AN EXPERIMENTAL STUDY OF THE ORGANIZATION OF THE RETICULOENDOTHELIAL SYSTEM IN THE RED PULP OF THE SPLEEN*

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The red pulp of the spleen is a concentration of reticular cells organized as sinal blood vessels and as cellular cords separating these vessels. In sinuses reticular cells are more or less flattened to endothelial form and, with the sinal reticulum, which is here a basement membrane, comprise the wall of the vessels.‡ The cords have traditionally been considered an extravascular tissue. Here, the reticular cells which are thought to have the same developmental capacities as those lining the sinuses, have been described as stellate in form (Maximow & Bloom, 1957). Most of the phagocytosis and a great deal of cytopoiesis in the red pulp occur in the cords. In histological sections of the spleen, blood is usually present in the cords; and it is a question whether it is there by artefact or pumped in directly through open-ended terminal arterial branches and through apertures in the sinal walls. These alternatives represent, respectively, the concepts of the 'closed' and 'open' circulation of the red pulp of the spleen.

In a study of the structure of splenic sinuses in man and the rat (Weiss, 1957), the present author suggested that collapsed splenic sinuses constitute splenic cords. His conclusion was based primarily upon the observation that cords appeared lined by an endothelium identical in appearance to that lining the sinuses. Moreover, the endothelium of the cords lay base to base with the endothelium of the sinuses separated only by a common basement membrane, the sinal reticulum. As a result the sequence of structure in the red pulp was: sinal lumen, endothelium, reticulum, endothelium, lumen, endothelium, reticulum, endothelium, lumen.... The vascular character of cords was masked because their endothelium, highly irregular and often folded upon itself, would virtually obliterate the lumen.

These observations have been extended to the red pulp of the rabbit's spleen; and it has been concluded that, in rabbits, as in rats, and human being, the cords do constitute blood vessels. The endothelium of the cords obtruded upon the lumen mainly because it was phagocytic and cytopoietic. As a result, cord tissue appeared as extravascular spaces made up of irregularly stellate cells, and not as blood vessels. The purpose of this paper is to present these additional observations and the results

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‡ I have chosen the term *reticulum* among several accepted synonyms to refer to the delicate argyrophilic (or PAS positive) fibres described first by Mall (1896). Recently this reticulum, where it occurs beneath an endothelium, has been recognized as a basement membrane (Bennett, 1958). Hence I have referred to the latticed reticulum of the sinuses as the basement membrane. I have chosen the term *reticular cell* to refer to the relatively undifferentiated connective tissue cells associated with the reticulum, rather than the equally acceptable term *reticulum cell*.

of experiments designed to test them. In these experiments the red pulp was dilated in an attempt to convert cord tissue to frank vessels. Conversely, phagocytosis and cytopoiesis were forced upon the red pulp in an attempt to induce the endothelium of frank sinuses to lose its vascular character and make the vessels appear as extravascular cords.

The methods and observations will be presented in three parts. These are: (I) the red pulp of normal rabbits, rats, and human beings; (II) the red pulp of the spleen of rats and rabbits dilated by perfusion, by passive congestion alone, and by passive congestion in eserinized animals treated with acetylcholine or in animals treated with sodium nitrite; (III) the red pulp of rabbits and rats in which phagocytosis and cytopoiesis were induced by the haemolytic effects of phenylhydrazine.

Observations were made, primarily, with the light microscope upon splenic tissue fixed in buffered osmium tetroxide, embedded in methacrylate, sectioned at a thickness of approximately 2 μ in a Porter-Blum microtome, and stained with the periodic acid-Schiff reagents. This routine afforded well-fixed preparations of optimal thinness in which the basement membrane was selectively stained. The blocks of tissue, however, could not practically be more than a few mm. in greatest dimension. Conventional paraffin-embedded blocks of much larger size were also prepared. From the tissue embedded in methacrylate a limited number of electron micrographs were made.

I. THE RED PULP OF NORMAL RABBITS AND RATS

Materials and Methods

Young adult New Zealand rabbits weighing 2–3 kg., or young adult albino rats of the Wistar strain weighing 175–300 g., were placed under surgical anaesthesia with subcutaneous pentobarbital (60 mg./kg.). The spleen was removed, and portions were fixed in osmium tetroxide, dehydrated, and embedded in methacrylate according to the routine previously used (Weiss, 1957). In most cases no attempt was made to prevent blood from escaping the organ. In a few animals the vascular pedicle was clamped and the spleen fixed whole without loss of blood. After fixation the superficial few millimetres of the spleen were cut into small blocks, dehydrated and embedded by the usual routine.

Sections about 2 μ in thickness were cut from these blocks in a Porter-Blum microtome and mounted upon coverslips. The methacrylate was removed by xylene, and the tissue was stained with the periodic acid-Schiff-haematoxylin technique (PAS-H) as previously reported (Weiss, 1957). For paraffin embedding ethanol, formalin and glacial acetic acid (90:5:5) was used as a fixative.

Observations were made upon the spleens of 8 rabbits, 10 rats and 6 human beings.

Observations

Patent sinuses were prominent in the red pulp of the rabbit spleen. They were branching vessels of large but varying diameter consisting only of an endothelium and a fenestrated basement membrane. The cord tissue was slender, in many places but 5–10 cells thick (Pl. 2, figs. 2, 3; Pls. 3, 4).

By and large, the endothelium of the patent sinuses in an adult rabbit spleen was

low and seldom showed evidence of phagocytosis or cytopoiesis. The lumen of the sinuses contained blood, often with a higher concentration of granulocytes than peripheral blood. Moreover, in places, loaded-down macrophages were free in their lumen. The basal surface of the endothelium displayed varyingly prominent, but on the whole usually slight, longitudinal ribbing (Pl. 2, fig. 3). The basement membrane, stained with the periodic acid-Schiff method, was a prominent structure, and on surface view had the form of a grid (Pl. 2, fig. 2). Infrequently, the substance of the basement membrane was not confined to its grid pattern but appeared diffused into the lumen of sinuses or into cords.

The cords in the rabbit were slender structures of varied composition. In simplest form they consisted of facing cell surfaces separated by a slit or lumen of variable width. These cells appeared to form a cordal endothelium, and they lay upon the obverse side of the basement membrane of the adjacent sinus with the result that the basement membrane was common to sinus and cord. The cordal endothelium could be identical to the sinal endothelium. More often the cordal cells had more voluminous cytoplasm containing phagocytized material. Occasionally, the basement membrane was absent beneath pronouncedly phagocytic cordal cells, even where the endothelium of the adjacent sinus showed no phagocytosis . In occasional places in the cords, nests of plasma cells or megakaryocytes lay in the lumen or actually appeared to replace the endothelial cells. In the latter case, again, the basement membrane could be absent. Varying numbers of blood cells were present in the cords.

Similar observations were made in the white rat. Here, however, the cords were much broader than the sinuses. They were filled with blood and contained plasma cells, megakaryocytes, and occasional immature blood cells. Their endothelium was evidently actively phagocytic.

In human spleen, the transverse strands of basement membrane were thicker and more nearly at right angles to the longitudinal strands, and the sinuses were more branched than in rat or rabbit spleen. The cords resembled those of rabbit more than of rat (Pl. 2, figs. 4, 5).

Arterial terminations. The arterial endings were different from sinuses and capillaries in having a wall often two cell layers thick and in having a greater concentration of endothelial cells. In perhaps 20-25 % of cases in the rabbit a direct union of arterial ending and frank sinus was observed. Otherwise, the arterial ending opened into the lumen of what would be recognized as a cord. In the latter case, the squamous endothelium of the arterial ending was continuous with the irregular surfaces of the reticular cells of the cords. Often the arterial terminations bifurcated shortly before connecting with sinuses or cords (see Pl. 4).

II. DILATATION OF THE RED PULP OF THE SPLEEN IN RATS AND RABBITS

Materials and Methods

In rats of the Wistar strain and in New Zealand white rabbits, the spleen was engorged by ligating the splenic vein in animals placed under surgical anaesthesia with ether or subcutaneous pentobarbital. In some rats sodium nitrite was administered

intravenously a few minutes before ligation (Farris & Griffith, 1949). Others were eserinized and then given 20 mg./kg. acetylcholine iodide intraperitoneally. The latter group shed bloody tears (Tashiro, Smith, Badger & Kezur, 1940). About 30 sec. to 1 min. after splenic ligation, the whole vascular pedicle was clamped and tied; and then the entire engorged organ was removed and fixed whole as suggested by Dr William Bloom. For paraffin embedding, Zenker's acetic acid mixture or ethanol, formalin, and glacial acetic acid (in the proportions 90:5:5) was used as a fixative. The latter was of practical use because it haemolysed the erythrocytes which otherwise were stained and obscured structures of greater interest. Tissue was fixed in osmium tetroxide and embedded in methacrylate as in Part I.

Serial sections were cut from the paraffin blocks and stained with periodic acid-Schiff reagents and haematoxylin. Occasionally, Bodian's silver stain (1936) or that recommended by Snook (1944) was used. From the methacrylate blocks thick sections were cut and stained with PAS-H.

The spleens of 5 rats and 3 rabbits were studied.

