of the sinusoids and venous sinuses. The appearance is further accentuated by the frequent presence of large amounts of

pigment in the narrowed pulp cords and in the lymphatic spaces around the arterioles and veins. Pigment is also prominent at the margins of the enlarged malpighian follicles in cases of old infection. The formation of structures containing giant cells supported by connective tissue and arranged in a lacunar fashion to replace the destroyed Billroth's cords is described (Marchiasava and Bignami, 1900) but we were unable to find examples of giant cells in our material. It is problematical whether the end result of chronic malaria infection without pigmentation could be considered a diagnostic picture, but with the experience of having seen these cases and a clue from the pigment present the etiology is readily apparent.

Liver. The liver, in such cases, is recorded as being markedly enlarged (3000 to 4000 gm.), firm and having a dark brown-black pigmented appearance. Hypertrophy is less prominent and frequently the liver is exceptionally firm. The cut surface shows the hepatic lobules to be clearly outlined by peripheral pigment deposits and the parenchymatous central portions bulge slightly (Marchiasava and Bignami, 1900; Deckerick, 1909; Marchiasava, 1931). Amyloidosis has been described in the liver and is discussed later.

The question of true cirrhosis of the liver resulting from chronic or repeated malaria infection is discussed in great length by Marchiasava and Bignami (1900). Basing their opinion on extensive histologic studies made in the Roman hospitals, they arrived at the following conclusion "... that malaria does not produce cirrhosis in general, and ordinary cirrhosis in particular. Nor do we believe that the chronic hepatic enlargement of malaria can be considered as the first stage in the cirrhosis of Laennec, because we have to do with two entirely different histological processes." They however, felt that grave atrophies of the liver may be directly or indirectly derived from malaria infections by thrombosis of the portal vein, or simple or marantic atrophy in the aged debilitated group. Chronic malaria of undesignated type has recently been considered to be an important agent in the production of cirrhosis of the liver. In Greece, Phokas (1937) reports that in chronic malaria infections a reversal of the functional impairment, as demonstrated by tests, did not follow adequate antimalaria therapy and felt that these cases progressed to permanent hepatic cirrhosis.

Histologic study of the liver in chronic infection shows increased periportal connective tissue with collections of lymphocytes in these areas (Marchiafava and Bignami, 1900; Gaskell and Millar, 1920; Pringault, 1921). Pigment deposits vary in relation to the last attack and when present are most prominent at the periphery of the liver lobule contained within the Kupffer cells as clumps or masses. The parenchymal cells have been described as showing cloudy swelling, fatty degeneration and atrophy with a compensatory hyperplasia of other areas (Marchiafava and Bignami, 1900) along with lymphatic dilatation and angiomatous formations. Marchiafava and Bignami differentiate malaric hepatomegaly from hypertrophic cirrhosis by the presence of scanty perilobular connective tissue, marked capillary dilatation, and the absence of biliary-channel changes and icterus.

Bone Marrow. The marrow in the ends of the long bones is red, hyperplastic and moderately firm, while the midportion usually retains its yellow appearance (Marchiafava and Bignami, 1900; Craig, 1909; Deaderick, 1909). Loss of erythroblastic hyperplasia with the marrow persisting as yellow (normal in appearance) has been described (Seyfarth, 1926) but is thought by some (Marchiafava and Bignami, 1900) to be present only in those cases showing a progressive pernicious anemia.

Microscopically the latter type marrow shows hyperplasia of the medullary cells with the formation of giant red cells or megaloblasts. In the red hyperplastic marrow, rich vascular medullary tissue is present with large numbers of normoblasts, lymphoid and myeloid cells. Parasites, when present in these cases, are found in the erythrocytes and small veins and never in the lacunae or foci of erythrocyte formation.

Hemal Nodes. Only one report (Warthin, 1913) is found describing hemal lymph nodes in chronic malaric infections (type unspecified). The histologic picture was proliferation of the endothelial cells of the sinuses with partial obstruction of the sinuses by large, pale epithelioid cells and numbers of pigment-containing phagocytes. The lymphoid follicles were atrophic or markedly depleted. It was felt that since this type reaction had been previously studied in other nonprotozoal conditions associated with blood destruction, the phagocytic reaction was largely concerned with removing damaged or destroyed erythrocytes. The large,

pale epithelioid cells were probably hyperplastic reticular cells transforming into macrophages.

