RETICULUM. ITS ORIGIN. THE OCCURRENCE OF RETICULUM FIBRILS IN CAPILLARY ENDOTHELIUM. A NEW METHOD OF DEMONSTRATION. II. THE FINER CAPILLARY BED *

JAMES F. RINEHART, M.D.

(From the Department of Pathology, University of California Medical School, San Francisco, California)

The present report is the first of a series of studies dealing with the derivation and differentiation of supportive substances from the mesenchyme as revealed by a new method of metallic impregnation described in detail below. Particular attention is devoted to the origin of reticulum and its occurrence in the mature organism. Other phases of mesenchymal differentiation are reserved for further study. In a preliminary way, fully realizing factors of incompleteness, a new concept of the finer capillary bed is presented.

A considerable literature has accumulated upon the subject of reticulum since Mall [1] in 1891 announced his discovery that the framework of many organs and tissues of the mammalian body is composed neither of white fibrous connective tissue nor of yellow elastic tissue, but of a third type of supporting substance composed of fine interlacing fibrils which not only differ from the white fibrous tissue in appearance, but are more resistant to acid and alkaline solvents, and are not so readily attacked by digestive ferments.

The introduction of the Bielschowsky method of silver impregnation stimulated renewed interest in reticulum. No attempt will be made in the present paper to review the rather extensive literature upon this subject. Some idea of its unsettled status may be gained by citing the contrasting concepts of Corner [2] (1920), and Mallory and Parker [3] (1927). Corner described reticulum fibrils arising in and extending from the capillary endothelial cells of the corpus luteum, adrenal, hypophysis and kidney. This author is one of the few making the distinct assertion that capillary endothelial cells in the adult organism produce a fibrillar substance, and identifying this substance with reticulum because of its affinity for impregnation with silver by the Bielschowsky method. Opposed to this view is

* Received for publication May 15, 1930.

that expressed by Mallory and Parker who deny formation of reticular fibrils by ordinary capillary endothelium, and assert that it is produced only by fibroblasts. The black impregnated fibrils are said to be fibrils originating in fibroblasts taking a black impregnation reaction due to a fine dispersion of the fibrillar substance.

Most of the other contributions deal with the occurrence of reticulum in various organs and tissues under normal and pathological conditions, without positive statement of its derivation.

Without further reference at this time to the literature, the method we have employed, our findings and their interpretation will be set forth.

The Methods Employed

Kinney [4] (1928) briefly reported the observation, that, in tissues fixed in a solution of 4 per cent formaldehyde containing 1 per cent sodium sulphantimonate, a substance was impregnated which she considered to be reticulum. This fixation was applied to tissues by the writer and a large variety of counterstaining methods applied. Results gave sufficient encouragement to pursue the subject further. Finally a silver ammonium carbonate solution was employed in tissues so fixed and a surprisingly sharp impregnation of reticulum was observed. Various refinements were devised. The following methods of fixation, impregnation and counterstaining were adopted which yielded the most satisfactory sections. These methods were employed in this study.

Fixation: Primary fixation in 4 per cent formaldehyde, preferably neutral, or Kaiserling's solution No. 1 is used. Following this, tissues are treated essentially as in the Kaiserling method of preservation for museum specimens. The tissues are washed in running water 24 hours, passed into 80 per cent alcohol for 24 hours, and then into Kaiserling's solution No. 3 for 3 or more days. Satisfactory preparations may be obtained in sections that have been long preserved in formalin or in Kaiserling's solution No. 3. Sections are taken from the Kaiserling solution and washed in running water for 12 to 24 hours. After washing, sections cut properly for paraffin blocks, 2 to 3 mm. in diameter, are mordanted or refixed in a freshly prepared 0.5 per cent solution of sodium sulphantimonate made in a 4 per cent solution of neutral formaldehyde. This refixation is employed for 24 to 48 hours. After this mordanting, sections are again washed

in running tap water for 24 hours, then passed into 80 per cent alcohol, dehydrated in the usual manner by running through 95 per cent and absolute alcohol, cleared in xylol and embedded in paraffin. Sections are cut the desired thickness and impregnated in the following manner.

Impregnation: Two methods of impregnation may be used, one involving gold toning, the other not. Both methods are detailed below. Either method gives satisfactory results. Toning transforms the golden brown reaction of collagen in silver sections into a rose red.

The silver impregnation method is essentially that devised by Foot for paraffin sections and detailed in McClung's "Microscopic Technique." [5] All preliminary treatment with sodium hyposulphite, potassium permanganate and oxalic acid, are, however, omitted. The gold toning employs a modification advised by Laidlaw [6] in the use of oxalic acid after the gold chloride toning bath.

Method I. Without Gold Toning

1. Remove paraffin and place sections in water in usual manner.
2. Impregnate with silver ammonium carbonate solution (prepared as described in footnote *) for 30 minutes in oven at 37° C.
3. Wash sections in distilled water — about 8 to 10 consecutive changes.
4. Immediately pour on 4 per cent *neutral* formladehyde and let stand for 5 minutes.
5. Rinse sections in distilled water several times.
6. Fix in 5 per cent sodium hyposulphite 2 to 5 minutes.
7. Wash at tap for 4 or more hours.

* *To Prepare Silver Ammonium Carbonate.*

1. Mix 12 cc. each of 10 per cent solution of silver nitrate and a saturated solution of lithium carbonate.
2. Wash precipitate 5 or 6 times with about 75 cc. of distilled water.
3. Add about 50 cc. of distilled water to washed precipitate and dissolve precipitate by adding ammonium hydroxide drop by drop until solution is almost clear. (Do not add too much — leave a few granules undissolved.) About 12 to 15 drops of ammonium hydroxide are needed.
4. Add distilled water to make solution of 85 to 100 cc. and filter.

Note: Solution must be prepared fresh for each batch and should be discarded after use. The solution is unstable and if exposed to the combined action of light and alcohol, there is danger of formation of an explosive mixture.

Staining Results: Mesenchymal fibrils range from yellow and golden brown to black. Reticulum fibers black. Collagen fibers golden brown (transition colors seen).

Method II. With Gold Toning

1. Remove paraffin from sections and place in distilled water in usual manner.
2. Impregnate with silver ammonium carbonate (freshly prepared each time and discarded) for 30 minutes in oven at 37° C.
3. Wash sections in distilled water about 8 to 10 consecutive changes.
4. Immediately pour on 4 per cent *neutral* formaldehyde, let stand for 5 minutes to reduce silver.
5. Rinse well in distilled water.
6. Tone in aqueous gold chloride solution 1:250 (acid yellow gold chloride) for 5 minutes.
7. Rinse well in distilled water.
8. Pour 5 per cent oxalic acid on sections for 5 to 8 minutes.
9. Wash well in distilled water.
10. Fix with 5 per cent aqueous solution sodium hyposulphite. Change the hyposulphite as many times as necessary until solution is clear.
11. Rinse in distilled water and wash at tap 4 to 6 hours before counterstaining.

Staining Results: Mesenchymal fibrils, delicate rose red to black. Reticulum fibers, black. Collagen fibers, rose red.

Counterstaining: Counterstaining by the May-Grünwald-Giemsa method has given the most satisfactory results. The method is essentially similar to that employed by Downey [7] in his studies of developing lymph nodes.

1. Place sections 15 minutes in dilute acetic acid (6 drops per 100 cc.).
2. Place sections 15 minutes in distilled water.
3. Stain in equal parts of saturated methyl alcohol solution of May-Grünwald eosinate of methylene blue, and distilled water 1 to 2 minutes.
4. Rinse sections in distilled water.

