VASCULAR PSEUDOHEMOPHILIA ASSOCIATED WITH CEROID PIGMENTOPHAGIA IN ALBINOS

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In 1959 Heřmanský and Pudlák [1] and others [2,3] reported a combination of albinism, hemorrhagic diathesis with prolongation of the bleeding time and the occurrence of atypical pigmentophages in the bone marrow not resembling any previously described cells of this type. The syndrome was considered to be on a congenital basis. The hemorrhagic condition was characterized as "pseudohemophilia," probably of vascular origin, since no changes were found in plasma coagulation factors or in platelets. One of the first reported patients died. It seems desirable to record the necropsy observations and to recapitulate the clinical data in this case.

CASE HISTORY

A 38-year-old children's nurse was hospitalized repeatedly because of a hemorrhagic condition present since childhood. This had been manifested by easy bruising and prolonged bleeding after small injuries; she had also noted occasional menorrhagia, intermittent hematuria, bleeding after tooth extraction and repeated epistaxis. Despite what appeared to be a practically healed pulmonary tubercular lesion, massive hemoptysis highlighted the terminal clinical course. The hemorrhages had recurred at irregular intervals from 1958 to 1962. During this period chronic hypochromic normocytic anemia was a prominent feature and a low plasma iron level (21 gm. per cent) was observed. Anemia was improved to a certain extent only with transfusions. On the day before death there was bleeding from injection sites, severe headache with nausea and vomiting and rapidly developing unconsciousness. A diagnosis of cerebral hemorrhage was made.

Physical examination showed incomplete albinism with oculogenic nystagmus. Laboratory examinations exhibited only a slightly prolonged bleeding time (maximal, 13 minutes, Duke's method). A host of other hemocoagulation tests and the biochemical investigations of the blood were normal.

In bone marrow smears a striking finding was the occurrence of atypical pigmentophages. Phagocytosis was apparent in some reticuloendothelial cells which contained digested neutrophil or normoblast nuclei and fragments of erythrocytes (Fig. 1). In addition, fine to coarse granules of an abnormal brown lipopigment were also noted. In Giemsa stained smears the pigment appeared to be markedly basophilic. In most of the large pigmentophages the deposit practically filled the entire cytoplasm (Fig. 2), and it was not possible to distinguish the remains of phagocytosed cells.

The necropsy examination was carried out 15 hours after death. A large subdural hematoma was found over the convexity of the left cere-

Accepted for publication, January 29, 1964.

bral hemisphere. The lungs were edematous, weighing 620 and 500 gm. respectively. In the middle lobe of the right lung a 1 cm. inspissated caseating tuberculous lesion was encountered. The bronchus to the right upper lobe exhibited fine anthracotic tattooing, and a contiguous lymph node was very anthracotic. No fistula was distinguished in the adjacent pigmented scar although this had been observed by bronchoscopy 3 years earlier.

The liver measured 29 by 23 by 12 cm. and weighed 1,900 gm. On section it was a brownish gray. The spleen weighed 350 gm. and on section had a brownish violet color. The kidneys were joined at the lower poles to form a horseshoe kidney which weighed 290 gm. The cortical parenchyma had a striking dark-brown color; the medulla was pale red (Fig. 3). There were no striking changes in other tissues, all of which were examined histologically.

Histologic examination was directed toward the characteristics of the pigmentation. The structure of the skin, iris and retina was quite normal but of albino type. The *nucleus niger* in the brain was pigmented normally, and pigment was also normally deposited in ganglion cells.

In the liver, Kupffer cells were enlarged and for the most part ballooned (Fig. 4) by granules of brown pigment, some attaining the size of the nucleus. Pigmentophages appeared in the porto-biliary spaces as well, singly and in groups. The pigment content of Kupffer and other endothelial cells of the sinusoids varied greatly from several granules to large quantities. Many pigmentophages were damaged and disrupted, as indicated by the accumulation of small mononuclear leukocytes and granulocytes in their neighborhood. Focally, nodules formed about the cell remnants with leukocytes "stuck" to them.

In the spleen, pigmentophages appeared singly, in clumps (Fig. 5) or filled distended sinuses and evidently arose from the sinus endothelium. Similar cells and clusters were also evident in the white pulp, mainly in reaction centers (Fig. 6) and in vessel walls, notably veins (Fig. 7). They formed clumps on the intima and immediately beneath it and caused considerable widening of this layer. In their vicinity connective tissue fibers of vessels were frayed and the vessel wall as a whole was much enlarged as though shaken up.

