RETICULUM *

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INTRODUCTION

In recent years one particular tissue, the so-called reticulo-endothelial system, has been attracting increasing attention both from the physiologic (immunologic) and from the anatomic point of view. Although this tissue has been extensively studied, the resultant views of the different workers in regard to it are by no means in agreement. The two distinctive elements of this system are the endothelial cells and the framework or reticulum of intercellular fibrils which support them. In this paper we are concerned only with the origin and nature of the reticulum. The two undecided questions with regard to it are these: 1. Is reticulum the same as collagen or is it a chemically different substance which may be transformed into collagen? 2. Is reticulum produced by endothelial cells, by so-called reticular cells, or by fibroblasts?

HISTORICAL

Under the name of *Gitterfasern*, Kupffer [1] in 1876 described the reticulum occurring in the liver.

Mall [2] in 1896, as the result of digestive experiments and chemical analyses, concluded that there were three kinds of connective tissue fibrils, elastic, collagenous and reticular. He felt that each of these was a distinct variety. He found much reticulum in the capsule and trabeculae of the spleen, but none in the pulp. The exact reverse of these conditions was obtained by later workers who used silver stains.

In 1908 Rössle and Yoshida [3] studied the reticulum of lymph nodes and other organs by means of the Bielschowsky-Maresch silver impregnation method and the Van Gieson stain, and decided that reticulum was closely related to collagen but not identical with it. They found that collagen and reticulum fibrils were often continuous with one another and they felt that the "*Gitterfasernbil-*

* Received for publication April 15, 1927.

dungzellen" were identical with the endothelial cells of the lymph sinuses, but at the same time thought that they might be fibroblasts. They also stated that reticulum was changed to collagen by a metaplasia under certain conditions.

In the same year and by similar methods, Russakoff [4] came to essentially the same conclusions.

Miller [5] in 1923 studied the reticulum content of tubercles in the lung and expressed the opinion that reticulum, while different from collagen, was apparently often changed into the latter.

This hypothesis was also upheld by Foot [6] in a similar study in 1925. Foot's opinion as to the formation of reticulum is that it does not exist as fibrils connected with cells, but is precipitated out in some manner in an intercellular secretion. He as well as several other pathologists apparently regard the reticulo-endothelial cell as the one which forms these fibrils because their diagnoses of the so-called reticulo-endotheliomas are based on the presence and arrangement of these fibrils in certain tumors.

Methods

In view of the differences of opinion in regard to the origin and nature of reticulum, it seemed to us worth while to undertake a comparative study of it by utilizing not only the silver stains but also all the other recognized staining methods for demonstrating intercellular substances.

We made use almost exclusively of tissues which were excised surgically in order to obtain them as fresh as possible. Thin sections of these tissues, not over 2 to 3 mm. thick, were cut and placed in Zenker's fluid within a few minutes after removal from the body and carried through in the usual way. Paraffin sections were employed and the following stains used on each specimen: Foot's modification of the Bielschowsky-Maresch silver impregnation method,[7] Van Gieson's picro-acid fuchsin mixture,[8] and Mallory's eosin or phloxin-methylene blue,[8a] anilin blue collagen,[8b] phosphotungstic acid and phosphomolybdic acid hematoxylin stains.[8c] In addition a limited number of sections were stained using Verocay's technic [9] and the different methods specific for elastic fibrils. Frozen sections of a few tissues fixed in formaldehyde were stained by the original Bielschowsky-Maresch method [9a] and by Perdrau's [10] modification of it.

In our work we chose tumors for examination because the amount of original tissue in them is obviously slight. Furthermore, tumors tend to grow as a single type of cell with only a supporting stroma of blood vessels and connective tissue. In describing our results we shall consider the various types of tumors studied and then the tissues of the reticulo-endothelial system.

With regard to the Bielschowsky silver method, it should be stated that it does not stain fibrin or neuroglia, fibroglia, myoglia or elastic fibrils. Moreover, the results obtained with this method at the edges of the sections where the fixative acted first are not reliable.

Phosphomolybdic acid hematoxylin, like the Verocay method, brings out quite distinctly all the recticulum and collagen in the tissues and has the great advantage of neither causing shrinkage nor freeing paraffin sections from the slide. The best formula for staining collagen seems to be the following:

Water	100 cc.
Phosphomolybdic acid	2 gm.
Hematoyxlin	1 gm.

The hematoxylin dissolves readily in the acid. The solution can be ripened for immediate use by the addition of five cc. of a one per cent solution of permanganate of potassium.

Stain paraffin sections of Zenker-fixed tissue for twenty-four hours in the cold, or, if a more intense stain is desired, in the paraffin oven at about 54° C. for two to three hours. Wash in water, dehydrate in ninety-five per cent alcohol followed by absolute, clear in xylol and mount in xylol balsam.

Leiomyoblastoma

Silver stains of a leiomyoma arising from the wall of a vein in the groin showed that each muscle cell, when examined in cross-section, was separated from its neighbors by a thin layer of delicate black-staining fibrils. In other words the muscle cells were completely invested in what is called reticulum. On staining sections, already impregnated with silver, by the anilin blue collagen method it could be seen that the myoglia fibrils were stained red by the acid fuchsin and lay inside of the black reticulum. Where the bundles of muscle cells were running lengthwise, the reticulum was disclosed as delicate wavy fibrils differing morphologically in no respect from the fibrils which form the bundles of ordinary collagen. It was no-

ticeable that the fibrils when occurring singly or in delicate strands stained black, but where they were compacted into bundles, as around blood vessels, they stained only a reddish color.

In a series of leiomyomas of the uterus the same condition was found. Where the layer of intercellular fibrils between the muscle cells was thin and delicate it was stained intensely by the silver method and appeared black. Wherever the muscle cells had died off and disappeared so that the intercellular fibrils were compacted, they no longer stained black but were colored reddish like ordinary collagen. It, therefore, seems reasonable to conclude either that the reticulum was originally collagen or that it had turned into collagen when the muscle cells had disappeared.

Even in the rapidly growing tumors of this type, the leiomyosarcomas, there is the same formation of a reticulum composed of delicate collagen fibrils which must be supplied by the fibroblasts of the stroma. No other cell in the body seems to make such demands for an intercellular substance to surround and support it.

A study of the smooth muscle cells occurring in blood vessels, the gastro-intestinal tract and elsewhere revealed the same peculiarity of staining of the surrounding stroma by the silver method. Where the stroma was in a thin layer it stained black; where it was abundant following the disappearance of the muscle cells, and the formation of patches of sclerosis, it stained light red.

The application of all the other stains for intercellular substances showed that reticulum and collagen stain in exactly the same manner. Morphologically they differ only in the compactness of the fibrils.

The foregoing observations show therefore that smooth muscle cells under all conditions tend to be invested by a thin layer of collagen fibrils which stain black by the silver method. Because of this staining peculiarity and the arrangement around the cells as seen in cross-section, the collagen fibrils are called reticulum. When the reticulum fibrils are compacted owing to the disappearance of muscle cells they stain like collagen fibrils with which morphologically they are identical.

Fibroblastoma

In rapidly growing fibrosarcomas, the collagen produced by the tumor cells is very slight in amount, and is stained black by the silver method. In cross-sections the bundles of cells are seen to be

surrounded by a delicate reticulum, but in longitudinal sections, the reticulum is resolved into delicate wavy fibrils. Stated differently, collagen is stained black by the silver impregnation method when it occurs in single fibrils, in very thin layers or delicate strands.

In more slowly growing fibrosarcomas and in fibromas, the collagen does not stain black, but has a reddish color. In a slowly growing fibromyxosarcoma, on the other hand, the collagen stains intensely black wherever it is separated into individual fibrils or fine strands by the homogeneous intercellular substance.

The tumors of this group, producing their own intercellular fibrils, demonstrate clearly that collagen stains specifically by the silver impregnation method only when it occurs in single separated fibrils or in fine strands or thin layers.

Carcinoma

We chose scirrhous cancers of the breast in order to study the stroma. The fibroblasts are always well developed with numerous fibroglia and collagen fibrils. In addition the elastic fibrils are often abundant in places. The silver preparations showed great numbers of black-staining fibrils around and often between the groups of tumor cells, in the masses of elastic tissue and in the stroma wherever there was edema or infiltration with leucocytes; but no black-staining fibrils were seen where the collagen occurred in coarse bundles or solid masses. Furthermore it was evident everywhere that the black-staining fibrils were always in direct connection with the lightly stained collagen.

With all the other staining methods for intercellular substances, collagen and reticulum reacted and stained in the same way.

Lymphoblastoma

The stroma of a rapidly growing lymphoblastoma consists of a delicate network of intercellular fibrils in the meshes of which lie the tumor cells. The finer threads of this network are stained black by the silver impregnation method, while the coarser are colored black to reddish according to their compactness. The network consists of collagen fibrils arranged in fine or coarse bundles. They are closely applied to the fibroblasts which form a syncytium. The collagen fibrils do not branch, but the bundles of them often seem to branch,

because they course in different directions over the surface of the anastomosing fibroblasts. In the scirrhous type of lymphoblastoma, the reticulum is smaller meshed and often contains many eosinophiles as well as other leucocytes in addition to the tumor cells. In a later stage, as the cells disappear owing to degeneration or emigration, the strands of the reticulum are approximated and stretched more or less definitely in one direction or another. Under these conditions they appear and stain as collagen.

The Reticulo-endothelial System

The information derived from the study of the staining reactions of the intercellular fibrils of certain tumors was applied to the organs of the reticulo-endothelial system.

The stroma of lymph nodes aside from the blood vessels consists of a network of anastomosing fibroblasts which form a syncytium. The collagen produced by the fibroblasts is arranged in a reticulum composed of strands of delicate fibrils which course over the surface of the cells in different directions. Endothelial cells are applied in places to the surface of the reticulum which stains black by the silver method. The lymphocytes are contained in the meshes of the reticulum.

When the axillary lymph nodes are invaded by a scirrhous carcinoma, the stroma cells react as fibroblasts and produce an abundance of collagen.

The capsule and trabeculae of the spleen contain much collagen, many fibroglia and elastic fibrils but no reticulum. The lymph nodules are like those in lymph nodes, and the arteries and veins are similar to the blood vessels elsewhere in the body. The peculiar and characteristic feature of the spleen is the presence of the blood sinuses. They are lined with endothelial cells resting on a stroma of delicate strands of collagen which, on account of its occurrence in this form, stains intensely by the silver method. The fibroblasts which produce the reticulum are characterized by the presence of fibroglia fibrils.