5. Giemsa stain diluted 1:15 for 15 minutes.
6. Wash in distilled water.
7. Differentiate in dilute acetic, 6 drops to 100 cc. for ½ to 2 minutes. (This differentiation should be watched under the microscope.)
8. Rinse in distilled water.
9. Dehydrate rapidly in acetone.
10. Clear in cedar oil, 1 minute.
11. Xylol.
12. Mount in thickened cedar oil.

A simpler, yet satisfactory counterstain, is the routine hematoxylin and eosin.

The above described methods have in the writer's hands given clean sections with clear differentiation. Connective tissue fiber substances including reticulum are completely impregnated and a satisfactory polychrome counterstain secured. In addition to applying the method to adult human tissues, both normal and pathological, the method has been employed in a small series of pig embryos of 7.5, 11 and 22 cm. This has greatly helped in achieving an understanding of reticulum in the normal fully developed human organism.

The Mesenchyme

The mesenchyme consists of cells with round, oval or slightly irregular nuclei usually surrounded by a small amount of faintly staining cytoplasm (the endoplasm) from which radiate delicate fibrils and fibers which are impregnated by the method employed. An exceptionally rich fibrillar mesh is formed, the fibrils of adjacent cells readily anastomosing with one another. These fibrils impregnated with silver alone range from a yellowish or golden brown color to black, and in toned sections from a delicate rose red to black. At nodal points where the fibers cross, the depth of the tone is increased. Mall [8] (1902) showed, that in the intestine, reticular fibrils develop in the cytoplasm of the mesenchymal syncytium and later Hueck [9] (1920) affirmed the same thing for mesenchyme in general.

An abundance of mesenchyme at this stage of differentiation is available for examination in the embryos studied. Phases of the differentiation of cartilage, fiber bone, skeletal and smooth muscle have

been traced with comparative ease using the method described. This will be the subject of a subsequent report.

Differentiation of Fibrous Tissue in the Mesenchyme: The morphological details of the differentiation of mesenchyme into fibrous tissue are so intimately associated with the problem in hand that an outline of this phase is deemed essential. As previously indicated, the delicate fiber substance of the simple mesenchyme shows shades ranging from yellow to black in untoned sections, and delicate rose to black in toned sections. With a condensation of fibrillar substance the color becomes more distinctly golden brown in simple silver prepared sections, and rich rose red if followed by gold toning (the color reactions of collagen). The transformation of simple mesenchyme into fibrous tissue entails a realignment of cells and fibrillar substance with the alteration in impregnation reaction indicated. The differentiation undoubtedly follows definite mechanical principles. For example, a bronchus enlarging in the mesenchyme acquires at its periphery a collagenous reacting mesenchyme—that is fibrous tissue. The fibers assume a radial arrangement about the expanding structures, the nuclei elongate in the direction of the fibers, the fibers now become more closely grouped, and the impregnation reaction becomes the distinct golden brown of collagen. The same phenomenon has been observed in the developing cuspid valves, in the corium and in other places where permanent fibrous tissue is being laid down. Fig. 1, a section of the skin and subcutaneous tissue of a 7.5 cm. pig embryo shows the transformation of the mesenchyme into fibrous tissue in the position of the corium. The loose underlying tissue is the fibrillated undifferentiated mesenchyme. Fig. 2 shows the same in greater detail.

The Development of Capillaries and Distal Lymphatics in the Mesenchyme: While not the primary consideration of this report, observations on the formation of distal lymphatics appear so clear-cut that brief attention will be given them at this time. Studied in the interlobular septae of the lung, these appear as simple clefts in the mesenchyme shown under low power magnification in Fig. 3. In Fig. 4 a portion of the wall of a lymphatic is shown in greater detail. The lining cells show fibrillated processes anastomosing freely with those of the surrounding mesenchyme. The nuclei flatten, paralleling the lymphatic wall and the fibers coarsen slightly by condensation.

Intimately associated with the problem under consideration is the question of the derivation of capillaries. The weight of evidence favors the concept that capillaries are formed *in situ* as a direct differentiation of the mesenchyme. Among those favoring this point of view Pulford[10] cites Reichert, Goethe, Felix, Rückert, Mollier, Maximow and Bonnet. In this country McClure,[11, 12] Reagan[13, 14] and Stockard[15] have contributed convincing evidence supporting the local origin of capillary endothelium. Hueck recently lends further support to this concept. The writer's study of pig embryo sections has convinced him that capillary endothelium is formed *in situ* by a very simple modification of the mesenchyme. This transformation is probably the simplest differentiation involved in the mesenchyme. The delicate fibers of this tissue are slightly rearranged and come to encircle the tube-like cleft in the mesenchyme. The new formed capillary is only readily recognized by its content of red blood cells. The cells lining it resemble those of the undifferentiated mesenchyme, and as a matter of fact, delicate fibrils can be traced from the lining cell into the undifferentiated mesenchyme. The cells lining the capillary possess the same delicate fibrillar processes as the mesenchyme. The fibrils stain darker, black or almost so. Reference to Fig. 4 and Fig. 2 will show this clearly. In Fig. 2 the small capillary in the mesenchyme underlying the corium shows distinctly the reticulum fibrils forming its wall. The nuclei in the primitive capillary wall are morphologically identical with those of the surrounding mesenchyme, and reticulum fibers extending from these nuclei not only surround the vascular channel but anastomose with the fibers of the surrounding mesenchymal cells. A capillary with the same cytological details remains in the fibrous corium above. Fig. 5 is a photomicrograph of subepithelial tissue showing an early phase in transformation of mesenchyme into fibrous tissue, containing two capillaries in which the fibered lining cells are recognizable.

In all capillary endothelium studied, both embryonic and adult, including practically all organs and tissues, reticulum fibrils have been demonstrable, thus confirming and extending the findings of Corner.

Demonstration of the delicate fibrils in mesenchyme serves to account for the reticulum fibrils in various tumors of mesenchymal origin as observed by Mallory and Parker. The very slight modifica-

tion of the mesenchyme involved in capillary formation leaves this cell not only as one producing reticulum, but with tremendous possibilities of differentiation, a very simple one being into fibrous tissue.

Evidence of the transformation of reticulin into collagen has been set forth by Rössle and Yoshida [16] (1909) in their studies of the reticulum of lymph nodes in normal and pathological conditions. Russakoff [17] (1909) likewise found little distinction in the chemical nature of reticulin and collagen. Miller [18] (1927) in studies of the reticulum in tuberculosis, although he did not assign a specific origin for reticulum, considered it a precollagenous type of connective tissue and observed this transformation in the healing process of tuberculosis. Foot,[19] in his excellent critical review of the endothelial phagocyte, gives an extensive bibliography touching many phases of the problem in hand and cites other evidence, including his own, dealing with the transformation of reticulin into collagen. The writer too, has observed the apparent transformation of reticulum fibers into fibers staining as collagen, under a variety of circumstances. Although a consideration of the rôle of reticulum in tuberculosis, as revealed by the present method of staining, will be the subject of a future report, some of the most convincing evidence of this change has been observed in this disease. In Fig. 6 is shown a tubercle in the liver in a case of miliary tuberculosis. At the periphery of the tubercle, it may be seen that the reticulum fibers lining the liver sinusoids have become thickened; with this thickening the fibers take the golden brown color of collagen in silver preparations and the rose red in gold toned sections. Other evidences of transformation of reticulin into collagen have been observed in tuberculosis.