Observations (see Pl. 5)

The entire spleen was engorged with blood, although the degree of engorgement varied from place to place. In some areas cords or sinuses were compressed between widely dilated sinuses whose endothelium was markedly flattened. In most places what had presumably been cord tissue was filled with blood. In normal rat spleen this tissue often differed from frank sinuses in that reticular cells in an endothelial position were often filled with phagocytized material and were stellate rather than flat. Their voluminous cytoplasm protruded so deeply into the lumen that in many sections the cells appeared free. In many areas, however, there was a succession of congested vessels, undisguised by phagocytic endothelium (Pl. 5, fig. 16).

The movement of basement membrane from its netlike pattern into the sinuses and cords was marked in the engorged spleens (Pl. 5, fig. 16).

III. INDUCED PHAGOCYTOSIS AND CYTOPOIESIS

Materials and Methods

Normal rabbits and rats and argyric rats were treated with phenylhydrazine. The rabbits were young adult New Zealand animals weighing 2–3 kg. Each animal was given 48 mg. phenylhydrazine in saline, subcutaneously, each day for 3 days. Approximately 20 % of the animals failed to survive the week following the start of the experiment.

Normal Wistar rats and Wistar rats made argyric by the administration of $AgNO_3$ in drinking water (Gatz, 1949) for 12–24 months were given phenylhydrazine subcutaneously daily for 4 days as described by Smith & Stohlman (1934).

Observations were made upon the spleens of 21 rabbits, 10 normal rats, 10 argyric rats treated with phenylhydrazine, and 3 rats treated with $AgNO_3$ alone.

Observations

Rabbit

Following the start of phenylhydrazine more or less normal relationships of sinuses and cords persisted a few days. Then the spleen became congested. After this the sinal endothelium became phagocytic, the basement membrane disappeared, and the sinuses became indistinguishable from cords whose reticular cells had become more markedly phagocytic. Cords merged with sinuses; and as phagocytosis became more pronounced extensive stretches of red pulp were filled with huge, closely packed, swollen phagocytes, with no sensible vessels visible. The structure of the red pulp was massively deranged.

On the fourth day of the experiment, when the circulating haemoglobin was typically about 5 g. % and the reticulocytes 25–30 %, the frank sinuses and cord tissue were widely congested, even more uniformly and more pronouncedly than could commonly be attained by splenic vein ligation and arterial dilatation (Pl. 6, figs. 24, 25). The basement membrane was well stained. In the next day or two, congestion remained extreme and phagocytosis was only of moderate degree. But the endothelial cells and the basement membrane of sinuses were altered; huge, odd-shaped, vividly stained phagocytes lay upon the basement membranes and free in the masses of erythrocytes, and the continuity of the basement membrane was disrupted in many places (Pl. 6, fig. 27; pl. 7, figs. 28–32). On the sixth or seventh day phagocytosis was more pronounced in both the cordal and sinal endothelium, with the changes in the cords in advance of those in the sinuses. Two events associated with phagocytosis—the loss of material from the basement membrane, and of the squamous character of the endothelium in sinuses—made it impossible to distinguish cord from sinus in many places in the spleen.

At this stage huge mononuclear cells loaded down with broken red cells were crowded in the lumen of many sinuses and cords. In about 10 days the lumina of those frank sinuses which remained were relatively free of these phagocytes, while the now expanded cords were packed with them. The topography of the red pulp had become considerably changed from normal. The tissue was still congested, cords were greatly expanded, and fewer sinuses were present, having been absorbed into the broadened cords. Many of the sinuses had become irregular blood-filled clefts unlined by endothelium (which had presumably been swept away after becoming phagocytic) and unmarked by basement membrane, in a mass of huge, closely packed, swollen phagocytes.

It must be emphasized that the broadening of the cords was not due to expansion of existing cords with compression and apparent disappearance of sinuses lying between the cords. Nor was it due to stuffing of a sinus with sequestered phagocytes so that it appeared cordal. Rather, the sinuses lost their vascular character because their endothelium became phagocytic and their basement membrane disappeared. They then became indistinguishable from cord tissue and merged with pre-existing cords.

The larger sinuses, similar to those free of blood in Pl. 1, fig. 1, which became confluent with splenic veins, were unresponsive to phenylhydrazine. The changes described above were characteristic of smaller sinuses.

The arterial terminations in phenylhydrazine-treated rabbits were noteworthy for clusters of macrophages at the orifice of the ending. Not uncommonly, the endothelium of one portion of a sinal wall near which an artery ended had become phagocytic and irregular while the rest of the wall stayed flat (Pl. 8, fig. 37).

Rat

Both congestion and phagocytosis in the spleen followed a course of phenylhydrazine in the normal rat. As with the rabbit, phagocytosis occurred first in the cords but, possibly because the cords in rats are more capacious, seldom extended to the sinal endothelium even with very large doses of phenylhydrazine.

In silver nitrate-treated animals, on the other hand, several of the effects similar to those obtained with phenylhydrazine resulted from the administration of silver alone (Pl. 1, fig. 1). Many macrophages were filled, presumably with silver, and the reticulum, particularly in and somewhat beyond the marginal zone, was blackened. The cords had become more cellular, and in some silver-treated animals the number of recognizable sinuses was actually reduced. In these animals the conversion of what has presumably been frank sinal tissue to cords after phenylhydrazine was more nearly complete than in any other group (see Pl. 10). Typically, several high-power fields in succession consisted almost entirely of closely packed macrophages and blood without any sensible sinuses. The non-sinal tissue had the appearance of a meshwork of phagocytic reticular cells and blood. In addition, the endothelium of several sinuses had become phagocytic and, only by means of some persistent basement membrane and its luminal contour, could its sinal character be inferred.

Few infarcted areas were present either in rabbits or rats treated with phenylhydrazine.

DISCUSSION

The major interpretations of the structure of the vascular bed of the red pulp have been set within the conception of sinal vessels separated by extravascular cords (Björkman, 1947). The presence of blood in these cords has required the theory that blood may normally flow extravascularly or the belief that histological techniques have resulted in the breaking of sinal walls with artefactual extravasation of blood. The interpretation, presented here, of cords as blood vessels having a responsive endothelium whose activities may mask their vascular nature emphasizes the reactivity and potency of the reticuloendothelial system and harmonizes heretofore conflicting observations without requiring the singular theory of extravascular flow or the conclusion that histological technique produces major artefact.

The technique of fixation in osmium tetroxide, embedding in methacrylate, sectioning at 2 μ or less, and staining with periodic acid-Schiff and haematoxylin affords sections of optimal thinness, excellently fixed with little shrinkage—in many respects superior even to celloidin embedding—in which the basement membrane is selectively stained and the cells well rendered. The blocks may also be sectioned for electron microscopy.

Interestingly, by his injection methods, Lewis (1957) concluded that sinuses are but minor modifications of pulp spaces. Indeed a close relationship of cord to sinus

was established by Mollier (1911) and Koboth (1939). In the rabbit cordal vessels appear in the present work to be sinuses, but masked by the activity of their endothelium. In the rat and human spleens, however, cordal vessels appear somewhat different from simply masked sinuses. In the rat the cords are broader. In the human being the cords are slender, and the cordal reticulum may form a mantle about the sinus as described by Koboth (1939). Conversely, Snook (1950) has emphasized species variations in the structure of sinuses.

While reticular cells must be recognized as relatively undifferentiated cells, there is in these experiments no evidence that they readily undergo differentiation into erythroblasts in the spleen. In fact, only a few would appear to undergo this transformation, perhaps more a reflexion of the power of the bone marrow than on the limits of the spleen. Most erythroblasts in the spleen have been sequestered as indicated by their presence in the spleen only when they circulate in the blood, and the absence in the spleen of any forms less mature than in the blood.

Even the capacity of reticular cells to become phagocytic would appear to vary from place to place in the red pulp. The larger sinuses and those in union with veins, though made of endothelium morphologically similar to that of tributary sinuses, remained unmistakably patent vessels. The endothelium of the tributaries was phagocytic. Whether the endothelium of the larger vessels was intrinsically less responsive or whether the blood was cleared of damaged red blood cells before reaching their endothelium is not known. Biozzi, Halpern, Benacerraf & Stiffel (1957) demonstrated that the reticuloendothelial system may be stimulated to enhanced phagocytosis by preliminary phagocytosis of injected particles, and suggested that this may be due to new reticular cells produced by mitosis. I have noted greatly increased frequencies of mitosis among the reticular cells in sites where phagocytosis is marked.

Under experimental conditions, as in the undisturbed spleen, phagocytosis occurs preferentially in the cords. Several factors may enhance phagocytosis in an already phagocytic endothelium: viz. the surface of a phagocytic endothelium is irregular and would presumably slow down blood flow; more arterial terminations end in cordal vessels than sinal vessels; the possible enhancement of phagocytosis by new cells has been noted in the previous paragraph; in places the basement membrane is washed into the paludal spaces of the cords where it coats red cells and may facilitate their ingestion.