In the organization of infarcts of the spleen, the fibroblasts of the stroma of the pulp produce scar tissue containing much collagen.

Liver

The connective tissue of the liver extends from around the portal and hepatic vessels through the lobule in the form of delicate strands of collagen, which stain black by the silver method. These strands which surround the columns of liver cells lie between them and the endothelial cells lining the sinusoids. They are produced by fibroblasts. In certain types of cirrhosis of the liver, the reticulum of the stroma stains like collagen when it is compacted into patches of sclerosis as the result of degeneration and disappearance of liver cells. When the stroma of the liver is increased in amount as the result of infectious processes (tuberculosis, infectious cirrhosis), collagen is produced in abundance.

In blood vessels, reticulum is present beneath the lining endothelium and between the muscle cells and extends out around the capillaries forming a delicate sheath about them. Reticulum is not formed by the endothelial cells but by the fibroblasts of the stroma for the support of the vessels, in exactly the same manner that it is supplied for the support of smooth muscle cells wherever they occur in the body.

Discussion

The recognition of reticulum depends on two characteristics, structure and staining reaction. The name was originally applied to the fibrillar intercellular substance which forms a network composed of fine strands for the support of cells in various organs and tissues. Later it was discovered that the silver impregnation method, variously modified, stained reticulum black while collagen took on a light brownish or reddish tint according to the method employed. On this account it was generally assumed that the stain was specific, and that reticulum and collagen were chemically different substances. Therefore, the Bielschowsky silver stain in recent years has become the standard means for the recognition of reticulum and has proved a great stimulus to the study of its distribution. It should be noted, however, that reticulum and collagen react alike to all other stains for the demonstration of intercellular substances.

As stated in the introduction to this paper, there are different views in regard to the origin and nature of reticulum. The subject has been confusing, because of the evident close relation between reticulum and collagen, not only chemically but also morphologically, Both are composed of delicate fibrils which are often wavy.

The different views in regard to reticulum will be taken up seriatim.

1. *Is reticulum a precollagenous substance?*

In favor of this view is the early appearance of the reticulum in tubercles and in granulation tissue. In these pathologic conditions the reticulum is in direct continuity with the surrounding collagen into which it changes at a later stage of the process. On the other hand, reticulum persists for a lifetime around smooth muscle cells and yet apparently changes at once to collagen if the cells it surrounds atrophy and disappear as, for example, in leiomyomas. The only change which has happened to the reticulum is a physical one: its fibrils have been brought into close contact with one another; in other words, they have been compacted.

2. *Is reticulum produced by reticular cells?*

This conception would necessitate the recognition of a new type of cell which produces a fibrillar intercellular substance composed of delicate fibrils exactly similar to those produced by the fibroblast, which change to collagen when they are closely packed together.

3. *Is reticulum produced by endothelial cells?*

This view has attained great vogue recently and is strongly advocated by Aschoff [11] in particular. It might be conceivable if one were to limit one's studies to the liver, spleen and lymph nodes; but not if one were to consider all the other organs and tissues of the body. It would mean that the intercellular fibrillar substance in a rapidly growing fibrosarcoma, which is stained black by silver, is produced by the endothelial cells of the blood vessels and not by the fibroblasts of the tumor. In the more slowly growing tumors of this type, however, it is produced entirely by the tumor cells because only collagen is present. It would also indicate that endothelial cells in the stroma of a scirrhous cancer of the breast produced the reticulum around and between the epithelial cells of the tumor at a considerable distance from the blood vessels.

4. *Is reticulum produced by fibroblasts?*

There are several points in support of this view. In a rapidly growing fibrosarcoma, the fibrillar intercellular substance stains like reticulum. In the more slowly growing tumors it stains like collagen and yet the age of the intercellular substance evidently has nothing to do with it. If the fibrils of a slowly growing fibrosarcoma are

separated by fluid or mucin, the collagen fibrils in these parts stain black with silver and must be regarded as reticulum.

In the stroma of scirrhous cancers the fibroblasts produce abundant fibrillar intercellular substance which stains like reticulum or collagen according to whether the fibrils are separated or compacted.

The most important point, however, is this: In its active condition, the fibroblast is characterized by the presence of fibroglia fibrils which run along its surface and which can be demonstrated by means of phosphotungstic acid hematoxylin or the acid fuchsin-anilin blue collagen method. Study of the so-called reticular cells of the spleen, lymph nodes and other organs show that they possess fibroglia fibrils and that they, therefore, are fibroblasts. For this purpose absolutely fresh tissue, just removed from the living body, cut into very thin sections 1 to 2 mm. thick and fixed in Zenker's fluid, must be used.

From this presentation of the results of a comparative study of the fibrillar intercellular substances present in various tumors and organs, it appears to us that reticulum is produced by fibroblasts and is merely collagen occurring as separated fibrils or as delicate strands. Only under this physical condition do the fibrils stain intensely by the silver impregnation method. When, as in leiomyomas, the fibrils of the reticulum are brought into close apposition through degeneration and disappearance of intervening cells, they no longer stain like reticulum but like collagen.

The Bielschowsky silver impregnation stain furnishes an excellent method for the demonstration of the finest fibrils of collagen, but the pictures it presents should be viewed with discretion to avoid misinterpretation. The results obtained by it must be controlled by other, more reliable methods which stain all the collagen present, even if not so intensely.

Summary and Conclusions

All recent work on reticulum has been based on the use of silver stains, chiefly Bielschowsky's and modifications of it. Our results depend on the use of tissue fixed immediately after removal from the living body, and on a comparative study of the various stains for intercellular substances.

Smooth muscle cells in leiomyomas, in the wall of blood vessels and elsewhere are surrounded by delicate fibrils which are stained

black by the silver. When the muscle cells die, the black-staining fibrils around them disappear, but the collagen is increased in amount. The obvious inference is that separated collagen fibrils are stained by silver, but compacted fibrils are not. Myoglia fibrils are not stained by silver. This point can be demonstrated by using the acid fuchsin-anilin blue method on a section already treated with silver.

In fibrosarcomas the collagen is stained black by silver, if it is slight in amount. It is not stained when present in large amounts.

In cancers many loose fibrils surrounding and running between the epithelial cells stain black; the same is true of single fibrils or very small bundles of them embedded in elastic tissue. Stains for fibroglia fibrils following silver stains show that they are not colored by the silver.

The capsule and trabeculae of the spleen contain much collagen, many fibroglia and elastic fibrils but no reticulum. The stroma of the lymph nodules and pulp stains black. Stains for fibroglia fibrils show them to be present running along the surface of the reticular stroma. Hence the cells forming this network are fibroblasts.

It is generally agreed that physically reticulum and collagen are intimately joined together, being always continuous one with the other.

Many believe that reticulum changes to collagen. All reticulum stains by the methods used for collagen, namely, Van Gieson's, Verocay's, anilin blue and phosphomolybdic hematoxylin.

The silver stains are specific for collagen but only under certain physical conditions. Collagen must be separated into individual fibrils, or into very small strands and thin layers by cells, elastic fibrils or fluid.

Reticulum as a chemically distinct intercellular substance does not exist; it is collagen in separated form, rendered prominent by the silver stain.

All collagen is produced by fibroblasts.

There are no reticular cells other than fibroblasts.

Endothelial cells do not produce an intercellular substance.

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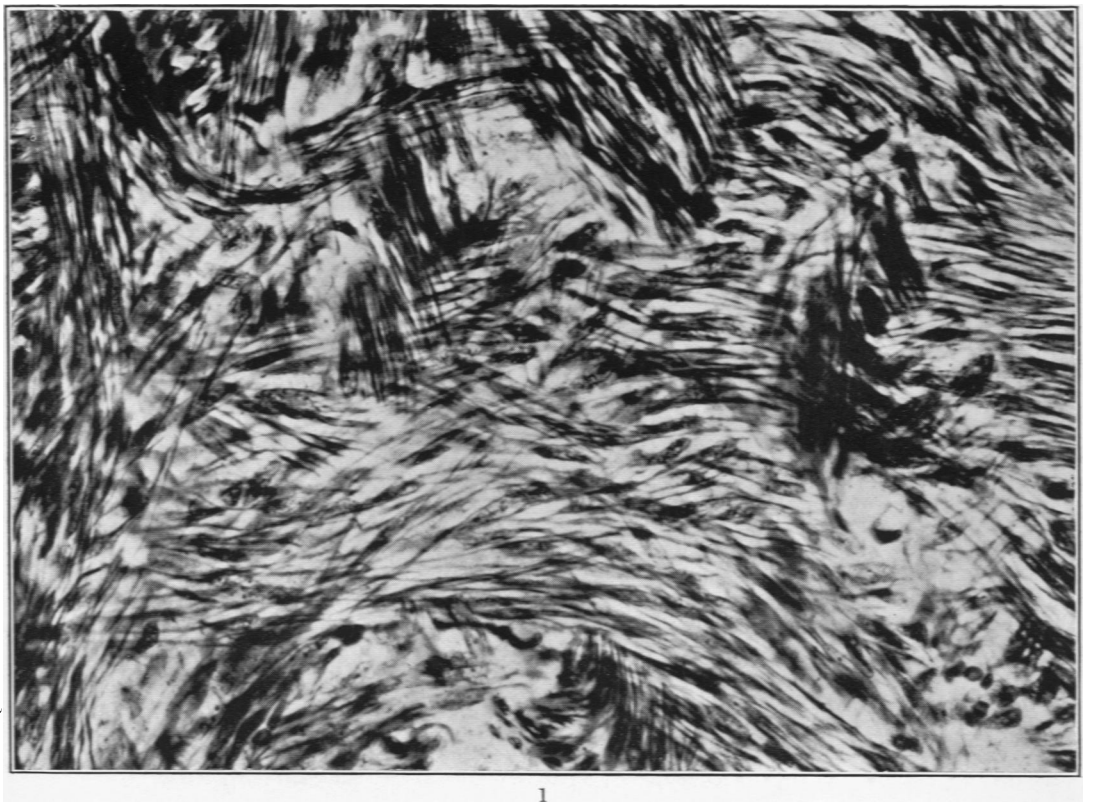
DESCRIPTION OF PLATE

All photomicrographs were made from sections of Zenker-fixed tissue, except Figs. 2 and 3; and stained by Foot's modification of Bielschowsky's silver method, except Figs. 1, 5, 16, 18 and 23.

PLATE 132

FIG. 1. Leiomyoma originating from the wall of a vein in the inguinal region. Stained with phosphotungstic acid hematoxylin to show the myoglia fibrils. × 500.