Reticulum in the Liver, Spleen and Lymph Nodes

The liver, spleen and lymph nodes form a special phase of the reticulum problem. Each has received considerable attention in the literature and the writer can only touch upon his findings. In the liver Kupffer [20] (1876) described the reticulum under the name of *Gitterfasern*. In well prepared sections, the reticulum fibrils of the liver are striking objects. Deep brown to jet black fibers lie against the columns of liver cells forming the immediate lining of the sinusoidal vascular channels. It is not uncommon to see fibers cross over from one side to the other. The fibers are frequently seen in

intimate association with, and radiating from cell nuclei projecting into the lumen of the sinusoid, that are considered to be the Kupffer cells. Fig. 6 illustrating the transformation of reticulin into collagen about a tubercle also illustrates the general cytology of reticulum in the liver.

From incomplete studies thus far made the writer feels that the pathology and pathological physiology of the spleen is to a large extent bound up with the problem of the fiber substance of the spleen. In spleens that can be considered essentially normal, reticulum fibrils are readily demonstrated in the capillary endothelium and in a portion of the intersinusoidal or so-called reticulum cells. Normally these are delicate fibrils and not over abundant. In Banti's splenomegaly the reticulum fibrils of the intersinusoidal cells are remarkably increased in number, coarsened, and many fibers transformed into a collagen-reacting substance, giving a golden brown silver reaction, and a rose red in toned sections. Fig. 7 illustrates such a spleen. In lymph nodes, reticulum is again demonstrable in the capillary endothelium. Rössle and Yoshida found so-called reticulum, stainable by the Bielschowsky-Maresch method, in the lymph sinuses, the lymphoid tissue and the capillaries. It was considered as a precollagenous substance and transitions into collagen were noted by them. They found no distinction between the resting reticulum cells and the so-called endothelium of the lymphoid sinuses. The writer's findings are in accord with the above and with the careful studies of Downey who derived the reticulum cells supporting the pulp cords and nodules, as well as the cells lining the lymphoid sinuses, directly from the mesenchyme, and demonstrated the capacity of both to produce reticulum fibrils. Fig. 8 shows clearly reticulum fibers extending from the endothelial cells of the lymphoid sinuses. Morphologically similar cells form the supportive stroma of the lymph cords. In other areas reticulum fibers are seen in the capillary endothelium.

Universal Occurrence of Reticulum Fibers in Capillary Endothelium

As examination of the accompanying photomicrographs and drawings indicates, fiber substance, reticulum, is demonstrable in all capillary endothelium. It would be beyond the scope of this paper

or the ability of the writer to trace the capillary formation in all organs, but an outline of two general concepts, it is believed, will explain the principles of capillary formation. In tissue directly differentiated in the mesenchyme, as the heart and skeletal muscle, undifferentiated mesenchymal cells remain lying against the differentiated structures, and being applied to adjacent fibers, a potential if not actual channel lined by fibrillated mesenchymal, now endothelial, cells exists. In epithelial structures such as the lung, thyroid and hypophysis, the epithelial cells grow into the mesenchyme leaving mesenchymal cells applied against the epithelial cell columns or alveoli, forming the lining of the finer vascular spaces. As previously indicated, Corner has shown reticulum fibrils in the capillary endothelium of the corpus luteum, adrenal, hypophysis, thyroid and kidney. Except the corpus luteum, which has not been studied, the writer has been able to confirm these findings. Fig. 9 shows an area of lesser reaction in the lung in a case of miliary tuberculosis. Here may be seen cells with distinct black fibers forming the immediate lining of capillary vascular channels. Fig. 10 shows the continuity of reticulum fibers and endothelial cells in the medulla of the adrenal. The same findings hold for the cortical endothelium shown in Fig. 11.

Fig. 12 shows the black fiber lining of the capillary endothelium of the anterior lobe of the hypophysis.

Fig. 13 shows the same for the thyroid. A single cell layer existing between the vascular channel and the epithelium precludes the possibility of this being a fibroblastic layer. The reticulum fibers are flattened down, due to the pressure of the contained colloid in the alveoli.

Fig. 14 shows two small capillaries in the brain, one containing red cells, the other smaller and empty. Here in vessels a single cell layer in thickness, are clearly shown reticulum fibrils in the endothelium. With complete impregnation of the fiber substance of the capillaries, combined with adequate counterstaining a more complete concept of the finer vascular channels has been achieved which is set forth below.

The Finer Capillary Bed: Fig. 15 shows a congested area in the cortex of the kidney of a child. Examination of this figure, which is a faithful reproduction of an actual microscopic field, readily shows the following: (1) continuity of delicate black fibers (reticulum

fibers) with the endothelial cells in a perfectly simple one cell layer; (2) the identity of the basement membrane with the above described fibers; and (3) in oval, round or angular spaces are red blood cells, *i. e.*, capillaries as seen in ordinary sections; further (4) there are distinct lines of continuity clearly marked out by reticulum fibrils connecting the capillaries seen, *i. e.*, intercapillary channels potential and actual. It is the writer's concept that such channels serve normally as finer nutritive spaces and may, under stress of circumstances, open into spaces capable of carrying blood corpuscles. In other portions of the same slide, single red cells may be seen lying flattened in narrow clefts in the intercapillary reticulum lines. Fig. 16 shows two such clefts in an intertubular reticulum line, each of which contains red blood cells.

The same concept is also well illustrated in a study of the pancreas in Fig. 17. Here again continuity of reticulum fibrils and endothelial cells is seen; reticulum fibrils form the immediate lining of the capillary wall and, intercapillary reticulum-lined spaces are readily demonstrable. The identity of the reticulum fibrils and the basement membrane is again shown. The capillaries of the islets of Langerhans (not illustrated) also show a fibered cytoplasm and in favorable sections surface views may be seen. Here, focusing high at the surface of the capillary, very delicate slightly undulating black fibrils paralleling the longitudinal axis of the vessels are seen. At the lateral borders they are viewed several layers thick and show as darker lines.

The gastric mucosa (Fig. 18) illustrates the same principles outlined for the kidney and pancreas.

It is in connection with study of the heart muscle that the most striking support of the concept of finer capillary radicles is achieved. Fig. 19 shows an area of heart muscle in tangential section. Here a number of reticulum-lined capillaries with contained red blood cells are shown in cross-section and the reticulum-lined, intercommunicating spaces readily seen. Continuity of reticulum fibrils and the capillary lining cells is demonstrated. Selecting an edematous and congested heart muscle, Fig. 20, we see many of the potential spaces opened, in fact a veritable reticulum mesh containing red blood cells at various points. The photograph lacks sufficient detail to show these clearly. The same principles apply to the circulation in the adrenal and hypophysis, but inasmuch as a sinusoidal type of circu-

lation is generally conceded for these organs, they were omitted from detailed consideration. A glance at the section of thyroid will confirm the same concept for its circuit.

These preliminary studies indicate that the capillary bed is tremendously greater than generally conceived. The unopened bed is outlined by reticulum fibrils of endothelial cells. Such lines of communication probably ordinarily serve as channels for the conveyance of non-corpuscular elements of the blood, and, it is believed, may open under effective stimulus into channels of sufficient size to carry corpuscles. The reticulum fibers are in intimate contact with the parenchymatous cells of the organ and are, in fact, identified with the basement membrane in the kidney, pancreas, adrenal and gastric mucosa. Corner identified these fibers with the basement membrane in the kidney. It will be seen, then, that the capillary circuit is of an absolute character.

The concept is set forth as a morphological basis for the well known physiological capacity, as demonstrated by Krogh[21] and others, of the capillary bed to increase suddenly in extent.