Motulsky, Casserd, Giblett, Broun & Finch (1958) have reported that blood, tagged with radioactive ^{51}Cr, normally flows rapidly through the spleen as through other organs. Therefore, in all likelihood it normally travels through frank vessels. For although the proportion of arterial terminations communicating with frank vessels is small, these vessels would appear able to convey most of the blood flow in the spleen because the number of fine vessels normally open in any capillary bed at any time is small and flow through frank vessels is efficient. Moreover, the observation that Kupfer cells remove most carbon and other particulate material given intravenously in moderate dosage, although the phagocytic prowess of the spleen is demonstrably greater (Biozzi *et al.* 1957), support the more direct measures (Motulsky *et al.* 1958) that the volume of the blood flow through the normal spleen is relatively low.

In abnormal spleens (for example those reacting to methylcellulose, in haemolytic anaemias, and presumably in the haemolytic anaemia after phenylhydrazine) the volume of blood is increased and blood flow is greatly slowed down (Motulsky *et al.* 1958). Here, in all probability, most of the blood flows through cordal vessels whose structure, as discussed above, prevents rapid flow and gives these vessels the deceptive appearance of extravascular tissue.

Lying between the endothelium of vessels made only of endothelium in a tissue made entirely of vessels, the basement membrane represents a continuous branching complex of fenestrated surfaces by which the disposition of sinuses and cords can be inferred. The fenestrations in the basement membrane are large, leaving a slender netted structure made up of regularly spaced strands. The opening in the basement membrane would appear to allow the endothelium of one vessel to present on the lumen of its neighbouring vessel, if that vessel's endothelium had been lost as a detached mononuclear blood cell, megakaryocyte or loosened phagocyte. Lymphatic capillaries in the rat diaphragm lack a basement membrane, and the abluminal surface of the endothelium may become actively engaged in pinocytosis, and transport great quantities of thorotrast or other materials into the lymphatic capillary from the extravascular spaces (Fraley & Weiss, 1959). Perhaps the absence of basement membrane from what is actually the greater part of the abluminal surface of the sinal endothelium permits such active transport across the sinal wall. The red pulp of the spleen, indeed, as in the case with other tissues provided with sinal blood vessels, contains no lymphatic vessels; their functions here may be subserved by the sinuses. With regard to the control of blood flow, the remarkable rectilinearity of the basement membrane suggests that this structure may constitute a grid or set of coordinates upon which the endothelium is aligned. Since the basement membrane may disappear as its overlying endothelium becomes phagocytic, self-regulation of blood flow may be inherent in the structure of the sinuses. For as the endothelium becomes phagocytic and moves from the wall and the basement membrane disappears in this system of vessels sharing common walls, a new entrance to the adjoining vessel is blocked, if at all, only by a motile unsupported endothelial cell. Since the basement membrane may be secreted by the endothelium and depends upon the endothelium for maintenance, all of the factors required for recasting their vessels appear to lie in the vessels themselves. In the process sinal vessels by reason of their responsive endothelium lose their frank vascular appearance and become cords of tissue having no resemblance to blood vessels and every characteristic of extravascular tissue except the presence of blood. Under appropriate conditions the transformed endothelium may be swept out (to be sequestered in other cordal tissue or liver or lung in the case of macrophages, or to the circulation in the case of lymphocytes or monocytes), the wall re-covered with new endothelium and the basement membrane restored. Perhaps the layout of sinuses and cords is worked and reworked in a manner similar to the Haversian systems of bone, responsive in this case to the requirements for phagocytosis, antibody production, platelet production, metaplastic cytopoiesis, sequestration of blood cells, and blood flow.

SUMMARY

Reticular cells in the red pulp of the spleen are organized as sinuses, and as cords separating the sinuses.

Splenic cords are here interpreted as unstable, responsive blood vessels whose vascular nature may be disguised by collapse of the vessels with apposition of the irregular endothelium and virtual disappearance of the lumen, and by phagocytosis and cytopoiesis in the endothelium with resultant loss in endothelial form. The endothelium of the sinuses is on the reverse surface of the basement membrane of the cordal endothelium. Thus the repeating sequence of structure in the red pulp is: endothelium, lumen, endothelium, basement membrane, endothelium, lumen....

Arterial terminations may empty in sinal or cordal vessels but many more empty into cords.

Cordal vessels may be experimentally dilated by congestion of the spleen. Their vascular nature is then revealed because collapsed vessels are opened and phagocytic endothelium tends to be pressed back into endothelial position. Sinuses may be converted to cordal tissue by forcing phagocytosis upon their endothelium by the haemolytic effects of phenylhydrazine.

The basement membrane appears to depend upon the endothelium for maintenance, and under certain conditions it may disappear or its substance appear to wash into the lumen of sinus or cord.

Thus the red pulp is almost entirely a vascular space whose vessels are unmistakably vascular, or masked as they carry out the functions of the spleen; viz. phagocytosis, cytopoiesis, sequestration of cells. As a result of the disposition of reticular cells into a responsive system consisting only of endothelium and basement membrane control of these reticuloendothelial functions would appear vested in the reticuloendothelial system itself.

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EXPLANATION OF PLATES

PLATE 1. Argyric rat spleen

A nodule of white pulp is surrounded by red pulp. The reticulum, including basement membranes, is vitally stained with silver and also stained with the PAS reaction. Macrophages, concentrated in the marginal zone and present elsewhere, are also doubly stained. Note the tendency to circumferential disposition off the sinuses in the red pulp just outside the marginal zone. From these vessels, branches spring in a radial direction. The netted appearance of the sinal basement membrane is rendered prominent by the silver. At the bottom of the photograph, a pulp vein enters a trabecular vein.

The tissue was fixed in alcohol-formalin acetic acid (90:5:5), embedded in paraffin, stained with PAS, and photographed × 300.

PLATE 2. Normal rabbit and human red pulp

Figs. 2, 3. Rabbit red pulp. These photographs are of the same field at different levels of focus. A sinus crosses the field from left to right. On the right side of each photograph, the section grazes the sinus's wall; and on the left it passes into its lumen. In fig. 2, on the right, the fenestrated basement membrane, selectively stained with the PAS technique, is in focus. In fig. 3 the basal endothelial surface is in focus. Note the endothelial nuclei and the more or less longitudinal orientation of the endothelial cytoplasm.

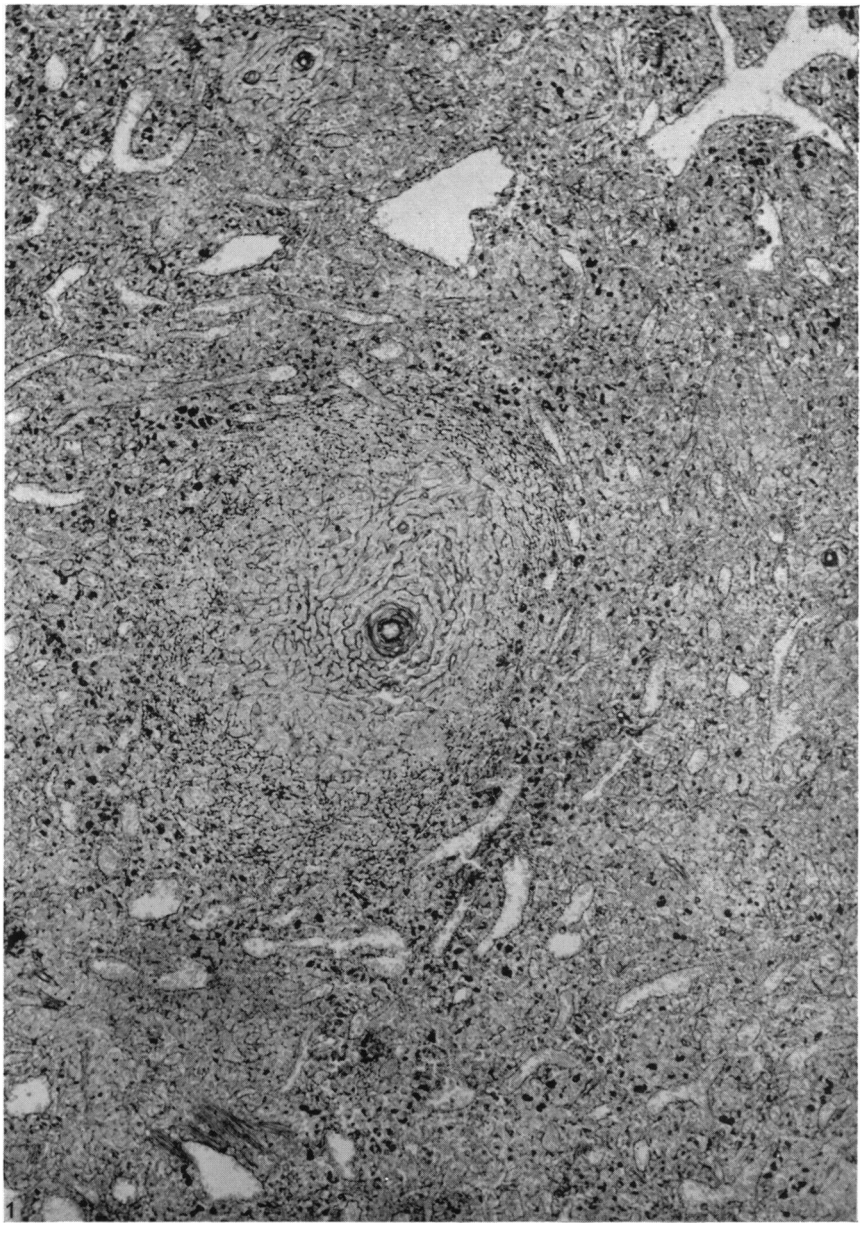
This vessel branches from its lower margin. Part of the basement membrane (see particularly fig. 2) and endothelial nuclei of this branch may be seen in figs. 2 and 3. Note a portion of lumen of this branch at the arrow in fig. 3. Its endothelium is phagocytic, as may be seen to advantage at the arrows in fig. 2.