FIG. 2. Cross-section of bundle of smooth muscle cells from the same tumor to demonstrate reticulum surrounding them. Formaldehyde fixation, Foot's silver method. × 1000.



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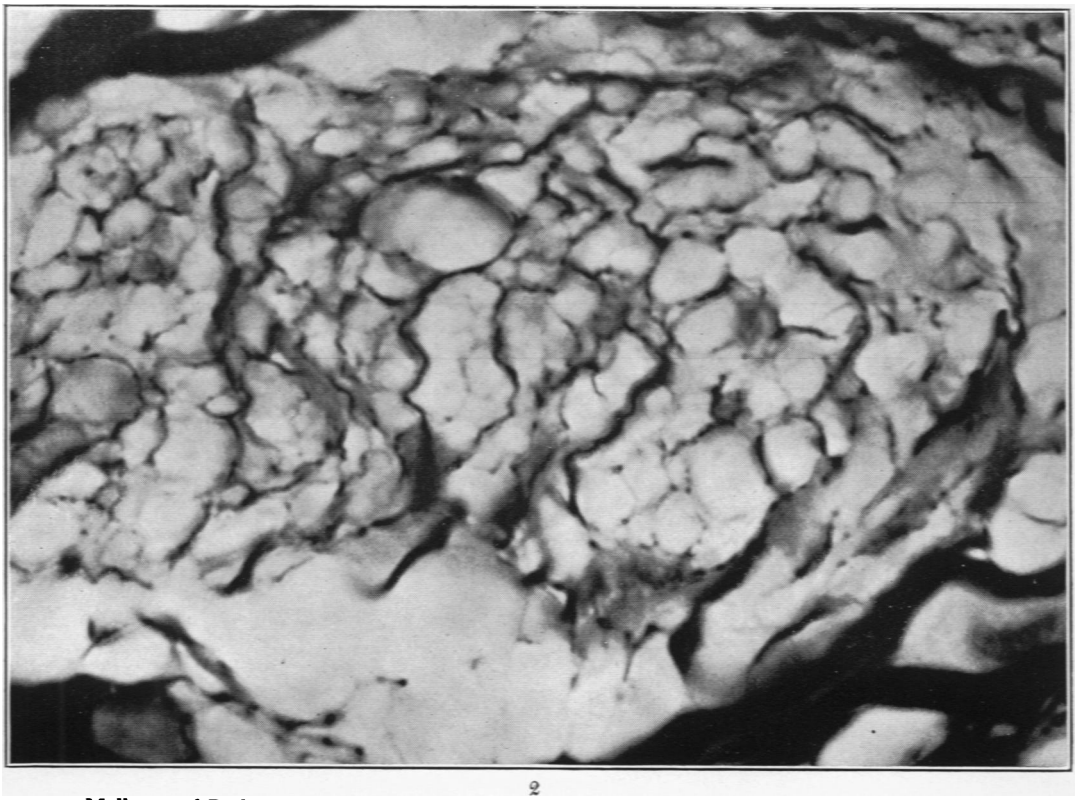

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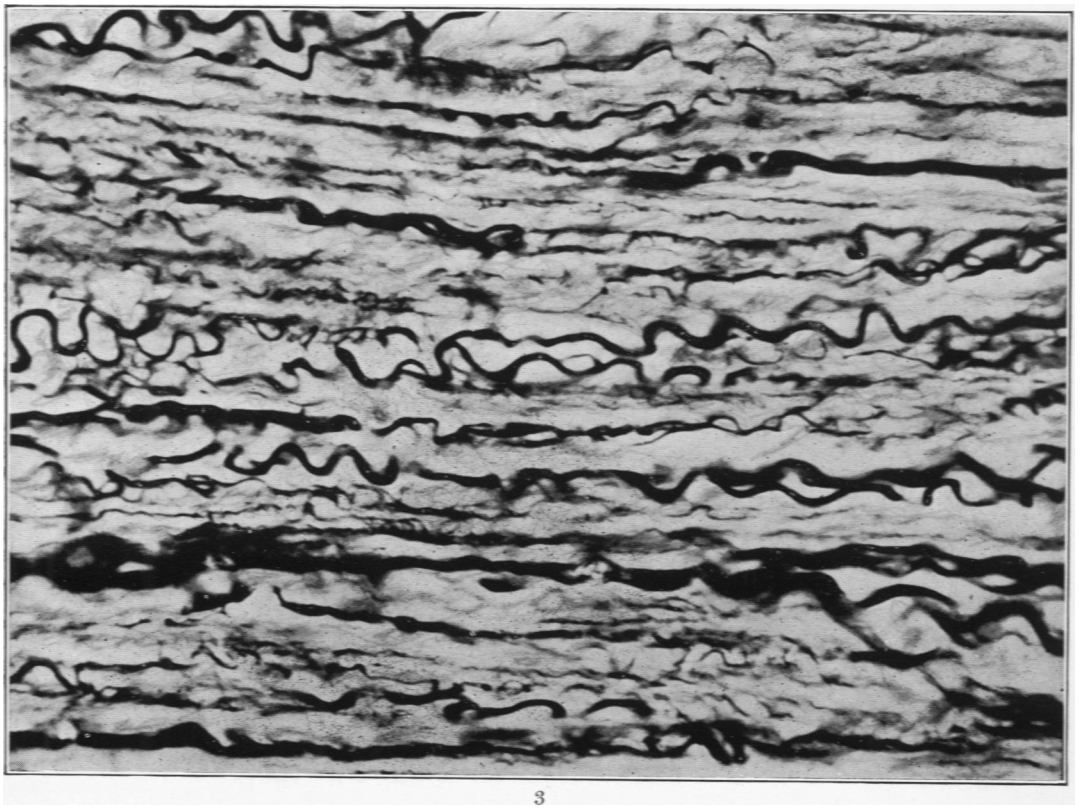
Mallory and Parker Reticulum

PLATE 133

FIG. 3. Longitudinal section of same leiomyoma (Figs. 1 and 2) to show reticulum resolved into wavy collagen fibrils occurring in single and in fine strands. Fixative and staining same as Fig. 2. × 1000.

FIG. 4. Leiomyoma of uterus. Reticulum around muscle fibrils. Where the cells have degenerated and disappeared the reticulum fibrils have been compacted into collagen and stain very slightly. × 1000.



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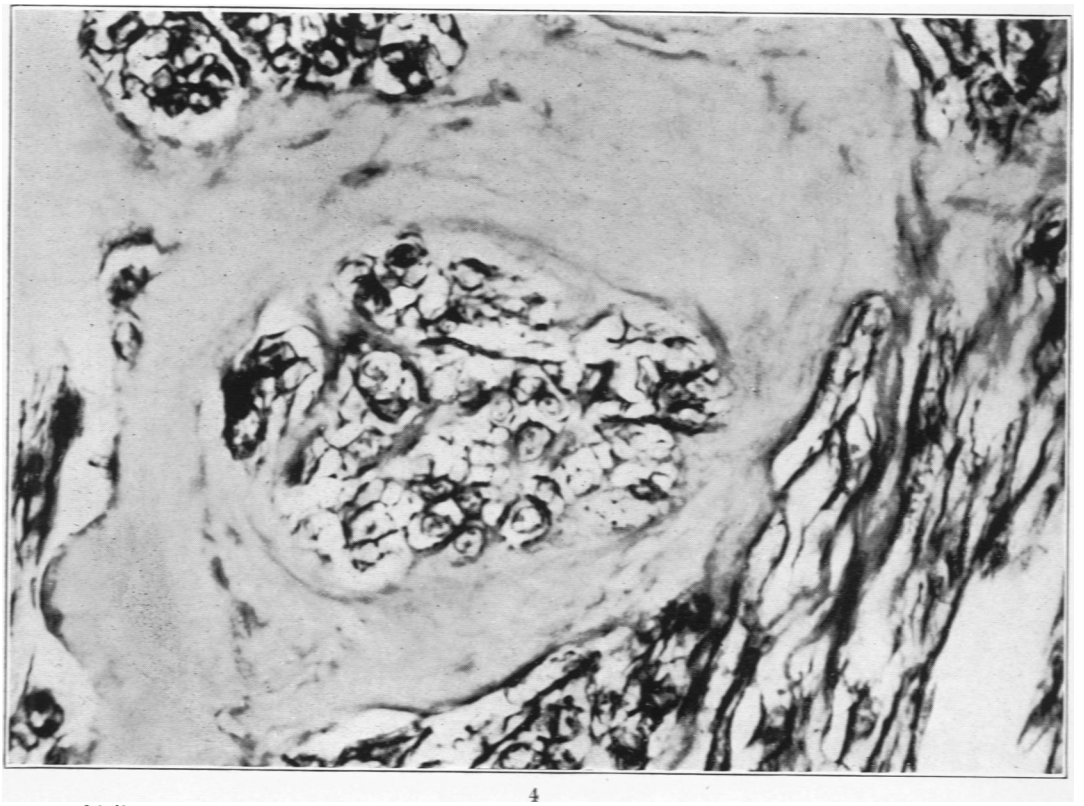