The capillary bed is conceived as one of an absolute character, *i. e.*, one of the highest efficiency, touching the surface of all parenchymatous cells. A morphological background is given for the well known physiological capacity of the capillary bed alternately to open and rest and, under effective stimulus, to increase greatly in extent.

Summary and Conclusions

A new method of metallic impregnation is detailed which yields complete impregnation of mesenchymal, reticulum and collagen fibrils. An adequate polychrome counterstain may be superimposed upon the impregnated tissues. The mesenchymal cells possess a rich delicate fibrillar cytoplasm; the fibrils are readily impregnated by the method employed. Morphological support is given for the generally accepted concept that capillaries are formed *in situ* by a direct differentiation of the mesenchyme. This differentiation of capillaries in the mesenchyme is of a very simple character. The capillary endothelial cell remains in the embryo as a fiber-producing cell and this property and capacity persists into the mature organism. Both reticulin and collagen are fiber products derived from a common fibrillar mother substance and are undoubtedly chemically similar.

Reticulum fibers are demonstrated in capillaries in a wide variety of tissues, sufficiently wide to justify the concept that they are of universal occurrence in the capillary endothelium. Otherwise stated, reticulum may be identified as the fiber product of capillary endothelium. Similar fiber substance is present in the endothelial and reticulum cells of the lymph nodes. These cells, as the capillary endothelial cells, are little differentiated, direct descendants of the mesenchyme. Reticulum fibers are also present in the intersinusoidal or so-called reticulum cells of the splenic pulp and line the sinusoids of the liver. Reticulum fibers are a little changed descendant of the mesenchymal fibers.

Brief evidence is presented favoring the ability of reticulin to be transformed into collagen.

Reticulum is the most widespread and important supportive substance in the body. It is the scaffolding of cells and cell units. It serves the double purpose of microscopic cell support and the lining of capillary vascular channels.

By identifying reticulum with the capillary endothelium and obtaining sufficiently clear sections, the finer structure of the capillary bed is revealed. Reticulum fibers form the immediate lining of capillaries and minute reticulum-lined spaces are shown extending between and connecting the small capillaries as seen in ordinary sections. Such channels are considered to serve normally for the transfer of elements contained in the plasma of the blood and to be capable of enlarging or "opening up" under effective stimulus to a caliber sufficient to convey corpuscular elements. The endothelial reticulum is identified with the basement membrane in the kidney, pancreas, adrenal and gastric mucosa; this probably applies to basement membranes in general.

The author wishes to thank Dr. G. Y. Rush and Dr. C. L. Connor for stimulation and assistance received in the conduct of this study.

REFERENCES

1. Mall, F. P. Das Reticulirte Gewebe und Seine Beziehungen zu den Bindgewebsfibrillen. *Abhandl. d. Math.-Phys. Classe Königl. Sächs. Gesellch. d. Wissensch.*, 1891, **17**, 229.
2. Corner, George W. On the widespread occurrence of reticular fibrils produced by capillary endothelium. *Contrib. Embryol.*, 1920, **9**, No. 272.
3. Mallory, F. B., and Parker, Frederick, Jr. Reticulum. *Am. J. Path.*, 1927, **3**, 515.
4. Kinney, Ethel May. A simple method for staining reticulum. *Arch. Path.*, 1928, **5**, 283.
5. McClung. Handbook of Microscopical Technique. Paul B. Hoeber, N. Y., 1929, 295.
6. Laidlaw, George F. Silver staining of the skin and of its tumors. *Am. J. Path.*, 1929, **5**, 239.
7. Downey, Hal. The structure and origin of the lymph sinuses of mammalian lymph nodes and their relations to endothelium and reticulum. *Hematologica*, 1922, **3**, 431.
8. Mall, Franklin B. On the development of the connective tissues from the connective-tissue syncytium. *Am. J. Anat.*, 1902, **1**, 329.
9. Hueck, W. Über das Mesenchym. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1920, **66**, 330.
10. Pulford, D. Schuyler, Jr. Neoplasms of the blood-lymph vascular system with special reference to endotheliomas. *Ann. Surg.*, 1925, **82**, 710.
11. McClure, C. F. W. The development of the lymphatic system in the light of the more recent investigations in the field of vasculogenesis. *Anat. Record*, 1915, **9**, 563.
12. McClure, C. F. W. The endothelium problem. *Anat. Record*, 1921, **22**, 219.
13. Reagan, F. P. Experimental studies on the origin of vascular endothelium and of erythrocytes. II. The local origin theory. *Am. J. Anat.*, 1917, **21**, 44.
14. Reagan, F. P. Vascularization phenomena in fragments of embryonic bodies completely isolated from yolksac blastoderm. *Anat. Record*, 1915, **9**, 329.
15. Stockard, C. R. An experimental study of the origin of blood and vascular endothelium in the teleost embryo. *Anat. Record*, 1915, **9**, 124.
16. Rössle, Robert, and Yoshida, Tanzo. Das Gitterfasergerüst der Lymphdrüsen unter normalen und pathologischen Verhältnissen. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, **45**, 110.
17. Russakoff, A. Über die Gitterfasern der Lunge unter normalen und pathologischen Verhältnissen. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, **45**, 476.

18. Miller, W. S. The reticulum of the lung. IV. Its presence in the reparative process of the tuberculous lesion of the lung with and without caseation. *Am. J. Path.*, 1927, 3, 217.

19. Foot, Nathan Chandler. The endothelial phagocyte. A critical review. *Anat. Record*, 1925, 30, 15.

20. Kupffer, C. Ueber Sternzellen der Leber. *Arch. f. mikr. Anat.*, 1876, 12, 353.

21. Krogh, August. The Anatomy and Physiology of Capillaries. Yale University Press, 1929.

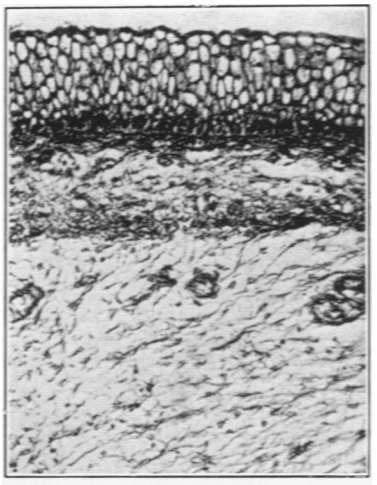
DESCRIPTION OF PLATES

PLATE 108

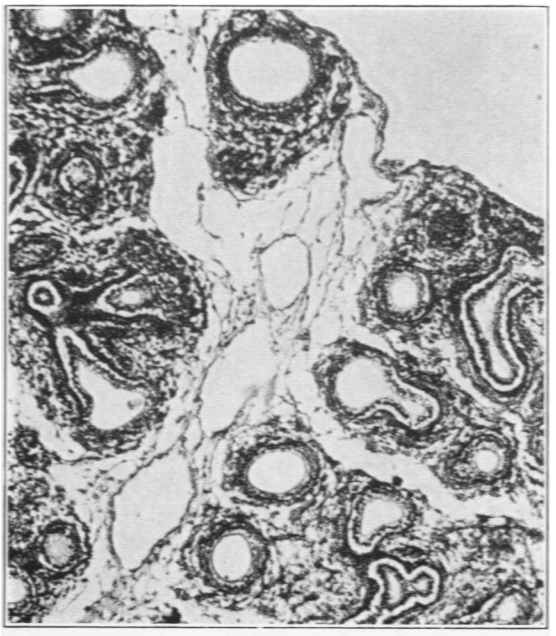
FIG. 1. Section of skin and subcutaneous tissue, 7.5 cm. pig embryo. Beneath the epidermis the mesenchyme is undergoing a transformation into fibrous tissue. The fibers have become rearranged longitudinally and now take the golden brown tone of collagen. The undifferentiated mesenchymal mesh is seen in the lower portion of the section. Stain: Method I. × 80.