Kidneys and Amyloidosis. Amyloidosis in chronic malaria is discussed by Marchiafava and Bignami (1900). They feel that amyloid degeneration, though not a characteristic finding in malaria, may on occasion be present in the organs of persons having never been exposed to other infections. Such individuals gave histories of long series of *P. falciparum* infections followed by cachexia and the rapid development of nephritic symptoms with death in a few months. At autopsy, grave anemia, a general marantic state of the organs, chronic nephritis, and diffuse amyloid degeneration were present. Amyloid was deposited, in order of frequency and intensity, in the kidney involving the small vessels, glomeruli and tubules; in the intestine; around the malpighian follicle margins in the spleen, and rarely in the liver. Similar deposits of amyloid in the kidneys are reported by Lorendo (1920). Amyloid deposits were not recognized in any of the malaria cases studied in this series or in other material available.

BLACKWATER FEVER

General Discussion. The exact etiology or conditions that are necessary to produce an attack of blackwater fever remain unsolved. The mechanism involved is a sudden intravascular hemolysis with coexistent or resultant degenerative changes in the renal tubules and to a lesser extent in the liver. Severe anemia may be produced by a single severe attack, but always produced by multiple paroxysms and jaundice is a frequent occurrence.

Various theories have been advanced in an attempt to explain the onset of hemolysis. Most observers agree that the condition does not exist without previous or present malaria infection, that the rare cases reported as occurring in the absence of any history of malaria infection must be regarded as instances of paroxysmal hemoglobinuria (Strong, 1944), and that *falciparum* infections are most frequently associated with hemoglobinuria (Marchiafava and Bignami, 1900; Whipple, 1909, 1927; Deeks and James, 1911; Thomson, 1924; Marchiafava, 1931). A single fatal case report of blackwater fever due to *P. ovale* is available (Fairley, 1939), and in cases reported as being the result of infections other than *P. falciparum* (Stephens,

1915) considerable doubt has arisen in regard to the possible presence of a mixed infection (Thomson, 1924). This doubt is strengthened by reports of cases showing only tertian parasites during life with an extremely rare *falciparum* parasite being identified in multiple smears at autopsy (Ewing, 1902) and in our autopsy material.

Giglioli (1930, 1932c), however, believes that the condition can occur in all types of malaria infections, and depends upon the predominant species in the area under consideration. He concludes that *P. falciparum* is most frequently responsible for blackwater fever, that the incidence of the condition is higher in such endemic regions, and the mortality is greater. Concerning *P. vivax* infections he states "What *P. falciparum* can produce by acute mass action, *P. vivax* can cause by a slower, more continuous process," and on this basis he explains the comparative mildness of symptoms and the persistence of *P. vivax* parasites in the peripheral blood throughout the attack.

Variations within the species of malaria parasites have been proved by immunity studies, and it has been suggested that blackwater fever may be related to particular strains, or biologic, or toxic varieties of the parasites (Marchiafava and Bignami, 1900; Whipple, 1927; Nocht, 1931; James, 1931; James et al., 1932; Giglioli, 1932b; Foy and Kondi, 1936). Quartan malaria hemoglobinuria occurring as a "house or family" condition has been stressed. Based upon the finding of over one-third of sixty-one cases being limited to eight families, the obvious conclusion was that the members received the same parasite strain capable of producing blackwater fever (Giglioli, 1932b).

The occurrence of blackwater fever especially in malaria patients having received quinine therapy has been frequently noted (Marchiafava and Bignami, 1900; Whipple, 1909; Deeks and James, 1911; Lovelace, 1913; Thomson, 1924; Fairley and Murgatroyd, 1940). Evidence of quinine per se producing hemoglobinuria except in rare cases is lacking, while the condition is found in natives never having taken a single dose of quinine (Giglioli, 1930). Giglioli (1932c) refers to the work of Nocht and Kikuth (1929) in which they found that quinine, in association with otherwise insufficient hemolytic substances, would produce hemoglobinuria. He concludes that quinine may act, along with chilling, exhaustion, etc., to increase the incidence of

this condition in areas where hemolytic strains of malaria parasites are endemic.