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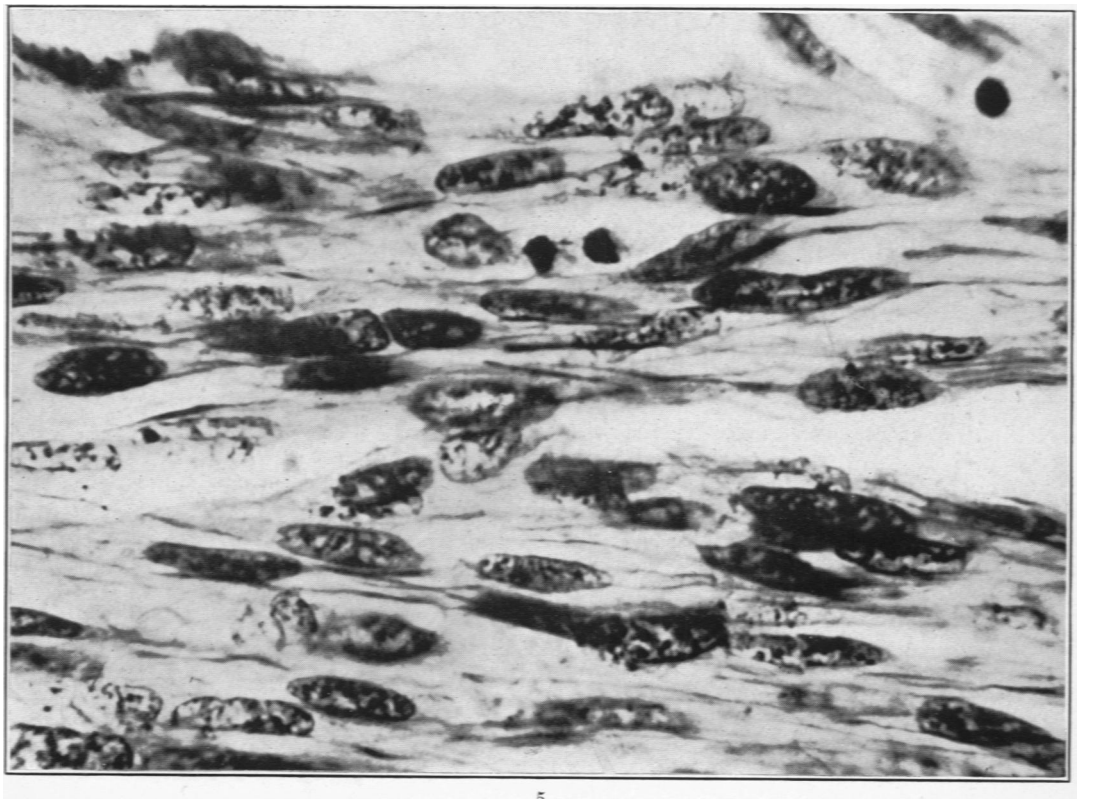
Mallory and Parker Reticulum

PLATE 134

FIG. 5. From a metastasis in a cervical vertebra of a leiomyosarcoma of the uterus. Stained with phosphotungstic acid hematoxylin to show the myoglia fibrils. One diaster present. × 1000.

FIG. 6. From a metastasis of the same leiomyosarcoma to the orbit to show the reticulum between the cells. × 1000.



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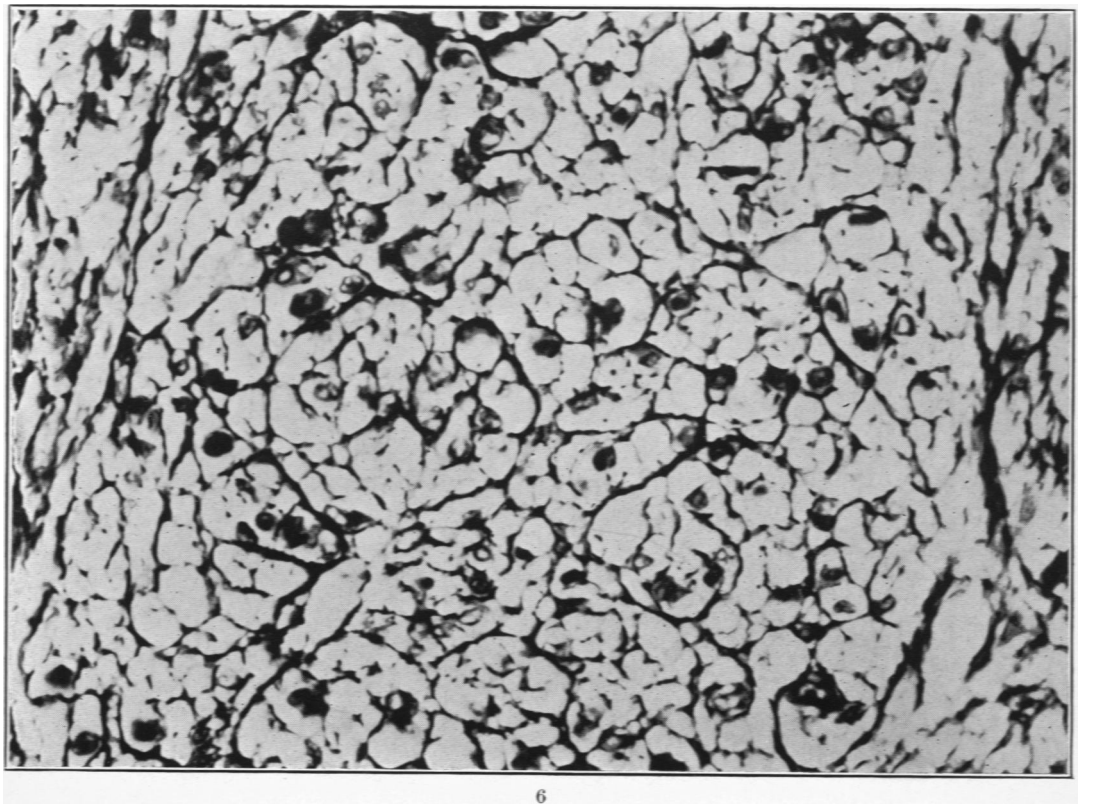

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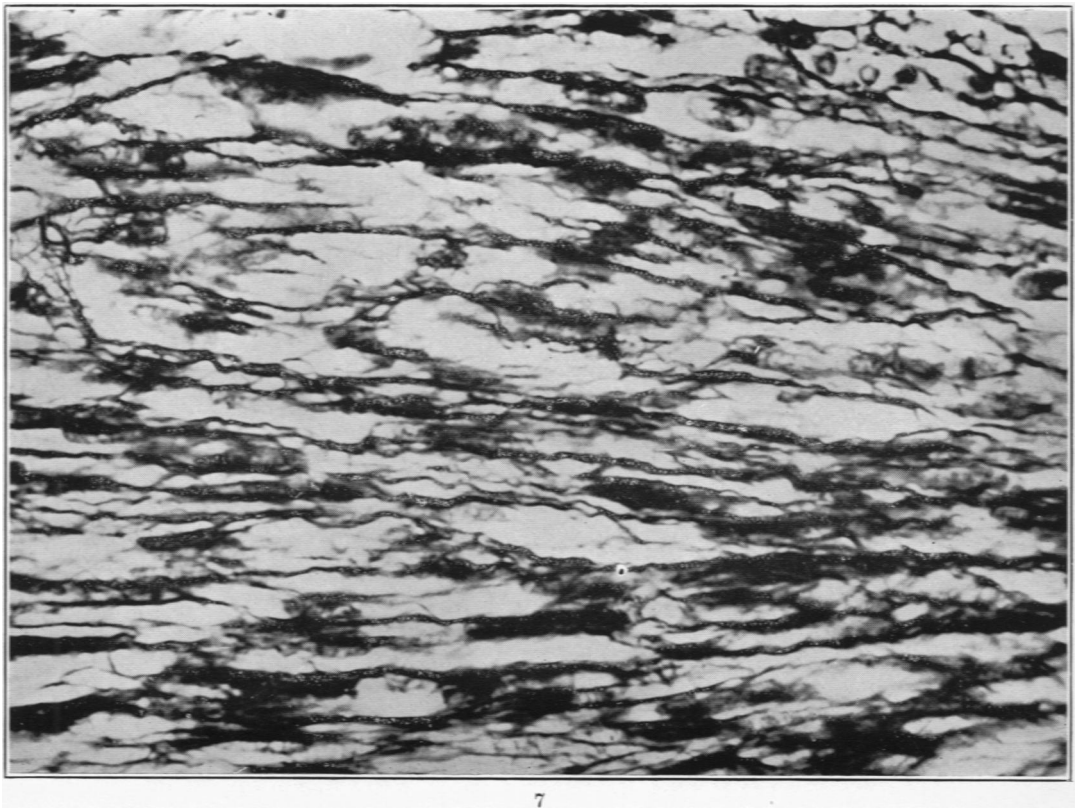
Mallory and Parker Reticulum

PLATE 135

FIG. 7. Longitudinal section of the metastasis (Fig. 5). The reticulum is seen here resolved into collagen fibrils. × 1000.

FIG. 8. A rapidly growing rhabdomyosarcoma projecting into a lymphatic. The fibroblasts of the stroma have furnished a reticulum surrounding single tumor cells or small groups of them. × 250.



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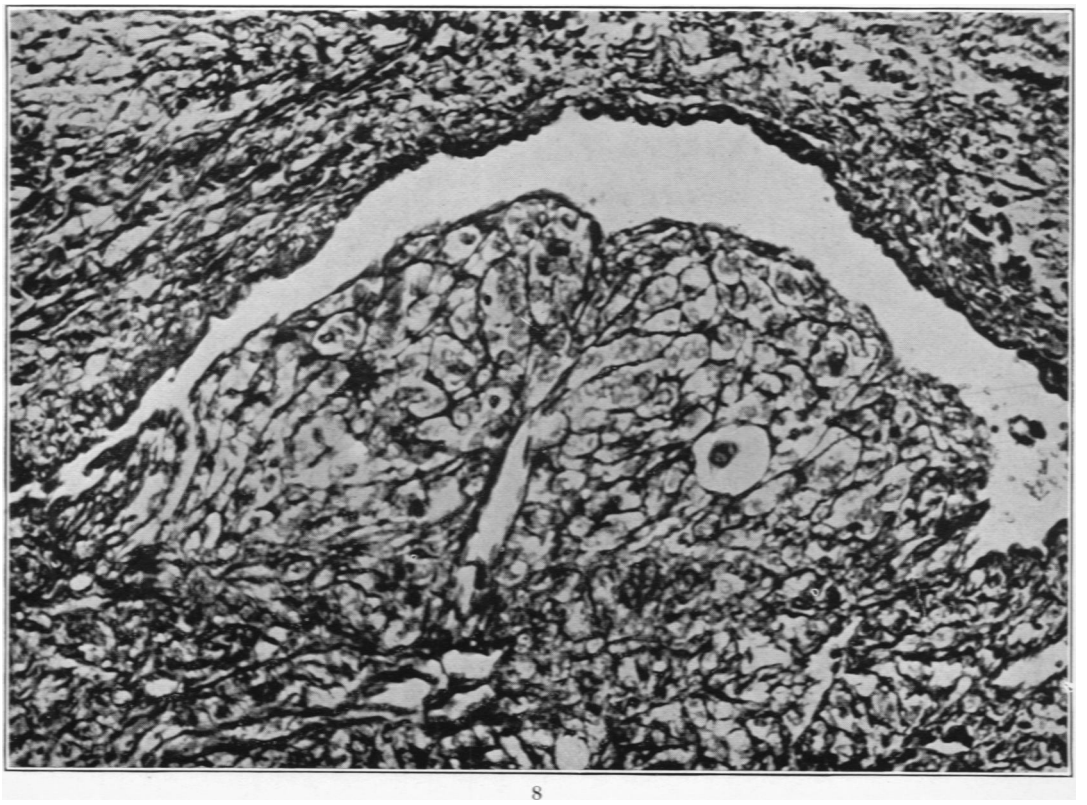