FIG. 2. Detail drawing taken from same slide as Fig. 1, showing the rearrangement of fibers in formation of the corium. The coarser longitudinal fibers take the rich golden brown tone of collagen. At the lower portion of the section is the looser fibrillar mesh of the mesenchyme. The wall of the small capillary in the mesenchyme is seen to be composed of cells, morphologically identical with those of the mesenchyme, the fibers of which not only form the wall of the capillary but anastomose freely with those of the surrounding mesenchyme. The fibers stain somewhat darker than those of the surrounding mesenchyme. If this capillary had "invaded" the mesenchyme it would have rearranged the fibers about it and certainly would not show delicate fibrils extending from its wall into the surrounding mesh.

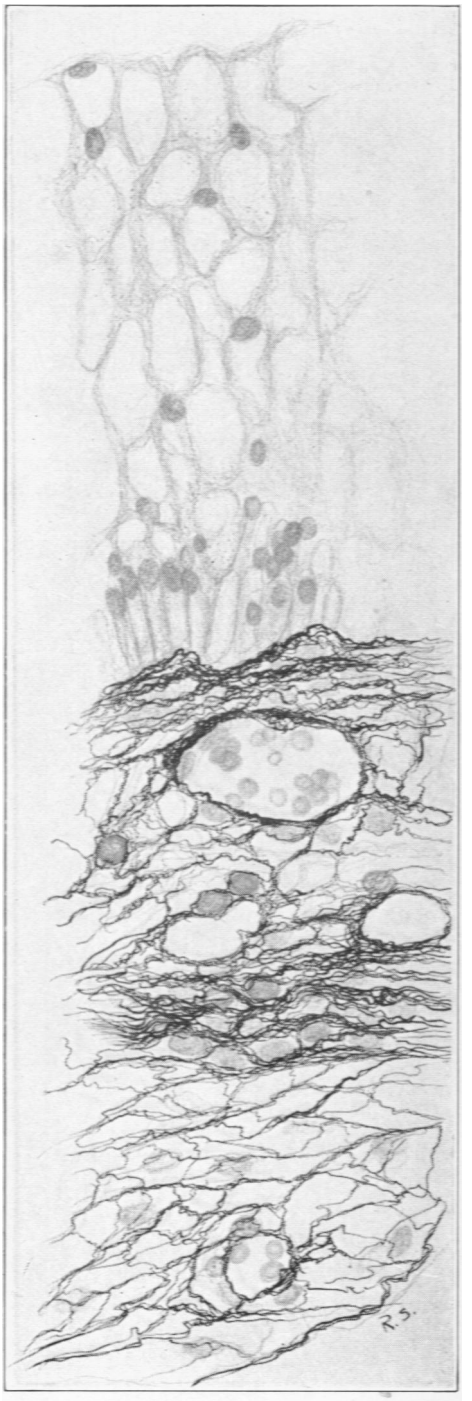
FIG. 3. Section of the lung, 11 cm. pig embryo. Lymphatic spaces appear as simple clefts in the mesenchyme in the interlobular septae. Stain: Method I. (See Fig. 4 for detail.) × 80.



1



3



2

Rinehart

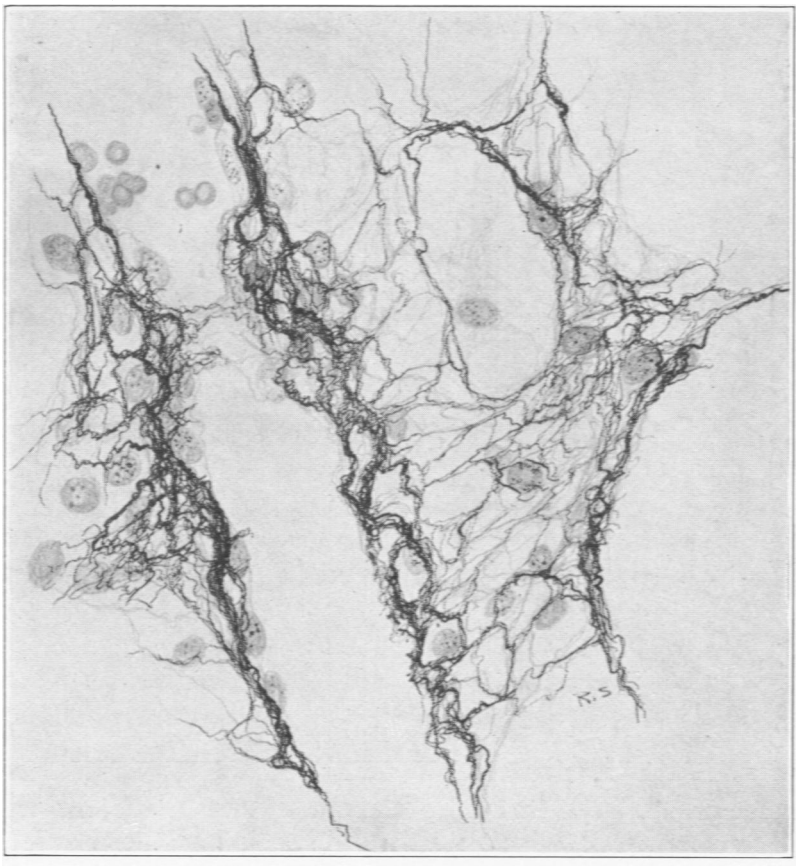
Reticulum

PLATE 109

FIG. 4. Drawing from interlobular septum of 11 cm. pig embryo showing development of a small blood vessel in the mesenchyme. The anastomoses of the fibers forming the wall and of the intervening mesenchyme is clearly shown. The vessel is destined to be one of larger than capillary caliber. The arc at the right is a portion of the wall of a lymphatic cleft in the mesenchyme. Staln: Method I.

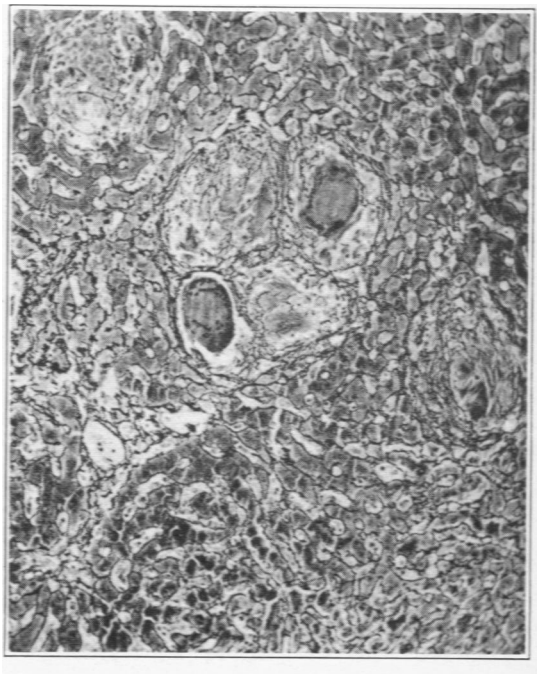
FIG. 5. Photomicrograph of two small capillaries in the corium in which the mesenchyme is being transformed into fibrous tissue. The fibers are assuming parallel arrangement and becoming coarser, and taking the deeper golden brown reaction of collagen. The fibers lining the two small capillaries are blacker than those of the surrounding mesenchyme. Stain: Method I. × 520.