The lymph nodes contained pigment in cells of the reticular stroma and occasionally in the sinus endothelium. Pigmentophages usually appeared singly and only rarely formed small clusters.

The kidneys contained considerable amounts of brown pigment in the tubular apparatus and in the interstitial tissue; the proximal convoluted tubules were most severely affected (Fig. 8). Smaller amounts of pigment were present in the lower portions of the nephron and single grains

were encountered also in the epithelium of collecting tubules. The pigment was always intracytoplasmic in the tubular epithelium, mainly beneath the nucleus. In the interstitial tissue the pigment was engulfed by phagocytes.

In the colon only the interstitial tissue of the mucous membrane was pigmented (Fig. 9). The granules were very coarse, deep brown and lay in large pigmentophages near capillaries in the vicinity of the mucosal lymphoid tissue. A section of appendix removed in 1960 showed identical features.

The bone marrow, as a whole, was normal, but pigment-laden histiocytes appeared in it singly and in clumps.

The brain exhibited focal rarefactions of perivascular glial fibers. The perivascular spaces were generally widened, and the adventitial tissue was thickened in the vicinity of large vessels and also about the capillaries. The adventitia of leptomeningeal vessels was also thickened. Here, clusters of pigmentophages of various sizes were present everywhere, obscuring distinctions between arterioles and veins. There were often accumulations of pigmentophages directly upon the endothelium (Fig. 10) or filling Virchow's spaces. Cerebral capillaries lacking a well defined mesenchymal sheath were also surrounded by large pigmentophages, which by their nuclear structure were thought to have been derived from oligodendroglia. Glial pigmentophages were detected in glial fibrillary network, also, near to or at a distance from vessels (Fig. 11) but without clear relation to them. The changes affected the entire central nervous system to essentially the same degree; only in the basal ganglia were the alterations somewhat more marked.

The alterations in the lungs were quite complex. The interstitial tissue exhibited slight hemosiderosis and anthracosis, independent of the posttuberculous scarring. Other pigmentophages resembling those encountered elsewhere were also present although in small numbers.

The adrenals contained occasional pigmentophages in the sinuses of the cortex and medulla. Other organs exhibited no significant alterations.

The anatomic findings established the cause of death as cerebral compression from a fresh left subdural hematoma.

The entire RE system appeared active and cells were filled with brown pigment, least striking in the lungs and brain, and most prominent in the kidneys and large intestine. The pigment was apparently distributed by the bloodstream and penetrated the intima of small vessels, particularly in the postcapillary portion. Vessel structure was altered by the accumulation of pigmentophages in different layers of the vessels, splitting and fraying the connective tissue fibers in the vessel wall. These vascular alterations and the disruption of ballooned endothelial cells were prob-

ably the cause of repeated bleeding, terminally manifested by subdural hematoma.

TABLE I

HISTOCHEMISTRY OF PIGMENT

Reaction	+	Remarks
Ammoniacal silver stain (Masson)	Result	Varied reaction, more feeble than with melanin
Schmorl method	+	Varied reaction
Ziehl-Neelsen	+	Varied reaction
Chrome alum hematoxylin	+	Varied reaction
Nile blue	+	Blue to bluish green
Hueck's method	+	Original bluish green color persists
Nile blue (Lillie)	+	Original bluish green color persists;
Nile blue (Lillie) + acetone	−	with extraction of dye, original color of pigment is evident
Fluorescence in ultraviolet light	+	Yellow
Bleaching in peracetic acid	−	Up to 18 hr.; after 24 hr. bleaching is more evident
Ferrous iron technique (Lillie)	−	Control melanin, +
Peracetic acid-Schiff	+	On frozen and paraffin sections (varying degree)
Periodic acid-Schiff (PAS)	+	
Diastase digestion, PAS	+	No digestion
Acetylation, PAS	−	Focally slightly ±
Toluidine blue	+	Blue to bluish green, depending on pH
Trypsin digestion-toluidine blue	+	No enhancement of staining
Azure A in 30% ethanol	+	Bluish green to green
Sulfation-azure A	+++	(Metachromasia also)
Methylation-azure	− (+)	Marked weakening compared to control
Deamination-azure	++	Enhancement, compared to control
Alcian blue	weakly +	Particularly on surface of granules and in young granules
Methylation-alcian blue	−	
Trypsin-alcian blue	weakly +	Like control
Acidified potassium permanganate aldehyde fuchsin	+	
Coupled tetrazonium reaction	+	Weaker than erythrocytes
SH groups (DDD)	weakly +	
Tryptophan (Adams)	weakly +	Especially young granules
Sudan black B	+	In frozen and paraffin sections
Oil red O	+	In frozen and paraffin sections; disappears after extraction of the stained section with acetone
Luxol fast blue	++ to +	Older granules unstained
Perls's method after 10% H_2O_2	− to very weakly +	Between granules of pigment in liver pigmentophages and in cells of renal tubules
Alkaline phosphatase	−	
Nonspecific esterase		
Using α-naphthyl acetate	+	
Using naphthyl AS acetate	+	Stronger in Kupffer cells than in cells of renal tubules
Using brom-indoxyl acetate	+	
DPN diaphorase	−	Singly among granules, +