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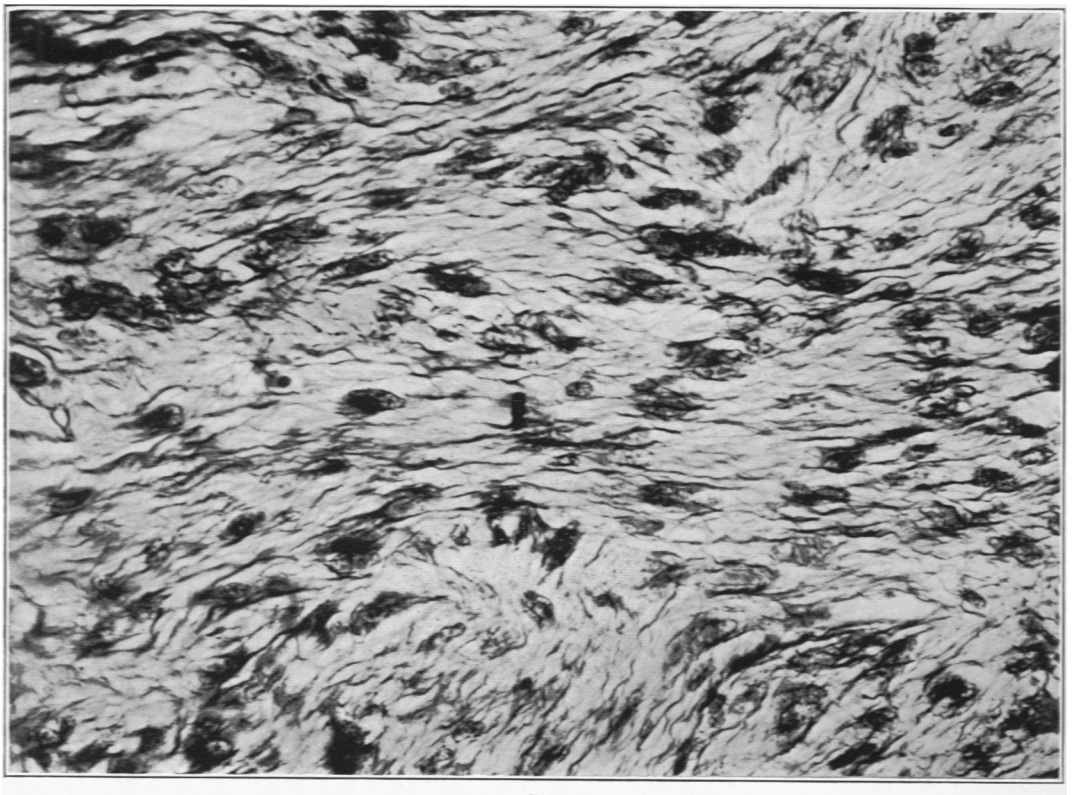
Mallory and Parker Reticulum

PLATE 136

FIG. 9. A rapidly growing fibrosarcoma of the kidney. The fibrillar intercellular substance is stained black by the silver method. One mitosis in the center of the field. × 500.

FIG. 10. Fibrosarcoma of the pectoral muscle in a child. The fibrillar intercellular substance is stained black by the silver method. × 500.



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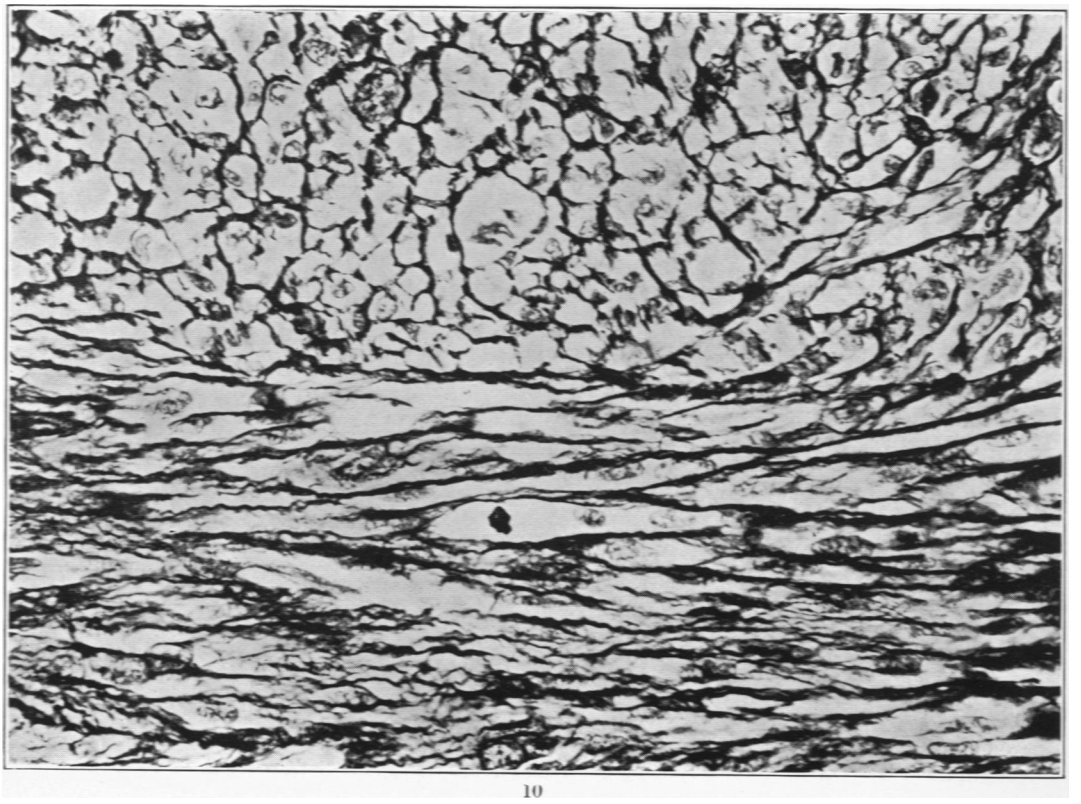

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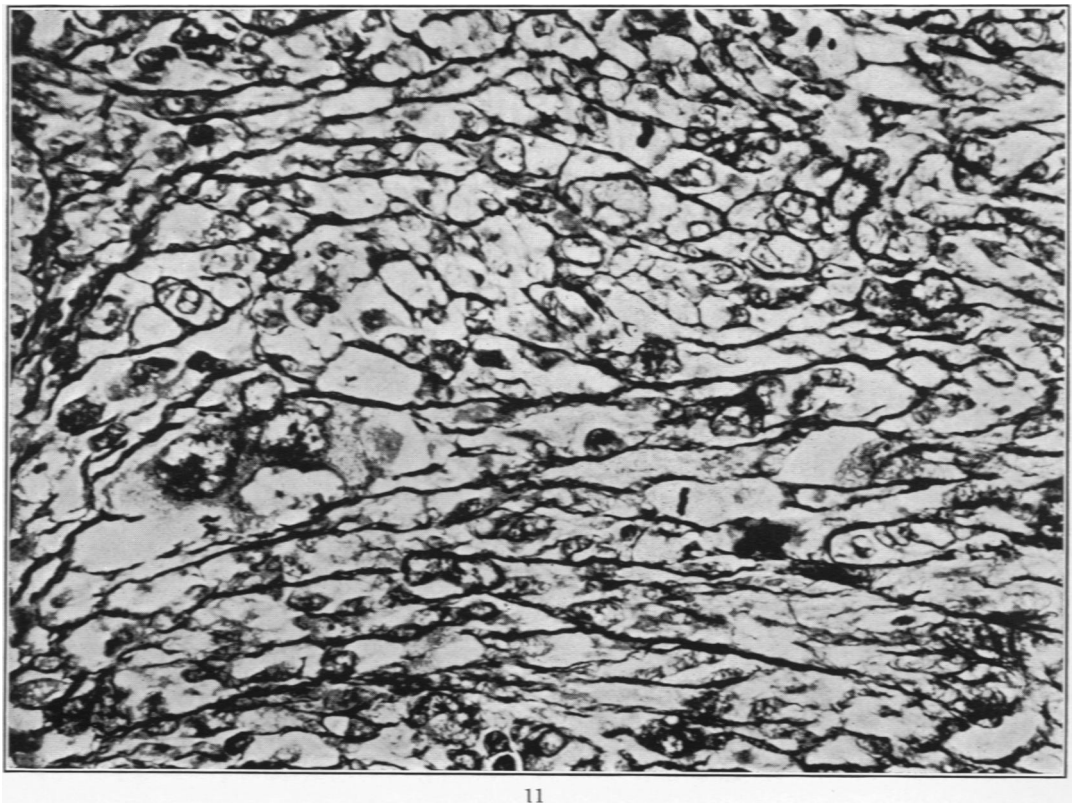
Mallory and Parker Reticulum

PLATE 137

FIG. 11. Same tumor (Fig. 10) showing mitoses and one tumor giant cell. × 500.

FIG. 12. A fibrosarcoma in which the fibrils run in all directions. All the finer ones stain black by the silver method, the coarser ones less intensely. The wavy fibrils in the center are in the wall of a dilated capillary. × 1000.