FIG. 6. Group of tubercles in the liver from a case of miliary tuberculosis. The general architecture of the reticulum lining the sinusoids is shown. At the periphery of the tubercles the sinusoidal reticulum is seen to be coarsened. In the original sections the color contrast is striking; the coarsened reticulum takes the golden tone of collagen, the reticulum away from the tubercles stains black. The coarsening of the reticulum is well shown in the sinusoids intervening between the tubercle at the right of the photograph and the group in the center. Stain: Method I. × 80.



4

5



6

Rinehart

Reticulum

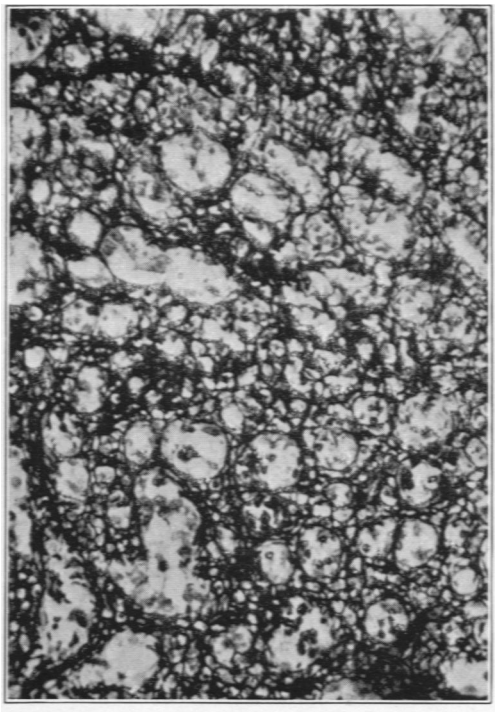
PLATE 110

FIG. 7. Section of spleen in case of Banti's disease. A marked coarsening of the reticulum is seen. The coarse black areas shown in the photograph give the golden brown reaction of collagen. Those less coarse maintain the black reaction of reticulum. Stain: Method I. × 150.

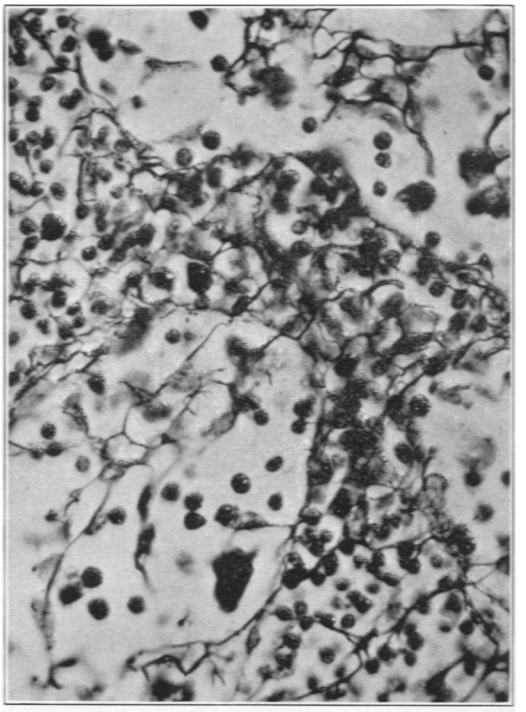
FIG. 8. Section of essentially normal lymph node showing reticulum fibers continuous with the cells, lining and extending into the lymphoid sinus. Morphologically similar cells form the supportive stroma of the lymph cords. Other areas show reticulum fibers in the capillary endothelium. Stain: Method II. × 520.

FIG. 9. Section of lung in an area of lesser reaction in a case of miliary tuberculosis. Reticulum fibers extending from cell nuclei and forming the immediate lining of two capillaries is well shown. One runs horizontally at the top of the photograph. The other in the upper right hand corner is cut transversely. Proliferating large mononuclear cells without fibers are seen attached to the capillary wall and free in the alveolar space. Stain: Method II. × 520.

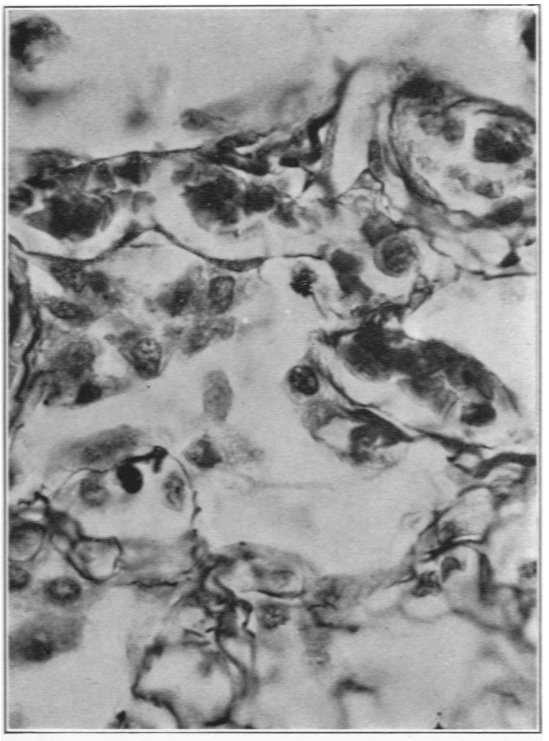
FIG. 10. The drawing taken from the medulla of the adrenal clearly shows the continuity of reticulum fibers with the endothelial cells lining the capillaries and extending as the basement membrane of the medullary cells. This figure also illustrates the concept of the closed capillary bed described in detail in the kidney, pancreas and heart muscle. A triangular cleft outlined by reticulum containing a single red blood cell is seen near the center of the field. At the upper left angle this cleft is closed but the reticulum fibers continue as the basement membrane.



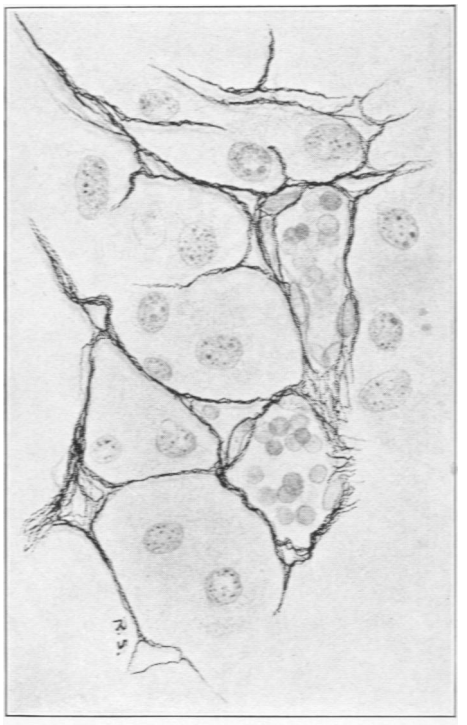
7



8



9



10

Rinehart

Reticulum

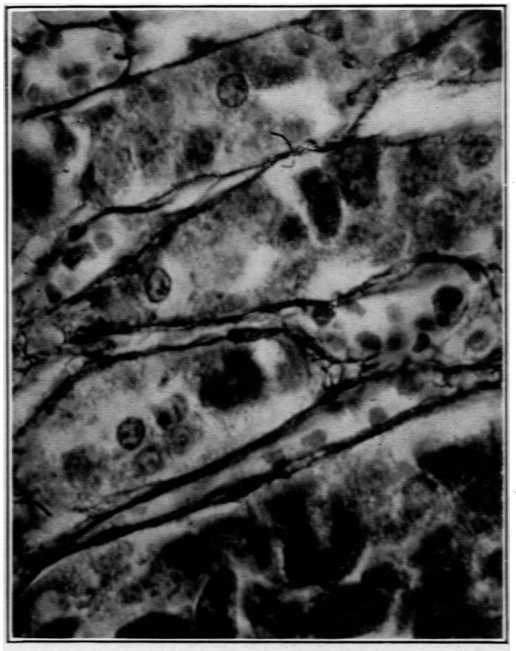
PLATE III

FIG. 11. Photomicrograph of the adrenal cortex showing continuity of reticulum fibrils and endothelial cells lining the capillaries. Stain: Method I. × 780.