A histochemical analysis of the pigment was made in part upon paraffin sections after fixation with formol. Other studies (Table I) were carried out on frozen sections, after 18 hours' fixation in cooled Baker's fluid. Evaluation showed that the deposit belonged among the lipopigments. Its nature was complex and it was composed of carbohydrate (other than glycogen), lipid and protein components. It appeared to be very similar to, if not identical with ceroid. This conclusion was in accord with its location in the RE system and its similarity to lipofuscin.[4-6]

We assumed that the carbohydrate of lysosomes[5,6] contributed considerably to the PAS positivity. The activity of lysosomal enzymes and PAS staining intensity increase with phagocytosis. Acid phosphatase and nonspecific esterase were particularly active in the pigmentophages; this might serve as further evidence that lysosomes took part in the production or storage of the pigment.

Positive staining with Luxol fast blue indicated the presence of choline-containing lipids; unsaturated fatty acids were suggested by the peracetic acid-Schiff reaction. The latter, however, also reflects the existence of disulphide bonds. Thus it was not possible to determine the extent of the reaction attributable to unsaturated fatty acid content or to the protein component.

Diazonium salts (in controls of the enzyme reactions where the incubation media did not contain a substrate) produced a color change in the pigment. This could have been due to coupling of diazonium salts with amino acids (tyrosine-tryptophan, histidine) but might well, however, be due to a catecholamine content. This probability could not be excluded, especially since Siebert and colleagues[9] assumed that the pigment observed in the Scholz sclerosis type of leukoencephalopathy was a sulphone complex of catecholamine and cerebroside. In our case a similar possibility existed.

The basophilia of the pigment, a reflection of the presence of $-COO'$ and $-PO_4'''$ groups, increased after de-amination and was suppressed by esterification.

One should emphasize that the pigment exhibited stages of development, since the reactions were not all of the same intensity. The strongest staining reactions appeared in renal epithelium.

Discussion

So far as we know, the anatomic observations in this case of pseudohemophilia are the first reported of this condition; thus there is no material for comparison. We were able, however, to make a comparison with necropsy tissue preparations from a male albino who exhibited no bleeding phenomena. There was no obvious pigment deposit in the RE sys-

tem, but the kidneys exhibited dark brown pigmentation. The microscopic features in the renal tubules were identical with those in our case. The albino patient died of miliary tuberculosis with bloodstream dissemination and a chronic post-rheumatic heart condition. A comparison of the two cases showed that despite the severe renal pigmentation in both, the RE system and the intima of blood vessels were not necessarily affected in albinos, nor were all evidences of pigmentophages invariably present. This has certainly been the case in the clinical experience of others.[10]

The relationship between pigmentophages in albinos and macrophages containing the "blue pigment"[11–13] (basophilia with Giemsa stain) found in bone marrow, spleen, liver and lymph nodes in individuals without albinism, remains unclear. Our own observations in similar cases have suggested that the substance probably represents the phagocytosed by-product of the incomplete decomposition of corpuscular blood elements.