At the upper right corner of each photograph is a portion of a frank sinus. Between it and the vessel crossing the field from left to right is cord tissue whose vascular nature is masked.

Figs. 4, 5. Human spleen. The sinuses branch and communicate, outlined by the selectively stained basement membrane. Fine basal endothelial striations, more highly developed than in the rabbit, are marked by arrows in transverse sections of a sinus or where the section cuts the basal surface of an endothelium. In fig. 5 the portions of sinus, each labelled *a*, are continuous with one another.

The deeply stained spherical cells in the sinal lumen are neutrophils rich in glycogen.

The spleen was fixed in osmium tetroxide, embedded in methacrylate, sectioned at about 2 μ, stained with periodic acid-Schiff and haematoxylin, and photographed × 1100.



WEISS—RED PULP OF SPLEEN

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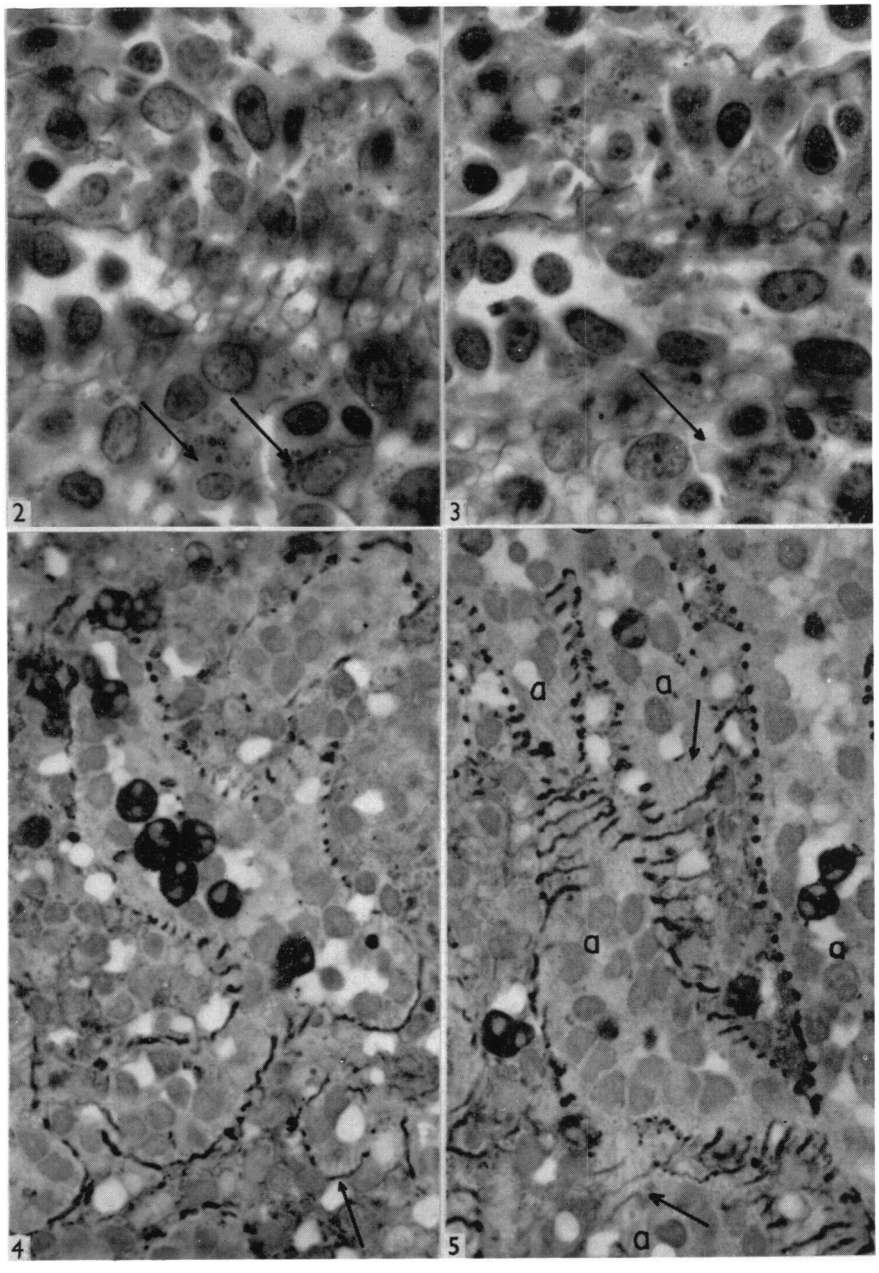
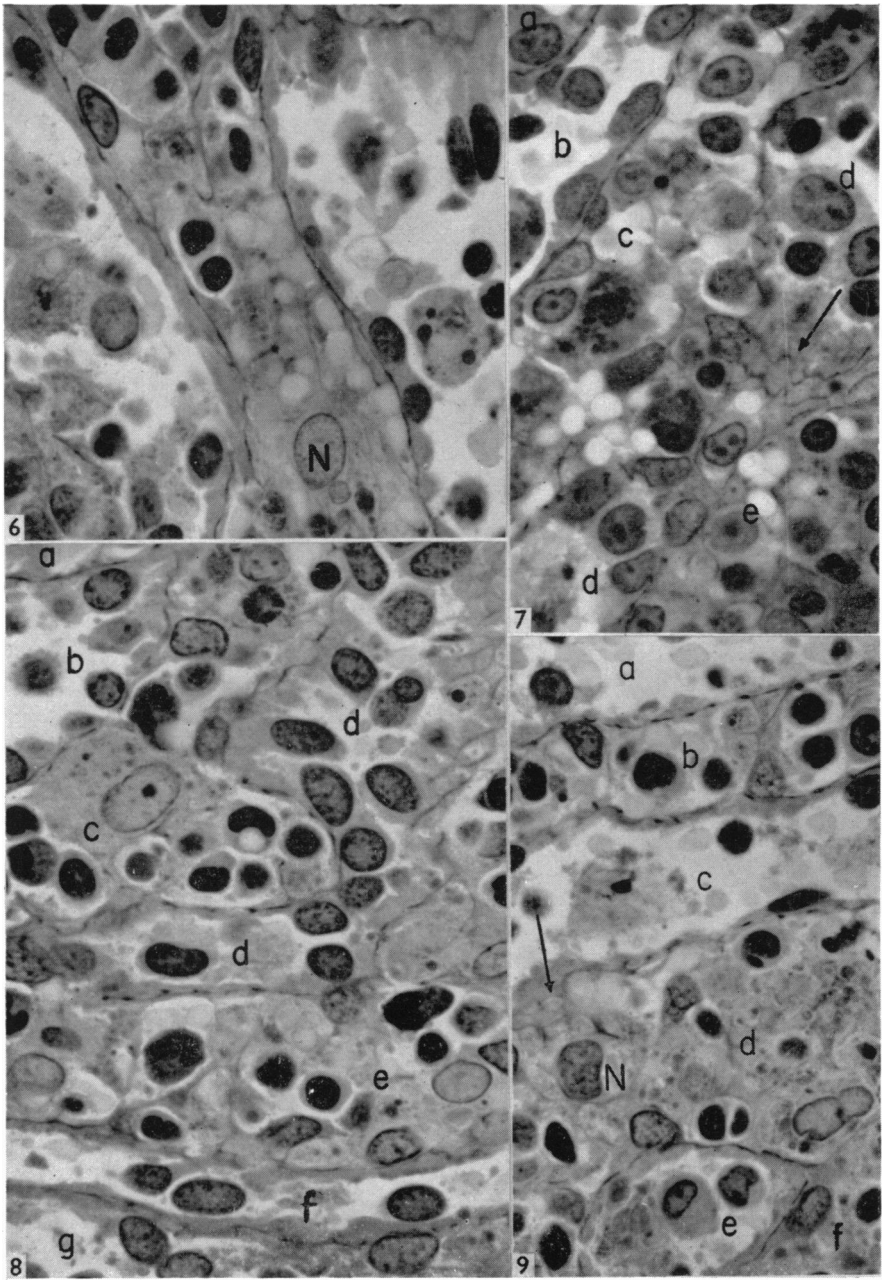