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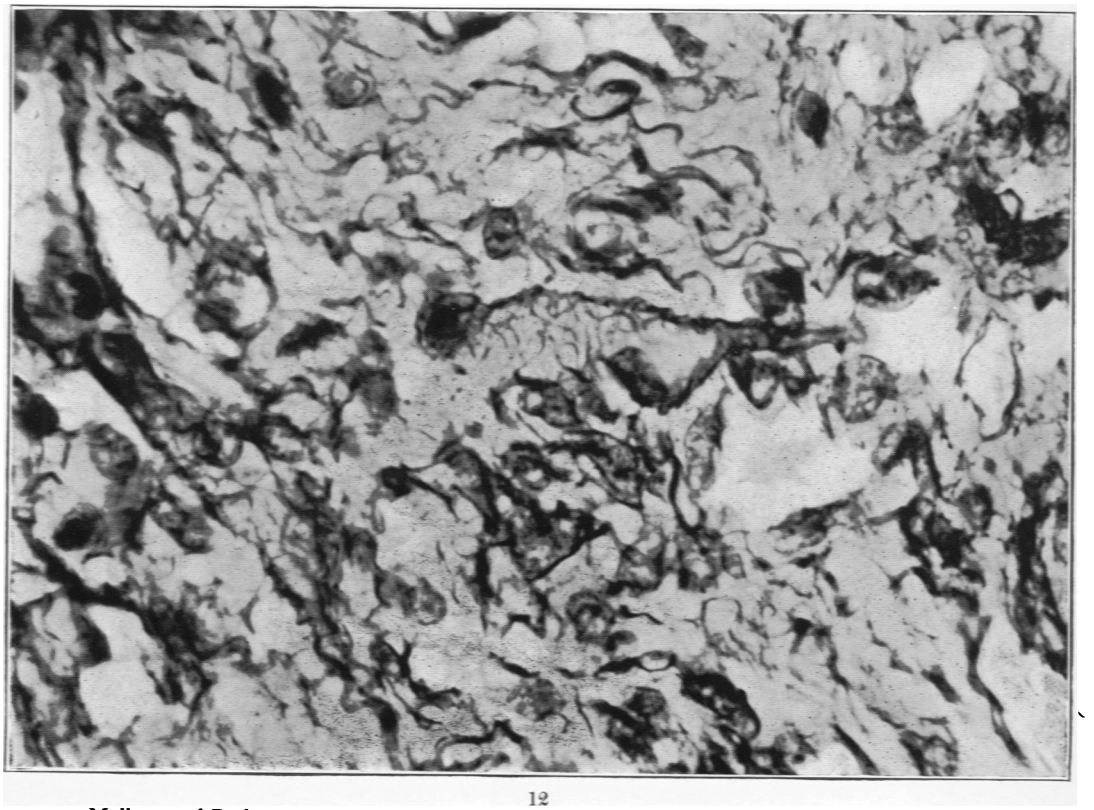

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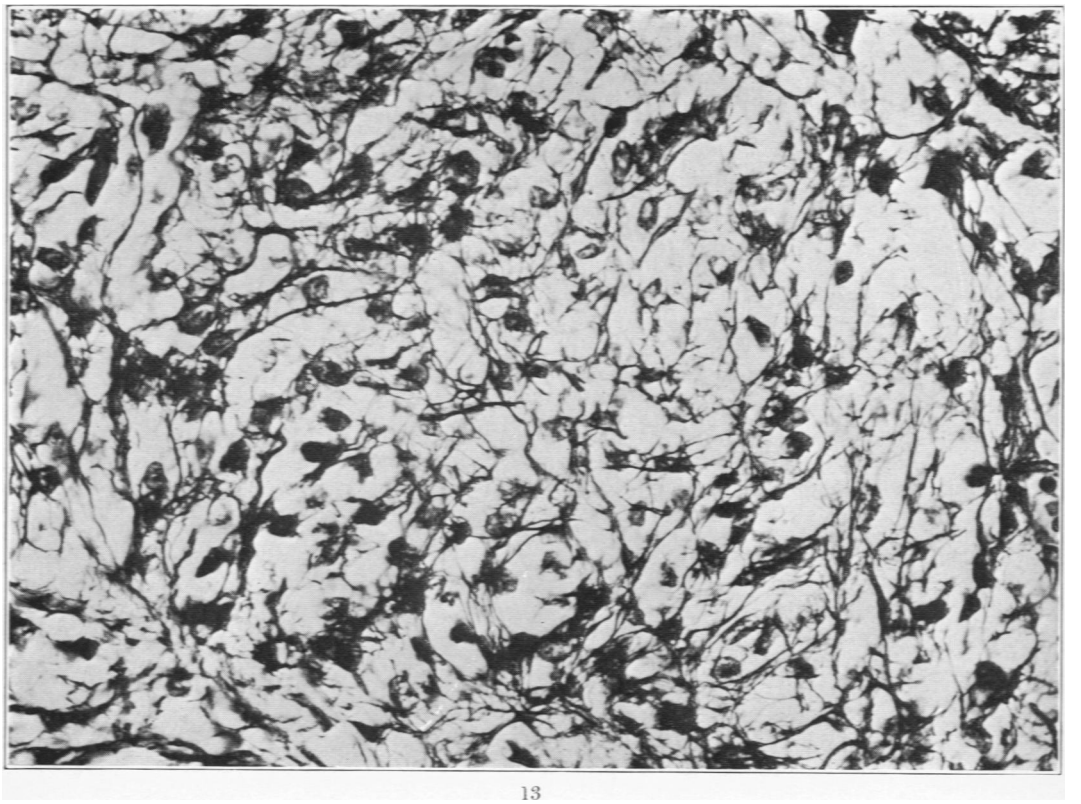
Mallory and Parker Reticulum

PLATE 138

FIG. 13. An edematous fibrosarcoma of the uterus. The separated fibrils all stain deep black by the silver method. × 500.

FIG. 14. Fibromyxosarcoma. The fibrils in the myxomatous portion stain black. × 500.



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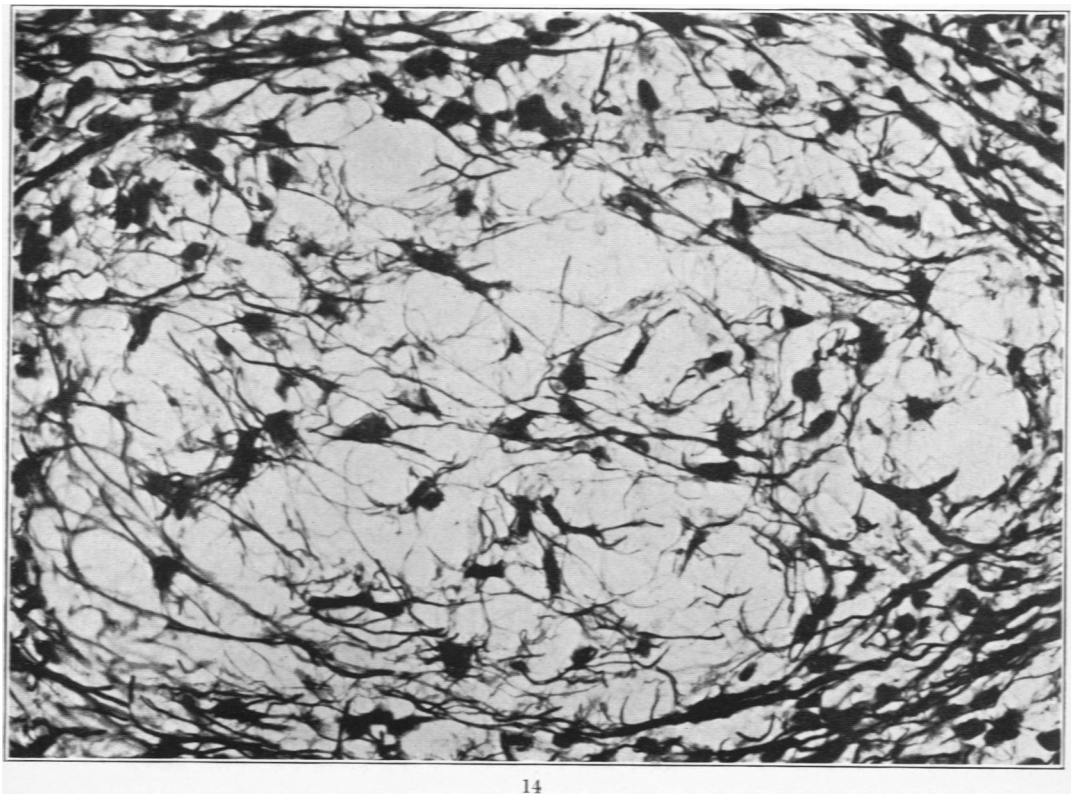

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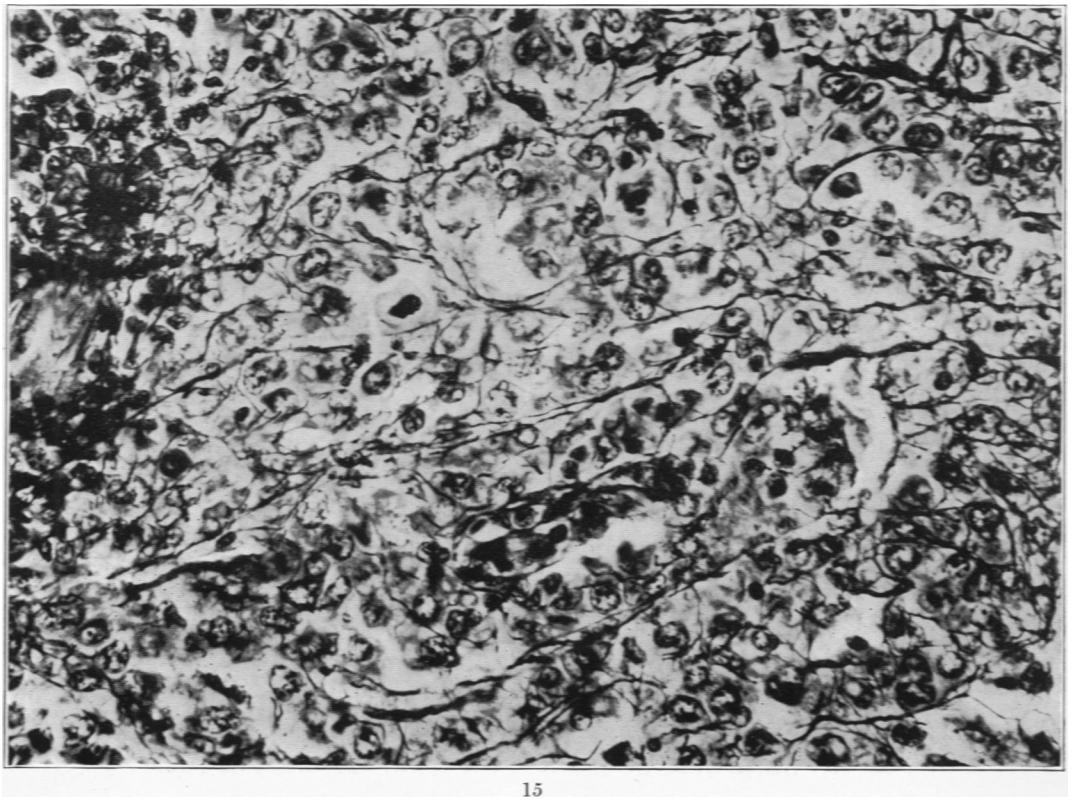
Mallory and Parker Reticulum

PLATE 139

FIG. 15. Lymphoblastoma. One mitosis present. The reticulum furnished by the fibroblasts of the stroma is stained black. × 500.