From the preceding résumé it is obvious that the mechanism by which hemolysis is produced remains unexplained, and the exact mechanism by which the hemolysis produces extensive kidney and liver changes is likewise unknown. An excellent discussion of the theories of formation and chemical properties of blood and urinary hemoglobin pigments formed is given by Strong (1944) and is beyond the scope of this study.

Pathologic Anatomy. In the records and material studied by us there were eighty-two deaths from blackwater fever. The racial distribution and incidence is given in Table 140. In a general way the majority of the autopsy findings are similar to those in cases of malaria with the added factor of hemolysis. Severe anemia is always present and jaundice was recorded in eighty instances.

Kidneys. The kidneys are always enlarged, and in forty-four selected adult cases showing no pre-existing changes they averaged 403 gm. combined (range: maximum, 470 gm.; minimum, 220 gm.). The capsule is loose, strips easily and reveals a mottled deep red-brown or mottled mahogany-red cortex. In some cases there are discrete cortical hemorrhages. Jaundice may be so pronounced as to give a yellow-green overall coloration. The cortex is not markedly widened but there is considerable edema and widening of the medullary portion. The pyramids are frequently the site of an intense brown-black discoloration, which with the congestion and occasional hemorrhage in this region may produce a purple appearance. Otherwise, and most frequently, the medullary portion is the same icteric mahogany color of the cortex. The pelvis and calyces may show spotty deposits of hemoglobin pigment over the mucosal surface and this is also occasionally present in the ureters. The urinary bladder during a hemoglobinuric paroxysm will show bloody or brown-black urine with deposits of hemoglobin on the mucosal surface. A constant finding in the urine of our cases was albumin and casts.

In selected cases, not having evidence of pre-existing pathologic change, the glomeruli show varying degrees of albuminous material around the tufts and occasional scattered brown-yellow crystals of hemosiderin and hemoglobin. The tubules show extreme changes which appear to

be selective. The proximal convoluted tubules show edema with some desquamation of the striated epithelial cells but rarely necrosis. The distal convoluted tubules show edema, some desquamation and a rare deposit of hemosiderin, or they contain hemoglobin. In the loops of Henle and collecting tubules typical cases show large hemoglobin casts obstructing the lumina with necrosis of the lining epithelial cells. The lining cells generally show granular and hydropic changes with areas of complete desquamation and other areas of regeneration. The interstitial tissue is frequently edematous and foci of mononuclear cells are occasionally present. In a rare case ending in complete anuria the entire distal portion of many nephrons will appear occluded by the hemoglobin casts and the nonoccluded nephrons show severe degenerative changes. In other cases it may be impossible to find a hemoglobin cast in any nephron although the clinical and laboratory evidence of hemoglobinuria having existed is indisputable. In these cases degeneration of the tubular epithelium with interstitial edema and mononuclear cell infiltrations is usually quite prominent and enables a positive diagnosis.

In a comprehensive study (Foy et al., 1943) of hemoglobinemia and hemoglobinuria in cases of blackwater fever, incompatible transfusions and crush injuries, an attempt is made to explain the anuria and oliguria on similar renal abnormalities in these examples. It was felt that the azotemia was of extrarenal origin and not the result of renal tubular blockage. Dehydration, acidosis, and decreases in blood volume, renal circulation and glomerular filtration were incriminated as producing tubular degeneration first, with the cast formations resulting from these changes.

Spleen. In our experience the spleen is larger in cases of blackwater fever than in those of acute malaria, with average weights in adults as follows: Negro, 367 gm.; foreign white, 629 gm.; and mestizo, 715 gm. This is to be expected since the condition usually occurs in persons with a long history of malaria (Whipple, 1909, 1927) and the racial variation in size also holds in these cases. The general gross picture varies in direct correlation to the chronicity and intensity of the infection. In very recent attacks the pulp is soft, congested and may be somewhat pigmented, while in the chronic cases congestion is less noticeable but pigmentation

is quite prominent. Thus in fifty-four of the cases in which the color was noted forty spleens are described as being pigmented but only fourteen are noted as being red or congested. As a general rule the capsules are described as thickened and showing adhesions and the trabeculae and malpighian corpuscles are prominent.