WEISS—Red pulp of spleen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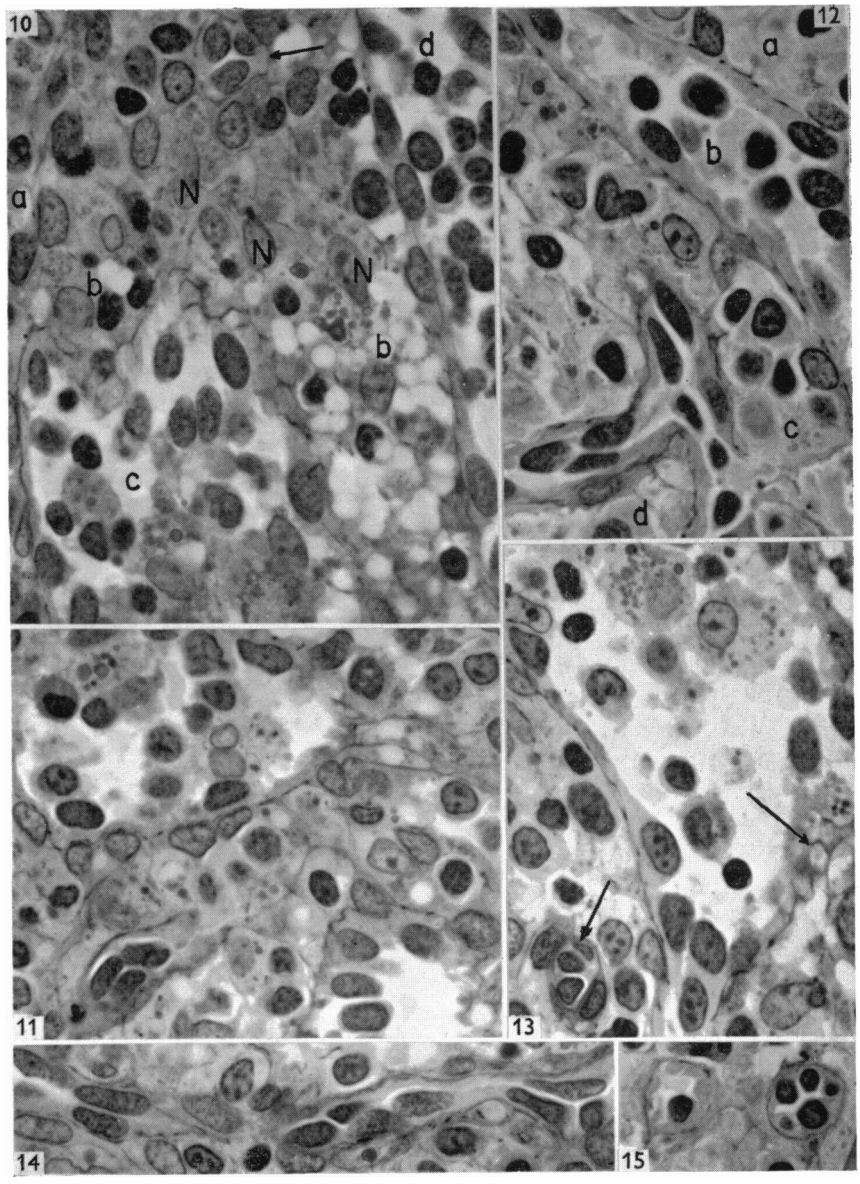
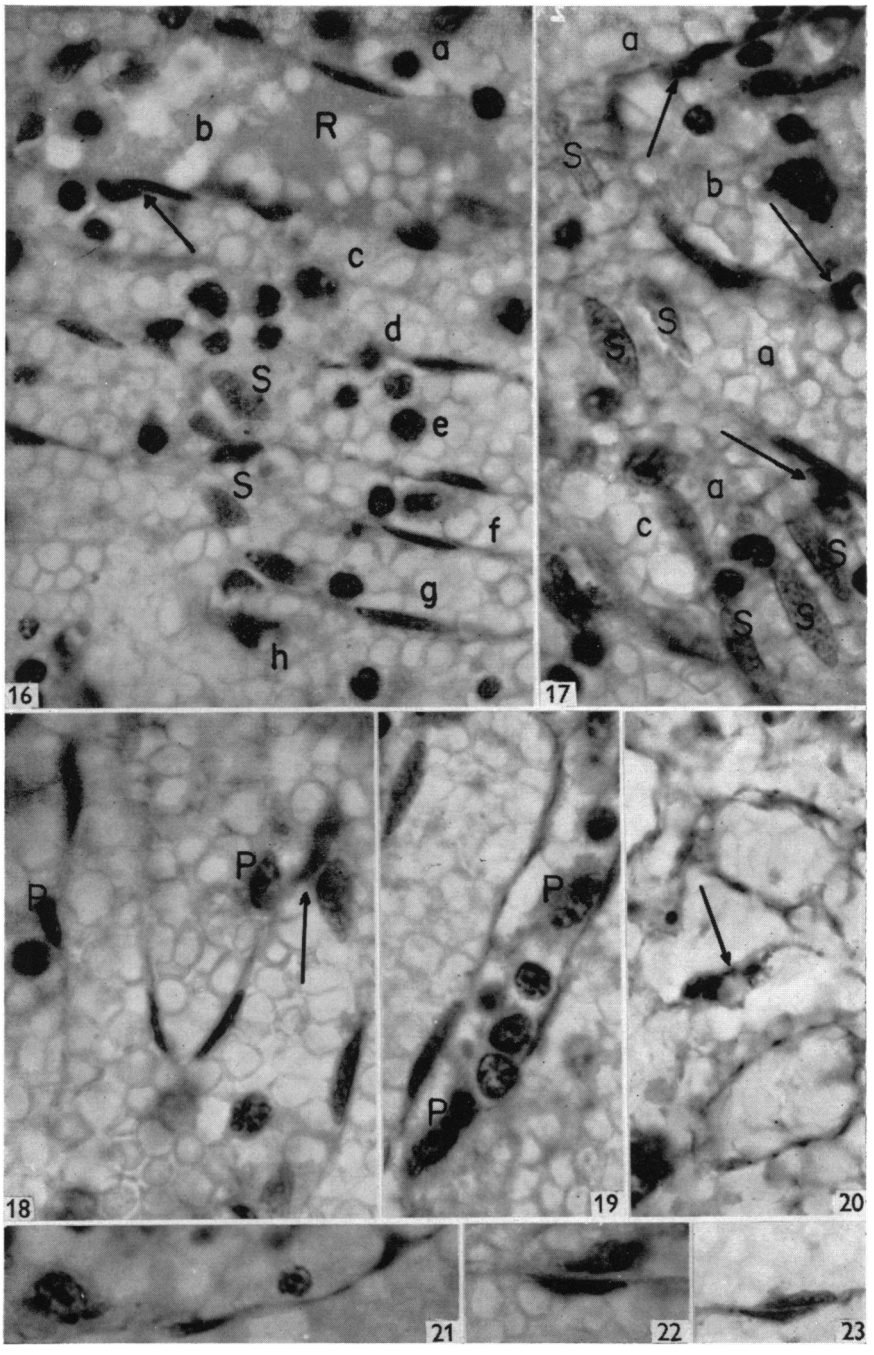