The relationship between our case and the Chédiak Higashi syndrome[14–18] is not certain. In the latter condition oxyphilic intracytoplasmic inclusions are evident in leukocytes. In our case leukocytes engulfed pigmented granules of disintegrating Kupffer cells secondarily. In the peripheral blood, however, leukocyte inclusions were not encountered. Moreover, in Chédiak Higashi syndrome pigmentophages are not observed in the bone marrow. There is no doubt, however, that in different cases the two syndromes may be closely related and they are probably both manifestations of a wider disturbance of lipid metabolism occurring in albinos. Landing's case[19] exhibiting susceptibility to infection and a hemorrhagic diathesis was probably a *"forme fruste"* of this category; no comment concerning the presence of albinism was made. Incomplete data also prevent comparison with "black kidneys" in cattle[20] and with several forms of "lipidosis" characterized by the accumulation of some lipids (sphingomyelin,[21] cephalin[22]) in the RE system and ceroid as well. The work of Oppenheimer,[23] who reported *thesaurismosis,* should also be mentioned.

The cause of the hemorrhagic diathesis in our case must be sought in blood vessel walls where, as a matter of fact, the alterations were very obscure. Many capillary endothelial cells were transformed into pigmentophages and occasionally became ballooned and disrupted. It appears to us that the existence of these two lesions in small vessels causes them to be highly susceptible to minor forms of trauma which may therefore result in rupture of these vessels. In small capillaries, swollen endothelial pigmentophages may occupy the entire circumference of the lumen, interfering with the proper maintenance of hemostasis. The tourniquet test was never positive, however.

In an attempt to explain the phagocytosis of ceroid and to determine the source of the pigment the following features seemed pertinent: (1) Unsaturated fatty acids are precursors to ceroid.[4,5] (2) The conversion of RE cells into pigmentophages was focal, suggesting phagocytosis of a corpuscular substance and no thesauration of a soluble material from the blood because thesauration is usually a general process. (3) Perls's stain gave a weakly positive reaction in the pigmentophages and pigmented renal tubular epithelium after unmasking of iron by treatment with 10 per cent H_2O_2. (4) Transitional forms between phagocytosed red cell fragments and pigment granules were observed, mainly in Kupffer cells and pigmentophages of bone marrow (Fig. 1). (5) The pattern of pigment deposition resembled that of erythrocyte fragments in paroxysmal nocturnal hemoglobinuria.[24] So far as we know, erythrocytes are the only elements to which all of the aforementioned features apply. This is further supported by experiments [5,25] in which ceroid was derived from erythrocytes.

The disturbance may be the result of alterations of the phosphatides in erythrocytes themselves, perhaps the consequence of interference by certain defective substances with the metabolism of phosphatides [26–28] in albinos. It may, on the other hand, be due to "indigestibility" of phosphatides in the face of an inadequate enzymatic equipment in the RE system of the albino. Which of these two eventualities pertains is not clear at the present time.

Summary

A 38-year-old albino woman suffered from vascular pseudohemophilia. Many ceroid-laden pigmentophages were encountered in blood vessel walls, particularly in the endothelium of capillaries and veins. In foci with large numbers of pigmentophages in vessels, there was a tendency for ballooned pigmentophages to rupture. The occurrence of bleeding in these patients may reflect the accidental rupture of such vessels.

The patient reported died as the result of a subdural hematoma. Pigmentophages were observed in the entire RE system and in the central nervous system glia, particularly with perivascular location. Maximal accumulation of pigment was found in the cytoplasm of proximal renal tubular epithelium. Pigmentation in the large intestine resembled that encountered in melanosis coli.

It is suspected that the ceroid deposit reflects a defect in phosphatide metabolism. This may result from the effect of catecholamine products derived from the abnormal melanocytes in albinism, or may constitute an "indigestible" substance resulting from defective enzymatic oxidation of lipids. The likely source of the ceroid is thought to be erythrocyte phosphatides.

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[*Illustrations follow*]

Legends for Figures

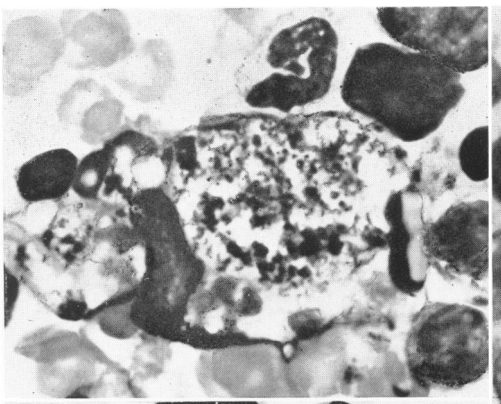
Fig. 1. Pigmentophages in a bone marrow smear contain ceroid granules and the remains of phagocytosed neutrophils and erythrocytes. Giemsa stain. × 1,200.