In general, the histologic pattern in the spleen is one of varying congestion, with constant hyperplasia of the malpighian follicles, white and red pulp. The evidence of exhaustion and degeneration present in overwhelming *falciparum* infections is usually absent in these cases. Phagocytosis of parasites is variable depending upon the degree of parasitemia present but malaria pigment was found in all of our cases. The lack of degeneration and exhaustion of the phagocytic cells in this condition is consistent with the findings in the spleen of chronic or recurrent malaria cases not showing hemoglobinuria, and is probably intimately related to the general adaptability and acquired immunity thought to be present in these individuals.

Liver. The liver is enlarged (1800 to 2400 gm.) and is swollen. The capsule shows no changes but the parenchyma is usually mahogany-colored with linear pigment deposits and frequently shows icterus. The cut surface is *glistening* and has a varying dark- and light-yellow appearance with small foci of necrosis sometimes present. The intrahepatic bile ducts are prominent, dark-black and dilated. The contents of the gallbladder almost invariably are inspissated almost granular black-bile.

The liver microscopically shows marked degeneration with focal areas of true necrosis. There are large deposits of hemosiderin in the parenchymal cells and the biliary channels are completely filled with green-black bile pigment. The Kupffer cells contain varying degrees of malaria pigment and large amounts of bilirubin. Phagocytosis of erythrocytes, with or without parasites, is a common finding in these cases.

Brain. The brain usually shows edema with flattening of the convolutions and an appreciable increase in the general consistency. In our cases there usually was ample evidence of recent or active *falciparum* infection, and jaundice was recorded in the brain in sixty-four cases, malarial pigmentation in eleven cases, and parasites or pigment were noted in the brain smears in twenty-four cases. In two cases

there were punctiform hemorrhages and abundant parasites were noted in these capillaries.

Bone Marrow. The bone marrow was usually recorded as hyperplastic and rather congested in appearance, and malaria pigmentation was recorded as present in fifty of the cases.

In our material the bone marrow smears and sections showed essentially the same changes as in chronic malaria infections, consisting of active hematopoiesis. Phagocytosis was variable in regard to malaria pigment but hemosiderin was constant.

Lymph Nodes. Considerable emphasis has been placed upon the gross and microscopic changes present in these structures (Whipple, 1909, 1927), and our material included the cases studied by him. The lymph nodes, especially in the portal region, are enlarged, soft and edematous and show an icteric coloring. Microscopically the subcapsular sinuses show large phagocytes (polyblasts) containing nuclear debris and erythrocytes. Malaria pigment is present in the medullary cords and the follicles are swollen and show some necrosis.

Other Viscera. Changes in the other viscera were not striking. In general, findings in patients showing severe parasitemia at or immediately before death corresponded to the findings in those with *falciparum* malaria, with no variations attributable to the hemoglobinuria.

TECHNICAL SECTION

The first requisite is that clean glass slides be used, and next that the tissue smears be crushed or drawn very thin. The thinness is important for visual clarity and for prompt drying and fixation. When completely dry the smears are further fixed in absolute ethyl or methyl alcohol for five minutes, and then washed in a gentle stream of water for several minutes.

Staining of smears is best performed in the same manner that thick blood drops are handled. We have found the routine use of Giemsa's stain preferable, and the results warrant the time expended in preparing the stain locally. However, satisfactory results can generally be obtained by using any of the so-called Romanowsky-type stains. Our experiences in using the rapid eosin-methylene blue stains recently proposed for blood films have not been satisfactory. For staining with Giemsa we prefer a Coplin dish, and use one drop of the stain per cubic centimeter of diluent. In most instances, ordi-

nary tap water is preferable to distilled water which frequently will be slightly acid. Staining time varies with each individual lot of stain but will ordinarily require from thirty to forty-five minutes for a well-balanced result. Startling demonstrations of the parasites and phagocytic cells may be obtained by treating the slides, following fixation, in a solution of 60 per cent alcohol saturated with picric acid for thirty minutes to one hour and then washing well before staining in the usual manner. The treatment removes the pigment of the parasites and other extraneous hemoglobin material and does not interfere with the cytologic picture.

Smears, regardless of preparation and staining, have a great tendency to fade especially in tropical climates. We have eliminated this by immersing the stained slides in xylol for a few minutes followed by toluol for several minutes and then coverslipping them using a neutral synthetic resin. Smears prepared in this fashion have not shown evidence of fading after being kept on top of an incubator at temperatures of 35°C. for six months, while ordinary slides will show fading when kept in the normal manner this length of time.