WEISS—RED PULP OF SPLEEN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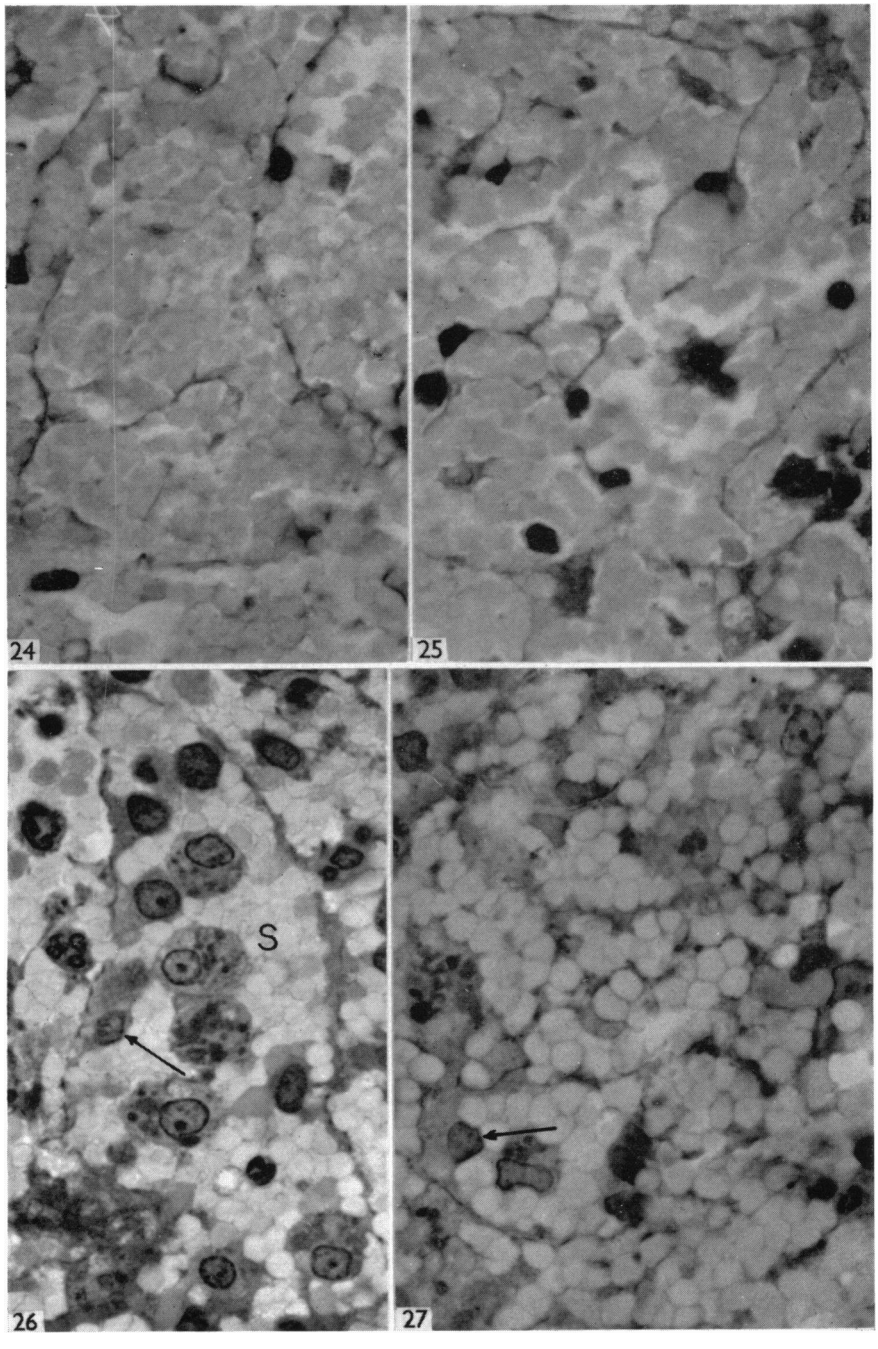
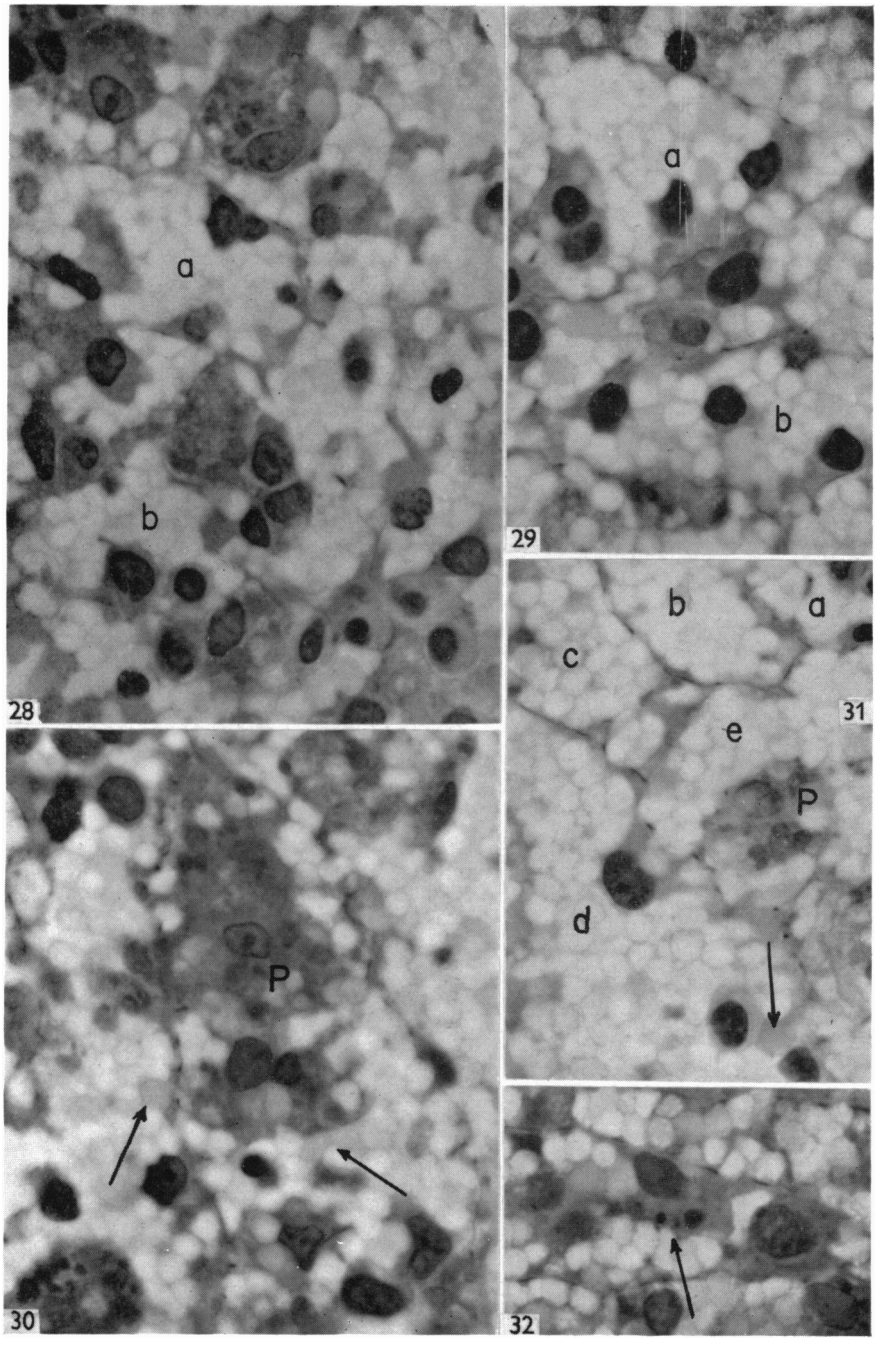
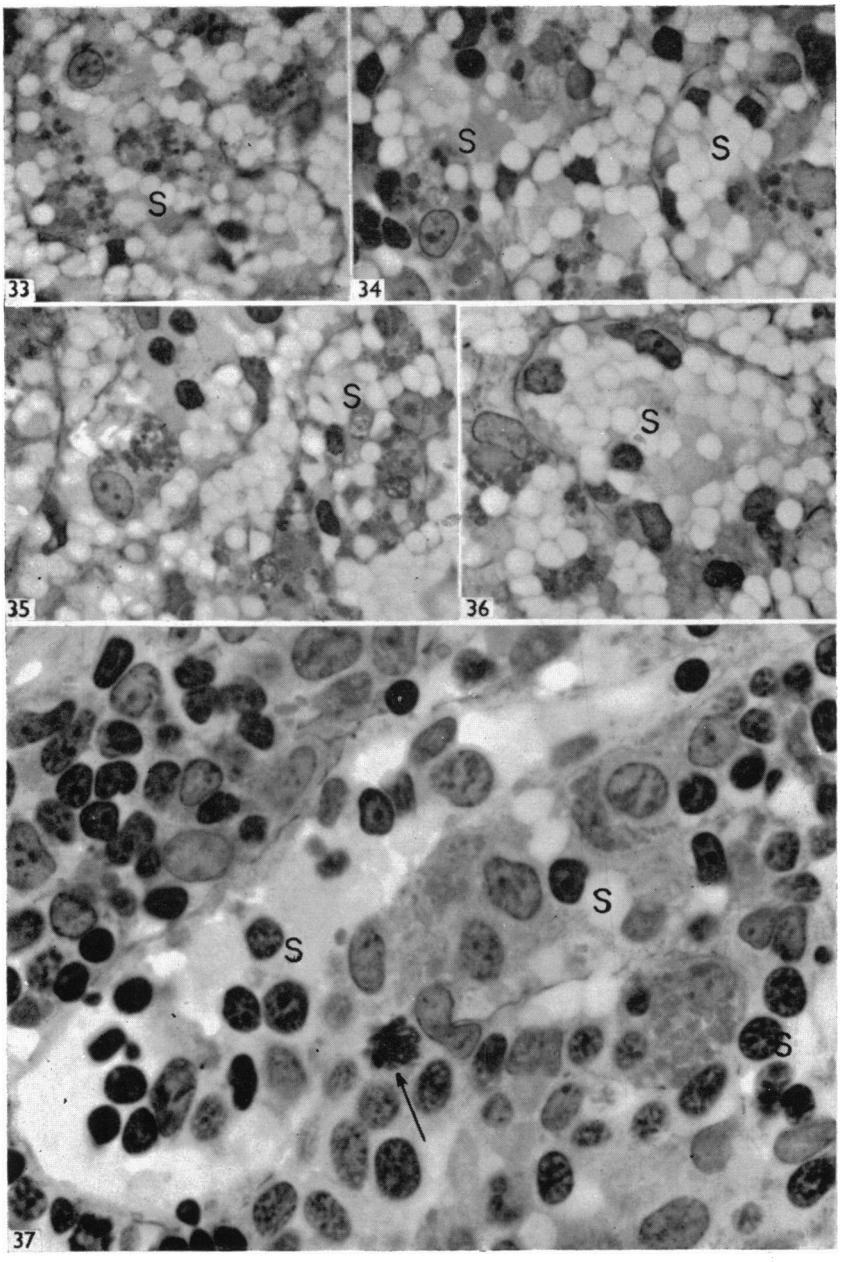
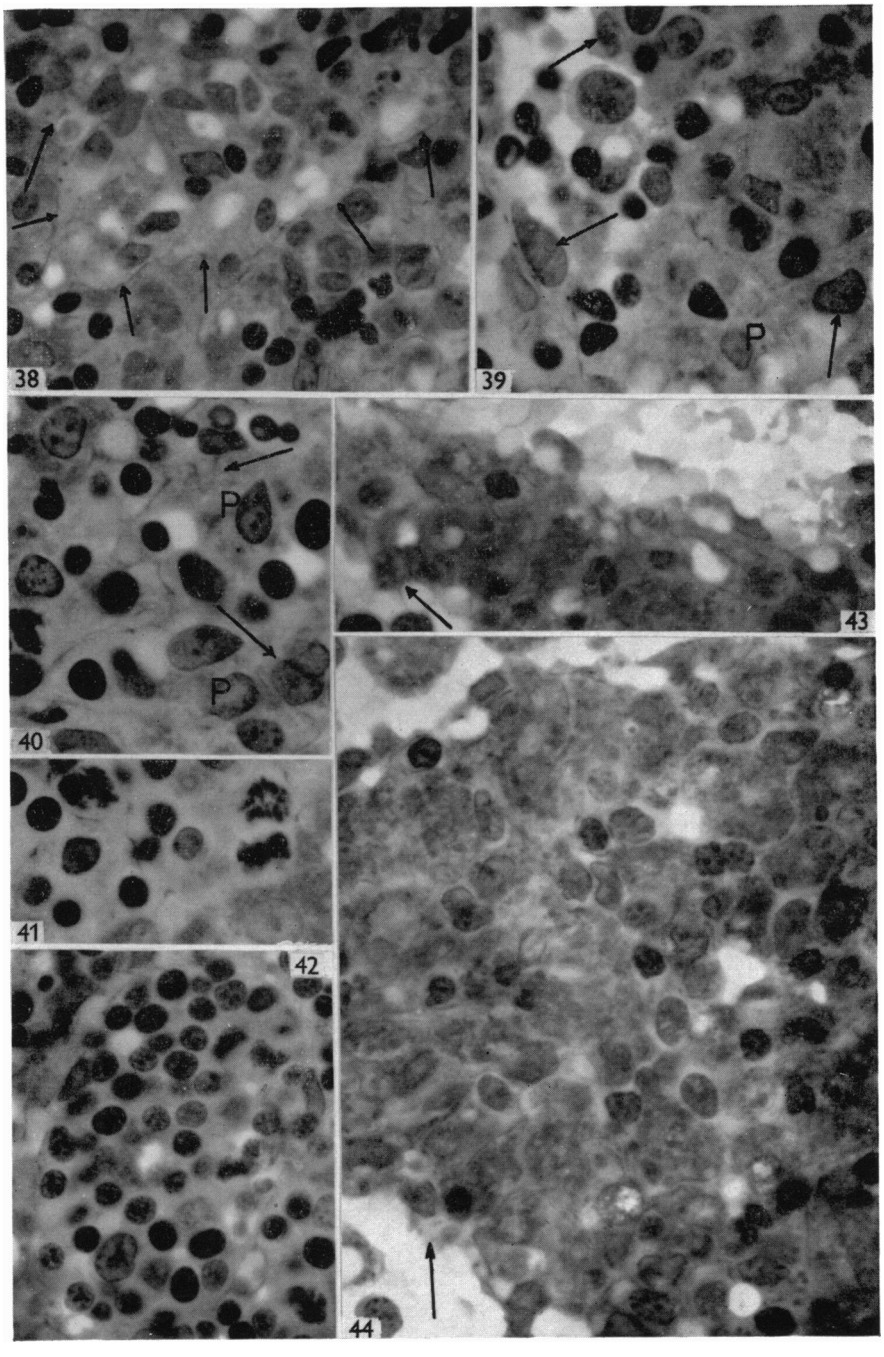