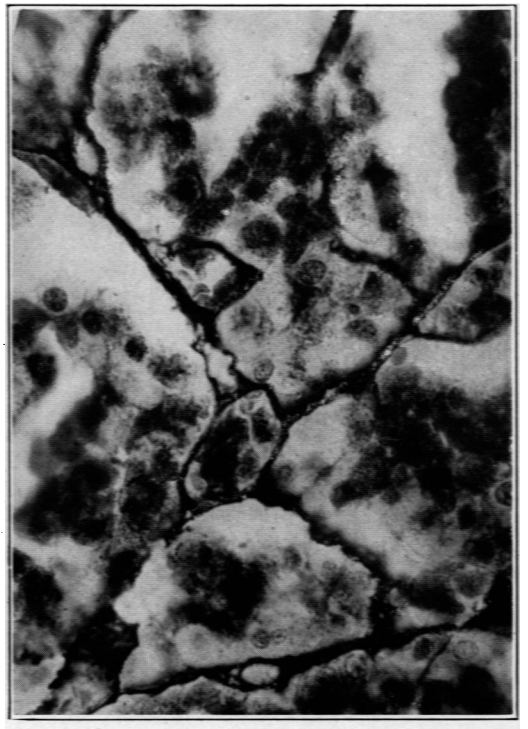
FIG. 12. Section of anterior lobe of the hypophysis showing the dense reticulum fibers lining the capillary spaces. Stain: Method I. × 520.

FIG. 13. Thyroid gland showing reticulum fibers lining open and closed capillary channels. A single cell layer existing between the vascular channel and the epithelium precludes the possibility of the reticulum being a fibroblastic layer. Reticulum fibers outline the open and potential capillary channels. Stain: Method I. × 520.

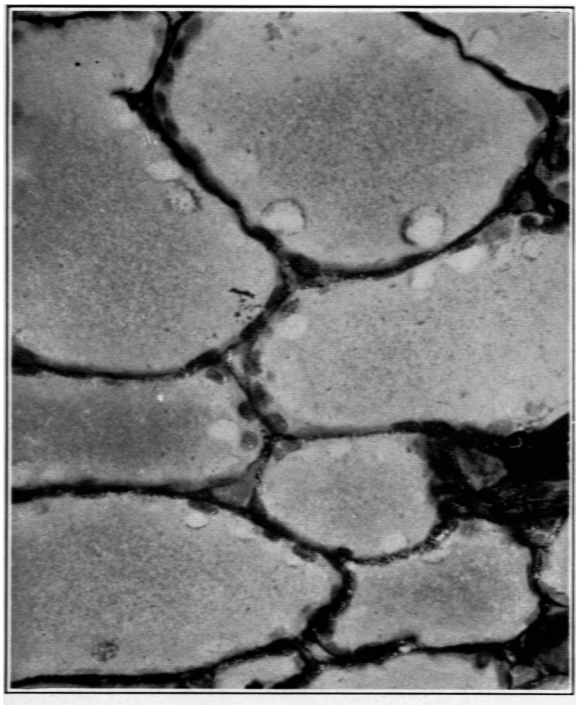
FIG. 14. Showing two small capillaries in the brain, one containing red cells, the other empty. Here in vessels, a single cell layer in thickness, are clearly shown reticulum fibrils forming the immediate vascular lining.