Fixation of Tissue. In preventing excessive hemolysis, prompt fixation of tissue is an important factor in preserving details for later study. Autopsies should be performed as soon as possible after death, and if possible the body should be refrigerated in the interval. One important factor is the immediate fixation of thin (1.0 cm.), small pieces of tissue upon removal of the organ and not at the completion of the autopsy. The choice of fixing solution is largely one of personal preference from experience. For most of our routine work we prefer a formal-alcohol solution prepared as follows: Ten parts of a 40 per cent formaldehyde solution are mixed with ninety parts of a 95 per cent ethyl alcohol solution, and the solution kept over marble chips to neutralize the formic acid that is released. This solution both fixes and partially dehydrates, and so is prone to accentuate shrinkage in the tissue. To obviate this change we routinely place similar portions of tissue in a solution made by substituting normal saline for the 95 per cent ethyl alcohol in the formula above. By gradual dehydration of this material, starting with 60 per cent alcohol, there is no shrinkage and the hemoglobin of the erythrocytes is not removed. Extremely satisfactory sections are prepared from tissue

treated in this manner. We have not found the use of Bouin's or Zenker's fluid staining agents to be of particular value for the study of malaria.

Section Preparation. In our studies we have routinely used the paraffin technic with sections being cut at 5 microns, or less when possible. The technical difficulty of preparing celloidin sections in humid climates constitutes a factor unjustified by the results, since equally good results are obtained by gradual dehydration following formol-saline fixation.

Section Staining. In general, human malaria parasites are very difficult to stain properly in tissue. It is frequently quite easy to recognize malaria pigment in the erythrocytes, and this has been used to corroborate the diagnosis. Giemsa's stain, in our experience with tissues, has given indifferent results and cannot be relied upon for routine use. Similar inconstant staining results in human malaria were obtained using phloxine-methylene blue or azure 11-cosin, alum hematoxylin methods and this led to the development of a toluidine blue, phloxine, orange G staining method which is reported in detail elsewhere (Tomlinson and Grocott, 1944). The illustrations presented in this section are from tissues stained by this method which is briefly outlined for convenience here.

1. Routine paraffin sections are carried through alcohols, xylols and alcohols into tap water.
2. They are stained for five minutes in a solution of phloxine (C.I. 4778) 1.0 gm., water 98.0 cc., and calcium chloride solution (1 per cent) 1.0 cc.
3. They are washed in tap water one minute.
4. They are stained for forty-five seconds to one minute, while being agitated, in toluidine blue solution (toluidine blue [C.I. 4923] 1.0 gm., lithium carbonate 0.5 gm., water q.s. 75.0 cc.) and allowed to ripen for three days. The volume is restored to 75.0 cc. and 20.0 cc. glycerin and 5.0 cc. dehydrated ethyl alcohol are added.
5. The slides are then rinsed in tap water for one minute, drained and placed in acetone for two minutes.
6. They are placed in acetic acid solution (glacial acetic acid 0.2 cc. in 100.0 cc. distilled water) until the clouds of blue stop streaming from the section (about one minute), and drained.
7. They are then placed in two changes of acetone for two minutes each.
8. They are transferred to differentiating solution for one to two minutes. The differentiating solution is to be made fresh each day by mixing 75.0 cc. of the stock rosin solution, 25.0 cc. of absolute ethyl alcohol and 1.0 cc. of the stock orange G solution. (Stock rosin solution is 5.0 gm. of yellow colorless rosin made up to 100.0 cc. with pure acetone. Stock orange G

solution is 1 per cent orange G [C.I. 427] in distilled water.)

9. Following differentiation the slides are placed in two changes of acetone for three minutes each.
10. Then dehydration is completed by changes of acetone-xylol and pure xylol, and the preparations are mounted in a neutral synthetic resin. Balsam will produce fading in time unless absolutely neutral.

The sections can be depigmented before staining by placing them in a 5 per cent ammonium sulfide solution (U.S.P.) for one to three hours or by using a saturated solution of picric acid in 60 per cent ethyl alcohol for approximately one hour. The latter solution is less harmful to the tissues in that they do not become released from the slides.

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