WEISS—RED PULP OF SPLEEN

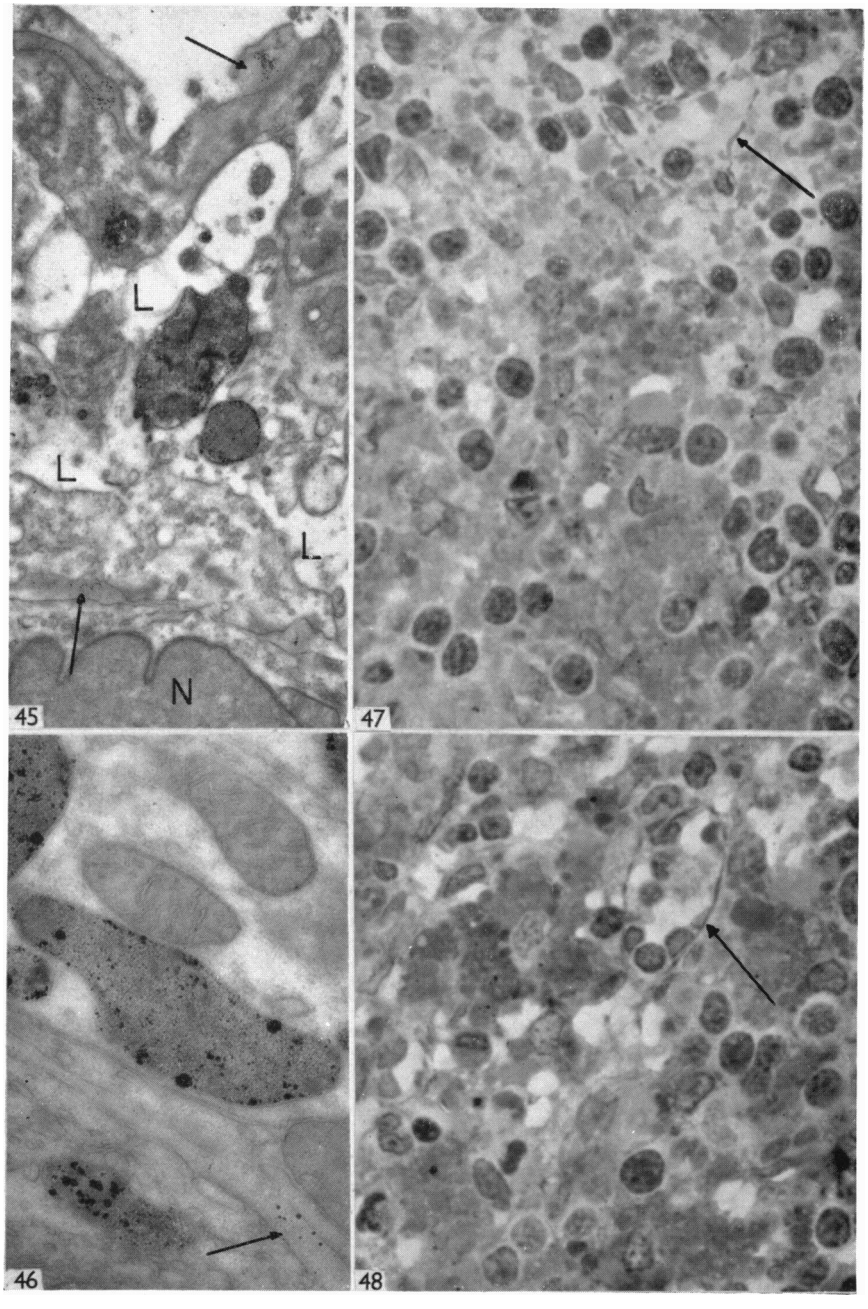

a
b
28
a
b
29
b
a
c
e
31
P
d
P
30
32



WEISS—RED PULP OF SPLEEN



WEISS—Red pulp of spleen



WEISS—Red pulp of spleen

PLATE 3

These photographs of normal rabbit red pulp illustrate relationships of sinuses to cords.

In fig. 6 a cord separates two frank sinuses which contain macrophages. Three endothelial nuclei are present in the cord; the lower one, cut parallel to the cordal surface, is phagocytic. It is labelled '*N*'.

Fig. 7 is a portion of the red pulp which may be analysed as a succession of vessels, indicated *a*, *b*, *c*, *d*, *e*, varying in endothelial reactivity, tortuosity, and luminal content, but alike in having an endothelium lying upon a basement membrane. The letter '*a*' touches an endothelial nucleus of a vessel largely out of the field. The letter '*b*' is directly over an endothelial nucleus and to the left and slightly below '*c*' is an endothelial nucleus. Similarly, an endothelial nucleus lies to the right of the lower letter '*d*'. Note that vessel *d*, running upward and to the right from the lower left corner narrows, is cut tangentially, and then, as the section passes again into the lumen, expands. An arrow marks the tangential cut and here the basement membrane may be seen on surface view.

In fig. 8, again, the field may be interpreted as a succession of vessels sharing common walls. The lumina are designated *a*, *b*, *c*, *d*, *e*, *f*, *g*. Vessels *d* and *c* have a highly irregular contour.

The field in fig. 9 contains three frank sinuses marked *a*, *c* and *e*, the latter oval in outline. The structures designated *b* and *d* are cords. Despite phagocytosis in *d* and possibly sequestration of cells in *b*, their vascular nature may be recognized by their configuration and the presence of endothelium. Note the surface view of the basement membrane in cord *d* at the arrow. As the section is followed in the direction of the arrow, it passes closer to the lumen, exposing an endothelial nucleus (*N*).

These tissues were fixed in osmium tetroxide, embedded in methacrylate, sectioned at about 2 μ, stained with PAS-H, and photographed $\times$ 1100.

PLATE 4. Normal rabbit red pulp

Examples of terminal arterial endings are present.