Fig. 2. Overloaded pigmentophages in bone marrow. Giemsa stain. × 1,200.

Fig. 3. The horseshoe kidney exhibits a deeply pigmented cortex.

Fig. 4. Hepatic Kupffer cells have been converted into pigmentophages. Ziehl-Neelsen stain. × 300.

Fig. 5. A clump of pigmentophages appears in the red pulp of the spleen. Hematoxylin and eosin stain. × 400.



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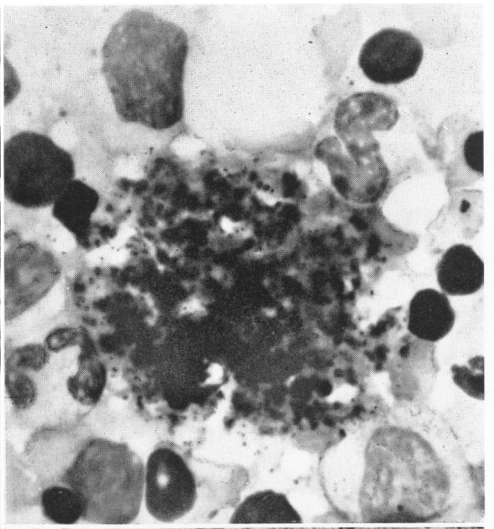

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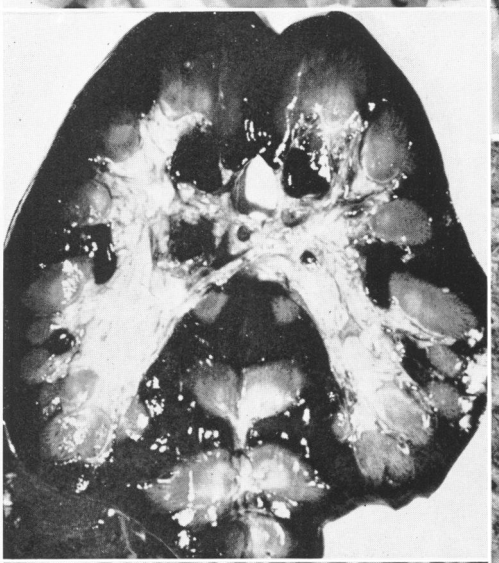

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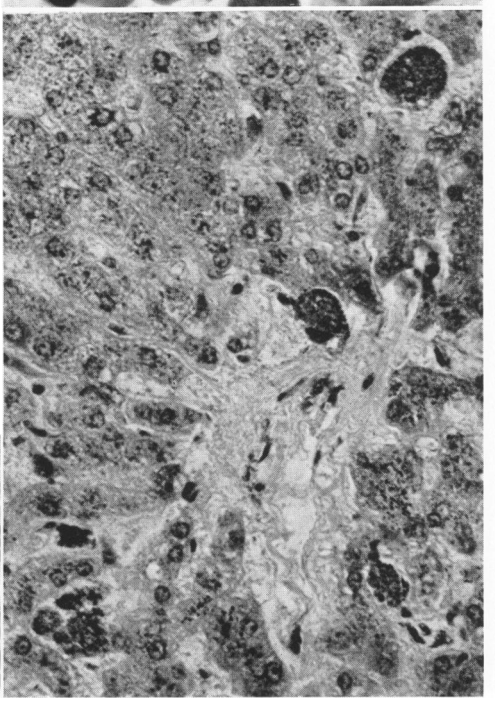

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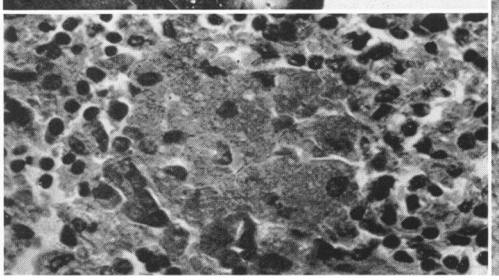