FIG. 16. A phosphotungstic acid hematoxylin stain of the same tumor to demonstrate the fibroglia fibrils of the fibroblasts. × 1000.



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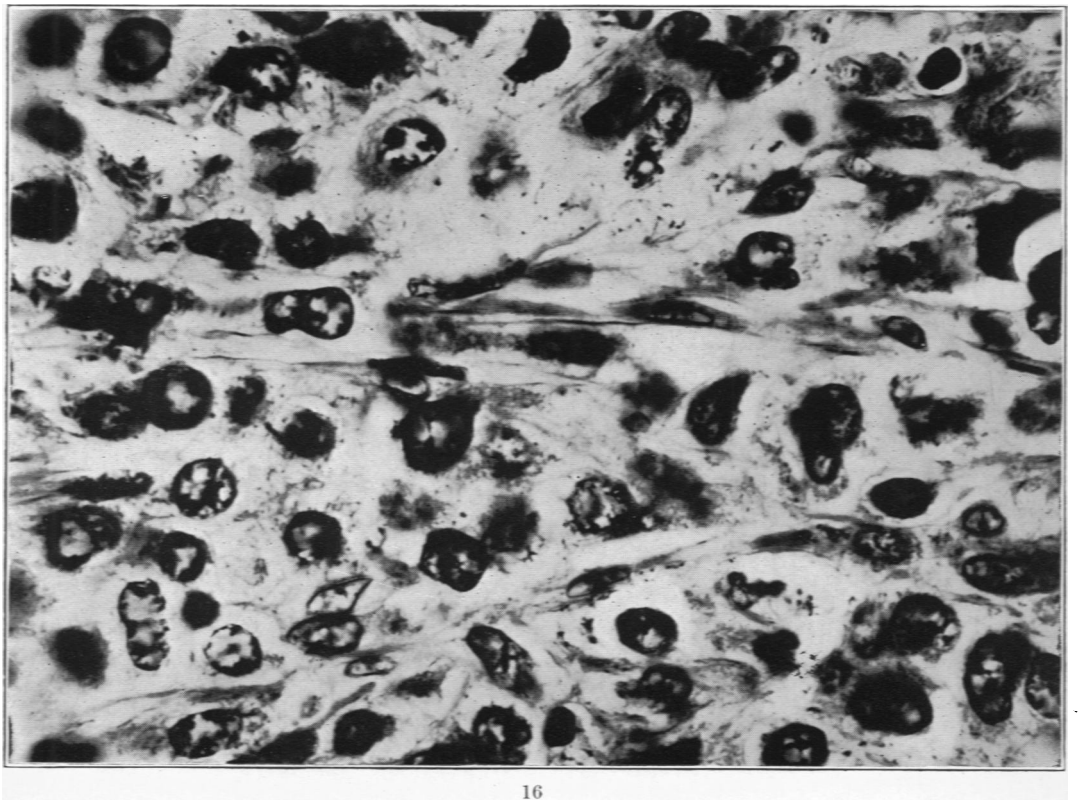

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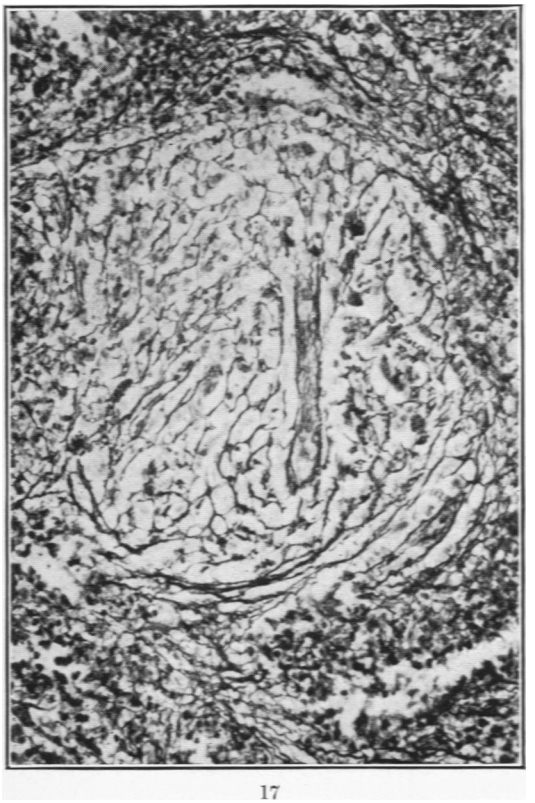
Mallory and Parker Reticulum

PLATE 140

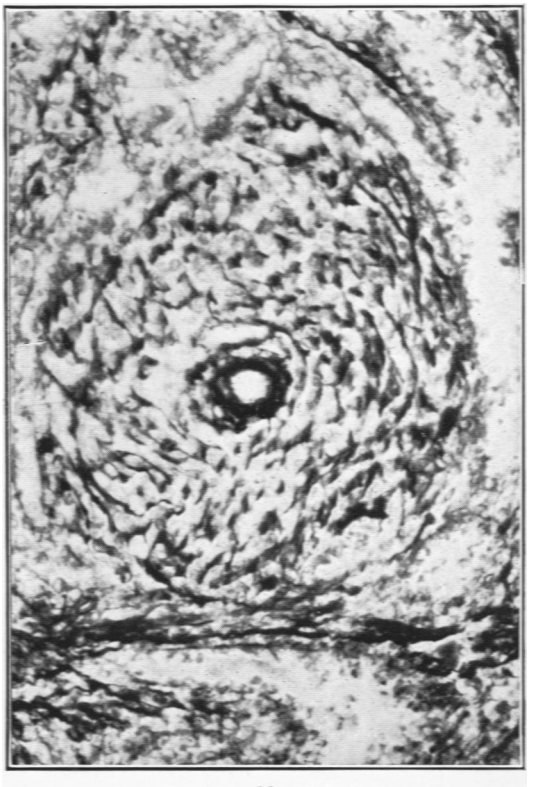
FIG. 17. A silver stain of the reticulum in a lymph nodule of the spleen. × 250.

FIG. 18. Another lymph nodule in the same spleen stained by phosphomolybdic acid hematoxylin. × 250.

FIG. 19. Cancer of the breast. The collagen fibrils running near and between the tumor cells are stained intensely black. × 500.



17



18

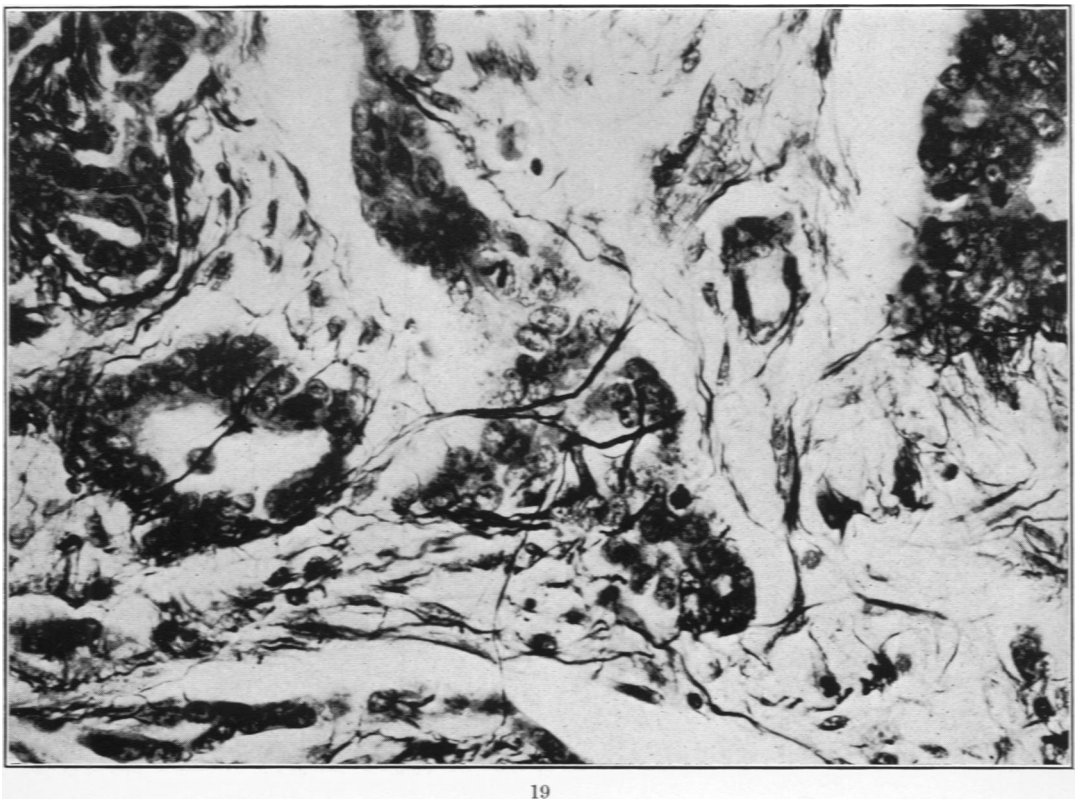


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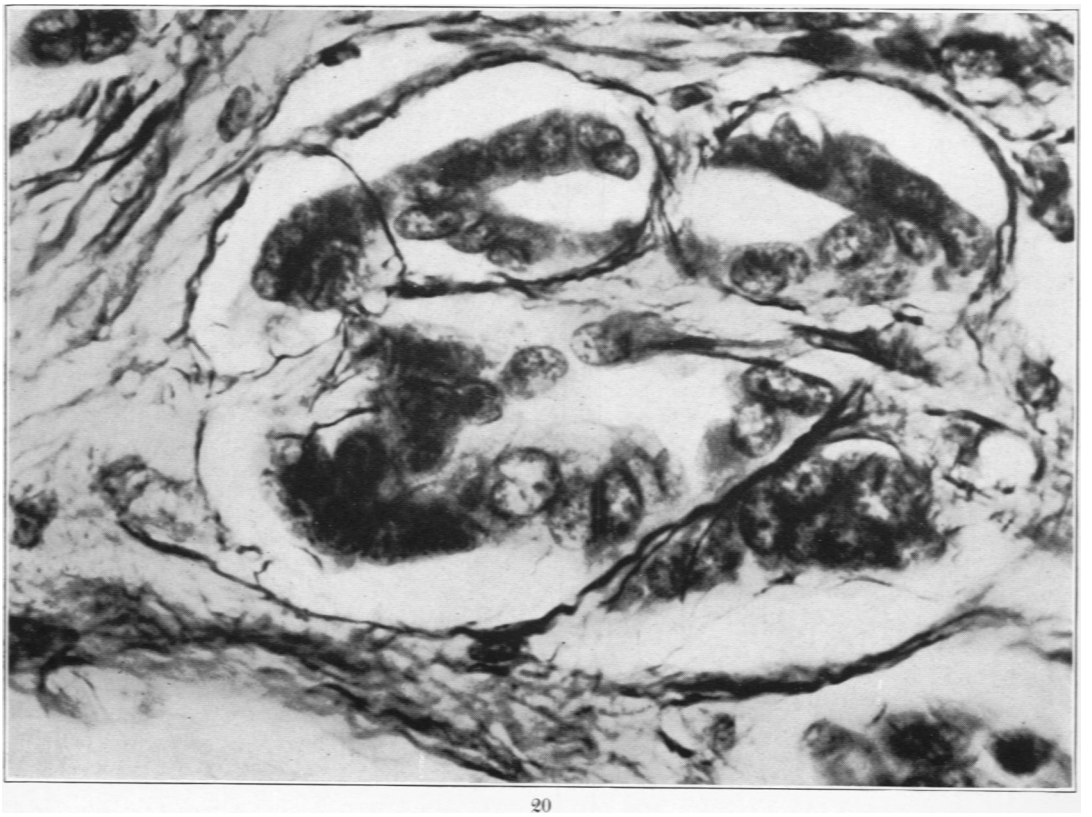
Plate 141

Fig. 20. A similar condition is shown in another illustration from the same tumor (Fig. 19). × 1000.