Some vessels designated *a*, *b*, *c* and *d* are present in fig. 10. *c* and *d* are frank vessels, *c* containing many macrophages. *a* is barely seen in the photograph. Vessel *b* branches, one limb to the right, clearly vascular, the other to the left, mostly out of the field. At the bifurcation, the section passes through the wall of the vessel, exposing endothelial nuclei (*N*) and delicate strands of basement membrane. This endothelium appears phagocytic. At the upper margin an arterial termination is present in cross section (arrow).

In fig. 11 frank vessels along the upper margin and at the right lower corner of the photograph are separated by a cord whose vascular nature may be inferred by the presence of endothelium. Marked phagocytosis is evident here. An arterial termination appears to empty into the cordal tissue.

Fig. 12 illustrates a common type of arterial ending. The terminal vessel bifurcates to form a T-shaped structure which ends in a cordal vessel. Again, note the succession of vessels *a*, *b*, *c* and *d*.

In fig. 13 an arterial termination ends in a frank sinus. Along the right margin of the sinus (arrow), the section verges to the tangential exposing the netted pattern of the basement membrane. Below and to the left (arrow), a terminal arterial vessel is cut in cross-section. Compare with fig. 7 of Björkman's work (1947).

In figs. 14 and 15 the terminal arterial segments cut in longitudinal and cross-section. The vessel dips in and out of the field in fig. 14. Note the difference in number of nuclei in the two segments of fig. 15.

These tissues were fixed in osmium tetroxide, embedded in methacrylate, sectioned at about 2 μ, stained with PAS-H, and photographed $\times$ 1100.

PLATE 5

The photographs are of rat spleen in which the splenic vein was tied off after the administration of sodium nitrite. The whole spleen was fixed in alcohol, formalin, acetic acid, embedded in paraffin, sectioned, stained with periodic acid-Schiff and haematoxylin, and photographed at a magnification of 1100.

The red pulp is markedly congested, the red blood cells unstained. Many nuclei are unusually flattened by the increased venous pressure. Compare these photographs with figs. 24–25.

In fig. 16 there appear to be eight vessels (*a–h*) running approximately parallel to one another. Note at the arrow the endothelial nuclei, back to back, separated by a basement membrane. (Additional examples are present in figs. 22 and 23. In fig. 23 one of the nuclei, presumably that of a dilated cord, is phagocytic.) The nuclei marked '*S*' are endothelial nuclei on surface view. At '*R*' the lumen is filled with what appears to be the substance of the basement membrane or reticulum washed from its basal location into the lumen.

In fig. 17, several limbs of vessel *a* diverge from the region marked by the topmost '*S*'. In several places the cut is tangential and the fenestrated basement membrane shown. Note the surface aspect of the endothelial nuclei at these places (*S*). At the lowermost arrow, the section includes a profile of a phagocytic endothelial cell which lines the lower limb of vessel *a* together with the tangentially cut cells marked *S*. A flattened endothelial nucleus, of the middle limb of *a*, lies on the other side of the basement membrane. Vessel *b* is presumably a dilated cord. Several of its cells are endothelial in position (arrows) in this section. Vessel *c* appears to be cut in oblique section.

A bifurcating vessel is present in fig. 18. Its right limb tapers to a point at the arrow. Phagocytic endothelial cells of the cordal vessels are labelled *P*. The cordal vessel in fig. 19 is compressed. It contains two phagocytic endothelial cells (*P*). Fig. 20 lacks the nuclear counterstain. The basement membrane is stained with the periodic acid-Schiff reaction. Again, note the succession of engorged vessels. At the arrow the countour of a vessel is completed by an endothelial cell whose phagocytized contents are stained.

A segment of basement membrane is present in fig. 21. A flattened endothelial nucleus is present on the lower surface and two endothelial cells (the one on the left obviously phagocytic) present on the obverse surface. In figs. 22 and 23 endothelial nuclei are present on obverse and reverse surfaces of basement membrane.

PLATE 6. The red pulp after administration of phenylhydrazine—early changes

The tissue in figs. 24 and 25 was fixed in Orth's fluid, embedded in paraffin, and stained with PAS-H. The red pulp is markedly congested with red cells, dilating cordal and sinal tissue. Surprisingly, few endothelial cells are present in fig. 24. Perhaps some of the endothelium came off the wall and was swept out. Compare with Pl. 5.

In figs. 26 and 27, normal sinal structure is disrupted. Note the endothelium in the sinus (*S*) of fig. 26 protruded deeply into the lumen. Several of the endothelial cells are phagocytic (arrow), and some free phagocytes are present in the lumen. At places the sinal wall is broken. Most of the erythrocytes are unstained, but several are coated with what appears to be the substance of the basement membrane. See also Pl. 7. In places this material is free in the plasma. In fig. 27 note the phagocytic sinal endothelial cell at the arrow.

The tissue in figs. 26 and 27 has been fixed in osmium tetroxide, embedded in methacrylate, sectioned at approximately 2 μ, stained with PAS-H, and photographed $\times$ 1100.

PLATE 7. The red pulp after administration of phenylhydrazine—early changes

The pulp is congested, almost all red cells unstained. Endothelial cells protrude from the walls of sinuses, the continuity of the walls is often broken, and in places the basement membrane has been washed into the plasma (for example, fig. 31, arrow) and on to occasional red cells (for example, fig. 30, arrows).

Disrupted sinuses may still be recognized at *a* and *b* in fig. 28; *a* and *b* in fig. 29; *a*, *b*, *c*, *d* and *e* in fig. 31. Note the endothelial cells which have become phagocytic, for example those labelled *P* in figs. 30 and 31 and at the arrow in fig. 32.

This tissue has been fixed in osmium tetroxide, embedded in methacrylate, sectioned at approximately 2 μ, and stained with PAS-H, and photographed $\times$ 1100.

PLATE 8. The red pulp after administration of phenylhydrazine—later changes

Many endothelial cells retain their attachment to the basement membrane, but their cytoplasm protrudes irregularly into the sinal lumen (*S*) and is loaded down with fragmented and whole red cells. By and large, the basement membrane persists, but in many places it is absent.

In fig. 37 an arterial termination (largely out of the field) enters a sinus at the right upper corner. The sinus (*S*) may be followed to the right and then down where part of its endothelium appears to have become phagocytic, although some of the phagocytes may have come away from the walls of other sinuses and been trapped here. Note the cell in mitosis (arrow) and to its right the constricted nucleus of cell, apparently squeezing through a narrow aperture in a sinal wall. Compare with fig. 7 in a previous paper (Weiss, 1957).

This tissue has been fixed in osmium tetroxide, embedded in methacrylate, and stained with PAS-H. Figs. 33–36, ×1100; fig. 37, ×1350.

PLATE 9. The red pulp after administration of phenylhydrazine—later changes

In fig. 38, endothelium of sinus is voluminous and occludes the lumen, rendering the limits of the sinus vague except where basement membrane (arrows) persists.

In fig. 39, a sinus runs from the left upper corner toward the right lower corner and then up. Several endothelial nuclei may be recognized (arrows). The basement membrane is present along most of the vessel's course but is not prominent. At one place a phagocytic endothelium (*P*) blocks the lumen. The arrows and the letter *P* lie in the lumen of one vessel.

Another sinuous vessel may be followed in fig. 40. Its basement membrane is indicated by arrows at two points. At the upper arrow the section grazes the surface of the basement membrane. Directly across the basement membrane from the lower arrow lie two nuclei of the endothelium of the adjoining sinus. Two phagocytic endothelial cells are marked '*P*'. The arrows and letters lie within the lumen of one sinus.

The number of mitoses, illustrated in figs. 41 and 42, in this material is considerably greater than normal. Note the sinus occupying most of the field in fig. 42 contains phagocytes and other, presumably sequestered, cells.

In figs. 43 and 44, phagocytosis is marked. Several sinuses have been incorporated into broadened cords, leaving no trace of endothelium and only short segments of basement membrane. In the sinuses that remain, the endothelium persists to varying degrees. At the arrows it is absent. Cell membranes are not sharp, possibly due to oedema.

These tissues were fixed in osmium tetroxide, embedded in methacrylate, sectioned at about 2 μ, stained with PAS-H. Figs. 38 and 42, ×950; remaining figures, ×1100.

PLATE 10. Argyric rat spleen

Figs. 45 and 46 are electron micrographs of argyric red pulp, ×13,000 and ×65,000. Note the dense particles of silver in the basement membrane (arrows). In fig. 45 note the order from above down: lumen, endothelium, basement membrane, endothelium, lumen (this one marked by 'L's'), endothelium, basement membrane, endothelium. The structure at the lower margin is an endothelial nucleus (*N*) against which, on the right, a mitochondrion lies. Within the broad luminal area in the middle of the photograph are numerous endothelial projections. In addition to its concentration in the basement membrane, silver is also concentrated in intracellular inclusions as in fig. 46. Sections were prepared with Porter-Blum microtome and photographed in RCA-ZE electron microscope.

Figs. 47 and 48 illustrate argyric rat red pulp after phenylhydrazine. In fig. 47 the field represents almost a pure concentration of phagocytes. A sinus, with phagocytic endothelium, persists, recognized by its basement membrane (arrow). Here and there short strands of basement membrane persist. In fig. 48 at least one sinus may be identified (arrow), but the reaction is similar.

The tissue in figs. 47 and 48 was fixed in osmium tetroxide, embedded in methacrylate and stained with PAS-H. ×1100.

Compare this plate with Pl. 1.