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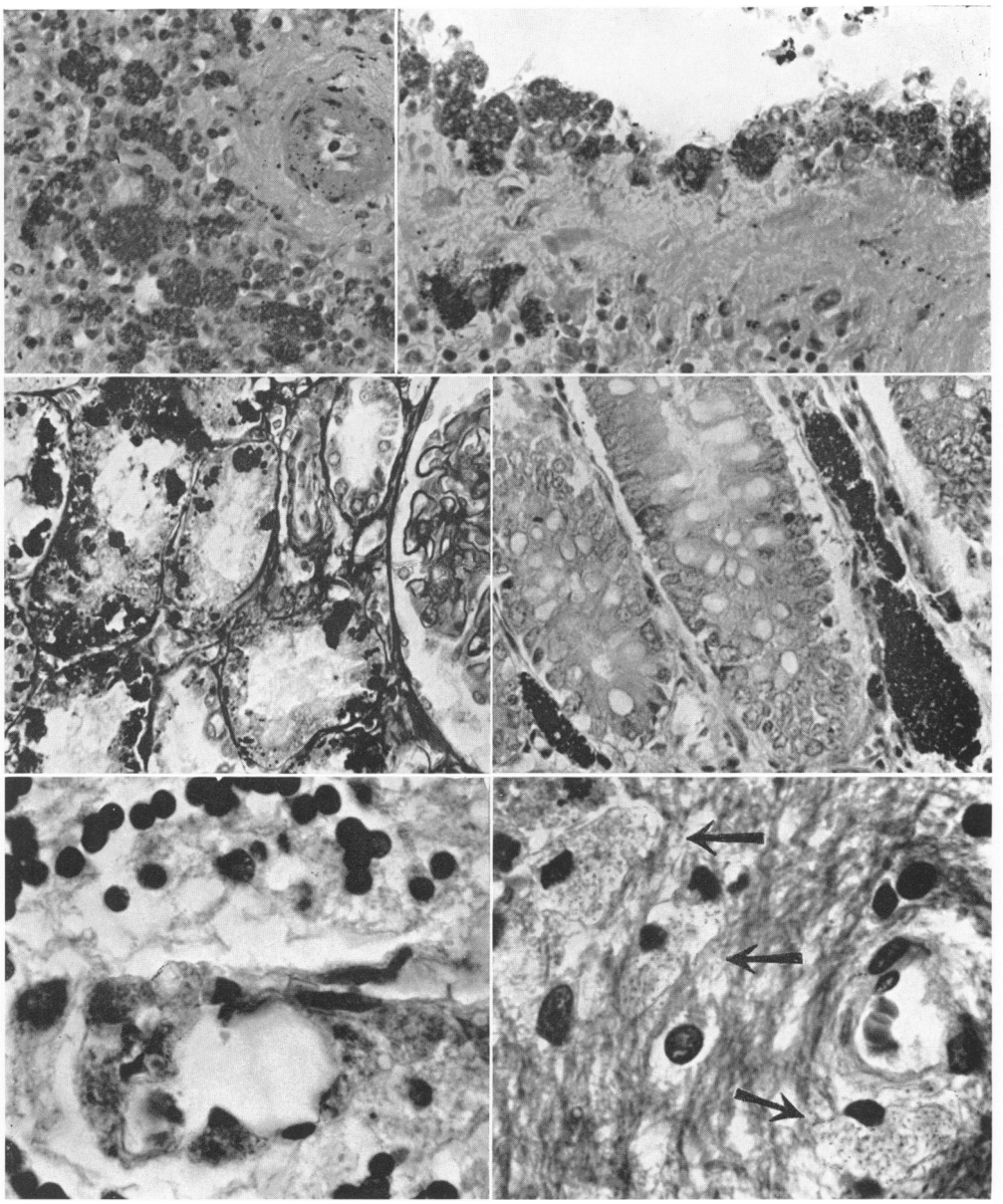

FIG. 6. Pigmentophages in the white pulp of the spleen. Ziehl-Neelsen stain. × 400.

FIG. 7. Pigmentophages may be seen in the intima of splenic veins. Ziehl-Neelsen stain. × 400.

FIG. 8. Pigment granules are evident in renal tubule epithelium, particularly in its proximal segments. Ammoniacal silver stain (Masson). × 400.

FIG. 9. Pigmentophages are encountered in the mucosa of the large intestine. Ziehl-Neelsen stain. × 400.

FIG. 10. Endothelial pigmentophages are observed in the cerebellum. Hematoxylin and eosin stain. × 800.

FIG. 11. Glial pigmentophages in the brain. Hematoxylin and eosin stain. × 800.