Fig. 21. In the same cancer the collagen fibrils, separated by elastic fibrils, stain intensely. × 500.



20

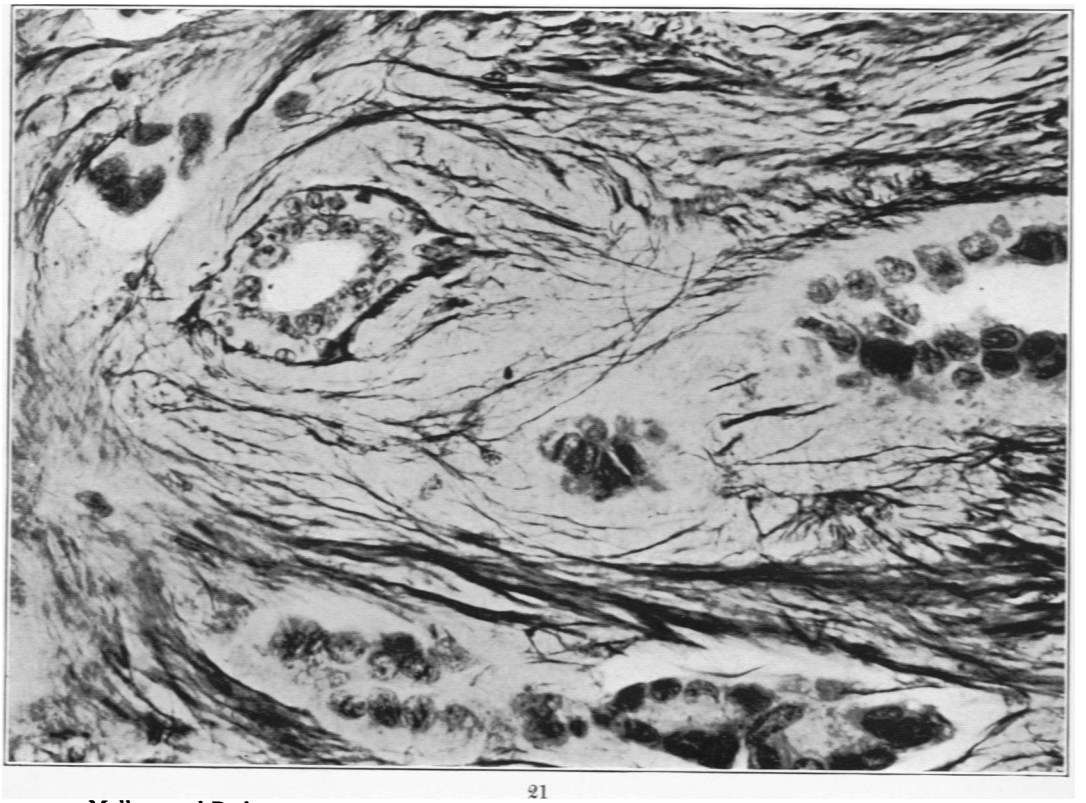


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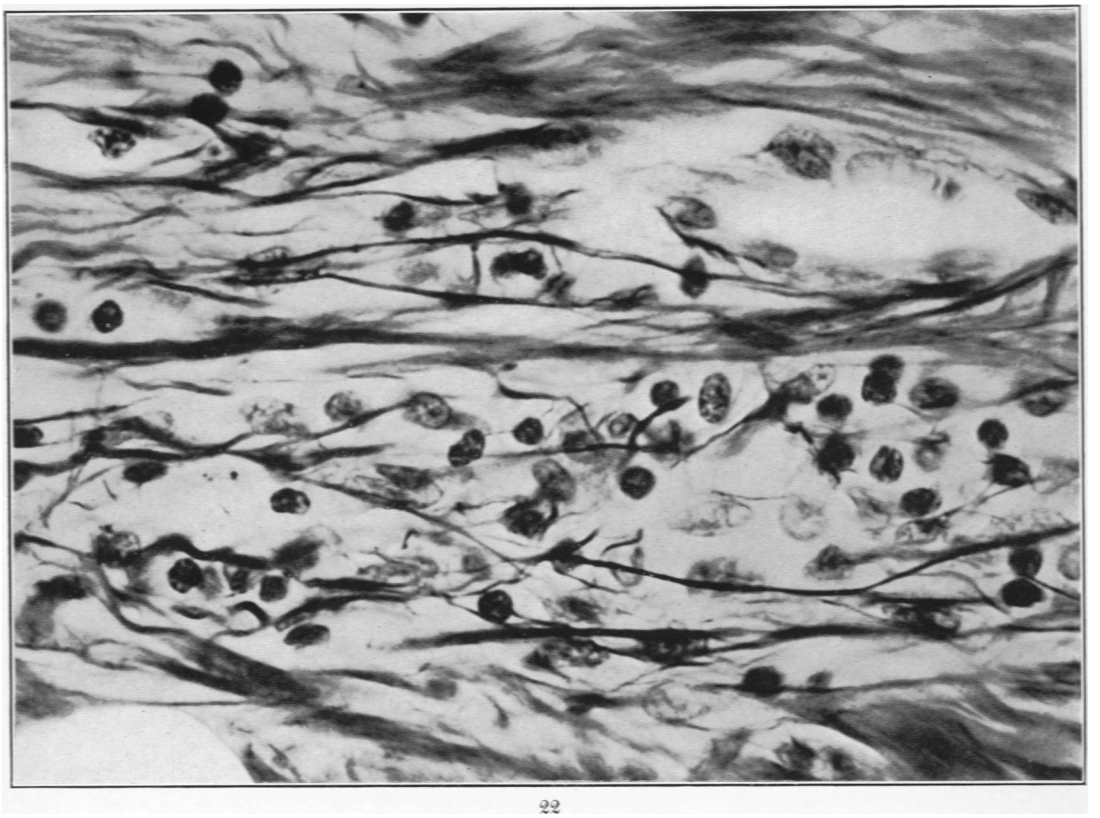
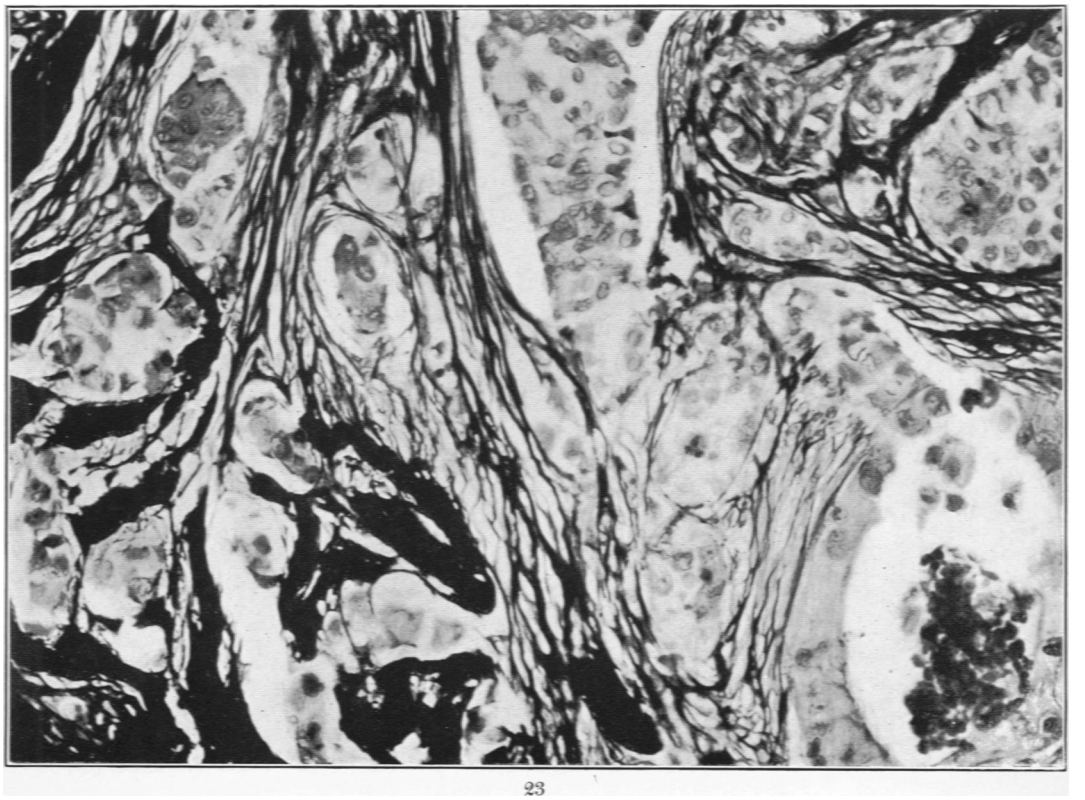
PLATE 142

FIG. 22. The same holds true of fibrils separated by an infiltration of lymphocytes. × 1000.

FIG. 23. Cancer of the breast stained by phosphomolybdic acid hematoxylin. All the collagen fibrils, both those that are separated and those that are compacted, stain deeply. × 250.



22



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