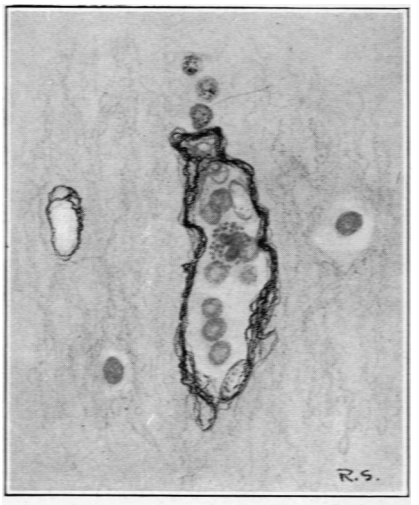
11



12



13



14

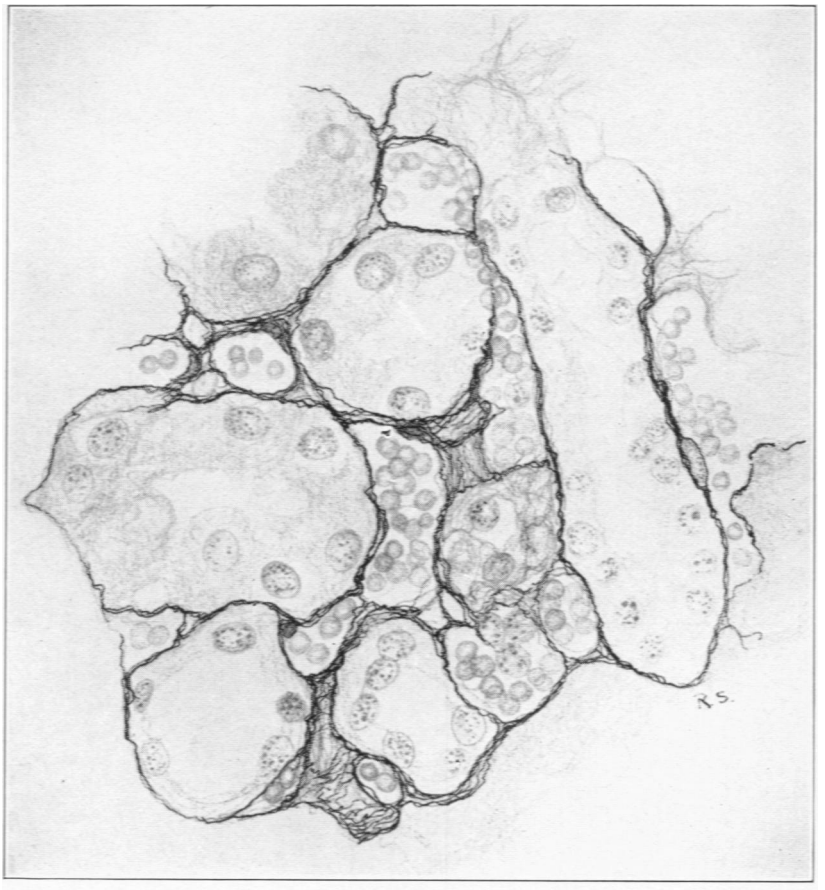
Rinehart

Reticulum

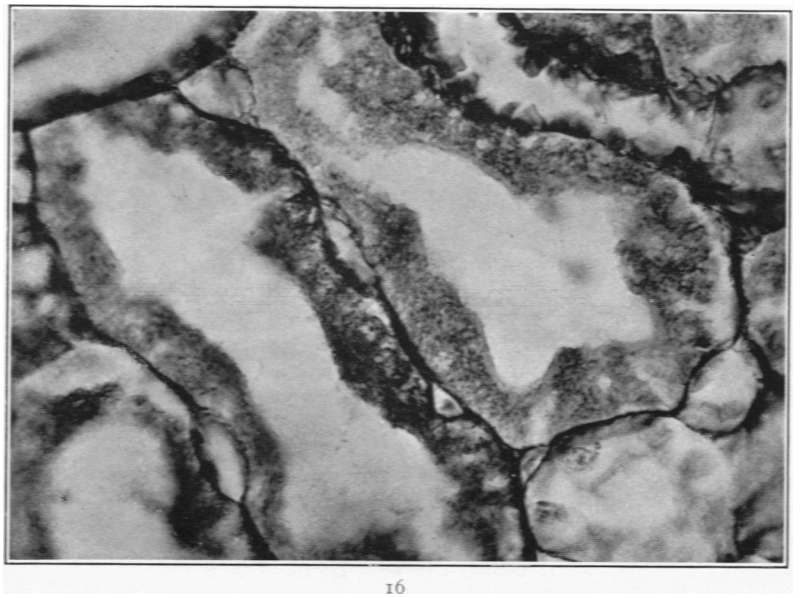
PLATE 112

FIG. 15. This drawing is taken from the kidney of a child. Here the following is shown: (1) continuity of delicate black fibers (reticulum fibers) with the endothelial cells in a perfectly simple one cell layer; (2) the identity of the basement membrane with the above described fibers; (3) oval, round and angular spaces, lined by reticulum fibers, containing red blood cells, capillaries as ordinarily seen, and, (4) connecting such spaces are fibers directly continuous with the capillary wall, forming lines of intercommunication between capillaries.

FIG. 16. Photomicrograph of the kidney illustrated in Fig. 20, showing two clefts in the reticulum "line" between two tubules. Red blood cells lie in each of these clefts. Stain: Method II. × 520.



15



16

Rinehart Reticulum

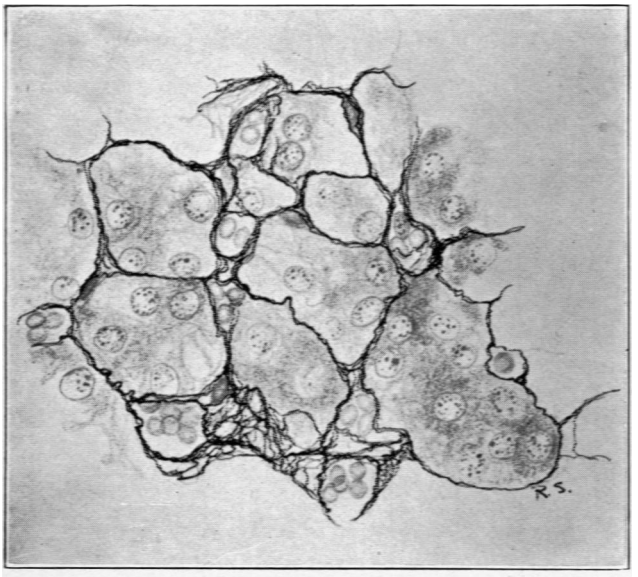
PLATE 113

FIG. 17. Drawing from pancreas of child, showing reticulum-lined capillary spaces containing red blood cells. The identity of the reticulum and the basement membrane is seen. Most of the capillary spaces in this field show only the reticulum lining, without the cells of origin. Endothelial nuclei with reticulum fibers in continuity are seen, lying between adjacent pancreatic acini, conceived as the lining of empty, collapsed capillary spaces. Potential but closed spaces are seen extending between demonstrable capillaries. Such fine channels are considered to serve for finer nutritive interchanges, and to be capable of carrying corpuscular elements of the blood under effective stimulus. Stain: Method II.

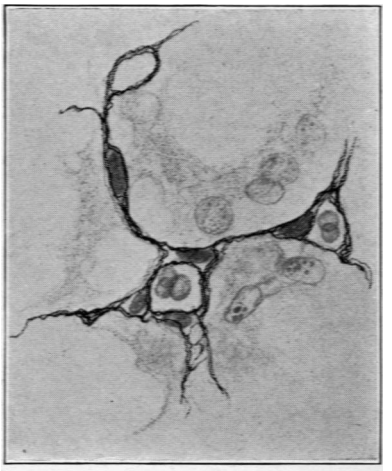
FIG. 18. Section of gastric mucosa, clearly showing reticulum fibers in the capillary endothelium and the identity of the latter with the basement membrane.

FIG. 19. Heart muscle in tangential section, showing continuity of reticulum fibrils with endothelial cells lining capillary spaces, and the continuation of these fibrils between capillaries forming minute passages for transfer of the blood plasma and conceived to be capable of "opening up" under effective stimulus for the conveyance of the corpuscular elements of the blood. Just above the center of the figure a single red blood cell is seen in a reticulum-lined endothelial cleft.

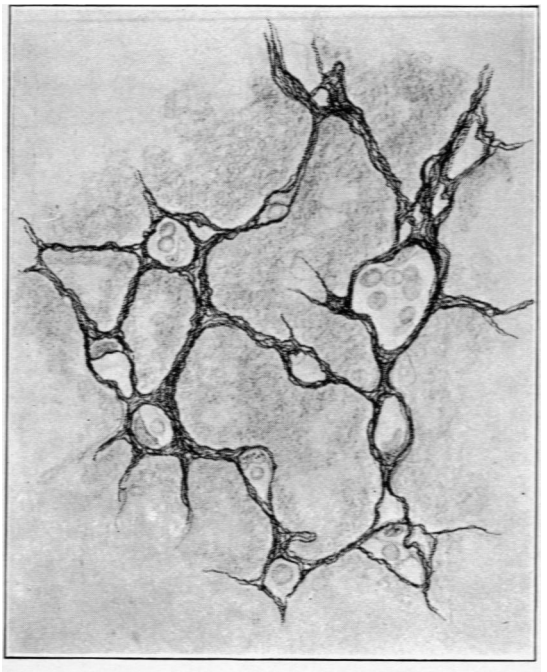
FIG. 20. Photomicrograph of a congested and edematous heart muscle, showing the reticulum-lined vascular spaces opened up. Although not showing clearly in the photograph, red blood cells are present at various points in the reticulum-lined capillary bed.



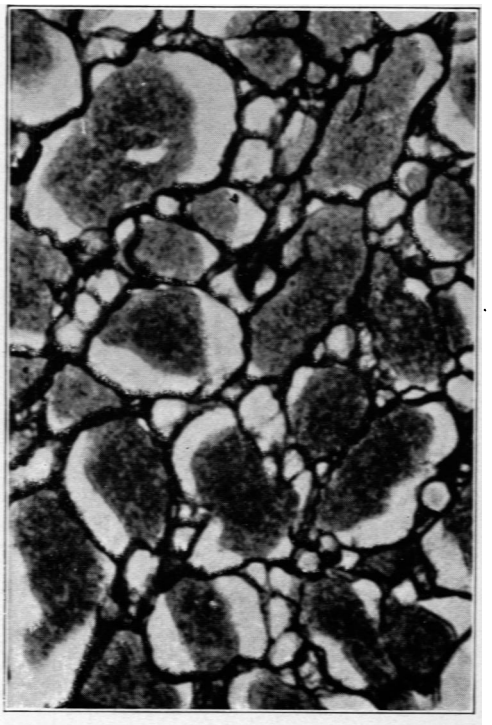
17



18



19



20