Thyroid Capsule Changes During the Development of Thyroid Hyperplasia in the Rat

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Young adult male Fischer rats were fed 0.25% thiouracil in a low-iodine diet to produce hyperplasia of the thyroid gland. The capsule of the thyroid gland increased in thickness from approximately one cell in controls to a substantial multilayered structure. Increase in capsule thickness was noted by 3 days. The cell population of the capsule was largely fibroblasts, but during a period within the interval from 14 to 28 days, the capsule tended to be exceptionally thick and contained many mononuclear leukocytes. At later times the capsule was not quite as thick and the leukocytes largely disappeared. Capillaries developed in the capsule probably by sprouting. The capsule growth was so extensive that certain neighboring tissues were often incorporated into the capsule, including arteries, veins, nerves, striated muscle, and lymph nodes. There was some regional specificity in the development of capsular hyperplasia. Connective tissue increased around the thyroid and parathyroid glands but not between them. Connective tissue in partitions with the thyroid gland also increased in thickness, although the extent of accumulation of cells and intercellular matrix was much less than in the capsule. (Am J Pathol 93:639–654, 1978)

THE HUMAN THYROID GLAND is surrounded by a substantial capsule.[1] In contrast, the capsule of the rat thyroid gland is barely visible in a histologic section of a well-fixed gland. It consists of a very thin layer of flat cells with widely spaced nuclei and of some extracellular material. During the development of thyroid hyperplasia, however, the capsule increases markedly in thickness. The present report describes the changes that occur in the rat thyroid capsule and in thyroid connective tissue partitions within the gland during the development of thyroid hyperplasia.

Materials and Methods

Male Fischer rats 5 to 8 months of age weighing 270 to 400 g were used. They had been fed Purina Laboratory Chow, but from the beginning of the experiment they were fed tap water and a low-iodine diet (Remington diet, Teklad Mills, Madison, Wis.) containing 0.25% thiouracil, a goitrogen. At each time interval (0, 3, 5, 8, 10, 14, 20, 65, and 100 days of thiouracil feeding), the thyroids of a minimum of 3 rats were examined; thyroids were also examined at other intervals. The rat was anesthetized with pentobarbital, an 18-gauge needle was inserted into the left ventricle of the heart, and the rat was perfused (at a pressure of 140 mmHg for normal and 95 mmHg for thiouracil-fed rats) with a solution of

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2.5% glutaraldehyde and 2.0% formaldehyde in 0.04 M cacodylate buffer containing 3% dextran (molecular weight, 35,000 to 50,000) as previously described.[2] Thyroids were excised and whole lobes were immersed in the same fixative for an additional 30 minutes to 3 hours. They were postfixed in 1% OsO_4 in the same buffer for 1 hour, dehydrated in a graded series of alcohols and propylene oxide, and embedded whole in Epon 812. To embed whole lobes, especially of the larger glands (those stimulated for longer than 10 to 15 days), lobes were allowed to incubate in unpolymerized Epon at room temperature (24 C) for an extended period, sometimes as long as 1 day. In general, lobes floated in the Epon when first immersed, and only after the lobes sank and remained submerged for 30 minutes were they transferred to the oven for polymerization. Thick sections (1.5 μ) were cut with glass knives using a Sorvall MT2 ultramicrotome. Whole lobes were sectioned from pole to pole, and slides were prepared with a series of serial sections on each, usually one slide every 0.3 mm. The sections were stained with alkaline toluidine blue.

Results

The capsule of the thyroid gland is here defined as a layer of connective tissue that is closely adherent to and envelops the thyroid gland. It may include elements in addition to connective tissue, eg, blood vessels, lymph nodes, and nerves. The latter were especially prominent features near the poles and at the side of the thyroid lobe near the boundary between the lobe and the isthmus.

In the normal control rat the capsule was very narrow, being approximately one cell thick (Figure 1). The cell itself was a typical fibroblast: long and narrow with a flat nucleus. Following thiouracil feeding, the capsule thickened. By 3 days the capsule width was noticeably greater (not illustrated), and it increased progressively but slowly for at least 10 days (Figure 2). During this time the shape and character of the cells forming the capsule were unchanged. However, between 10 and 20 days of thiouracil feeding there was generally a gross increase in capsule thickness (Figures 3, 7 through 10). Mitotic figures were observed but were relatively uncommon. Cells resembling fibroblasts were still present (Figures 3, 7, 9, 10), but there were also cells that were round or cuboidal with round or irregularly shaped nuclei (Figures 3, 7 through 10). Some cells had relatively dense-staining nuclei. Those with round nuclear profiles and little cytoplasm resembled small lymphocytes (Figure 8). Many of those with irregularly shaped or flattened nuclear profiles resembled monocytes (Figure 9). The distribution of these cells with dense nuclei was nonuniform, and the character of the thickened capsule varied markedly (Figures 7 through 10). Although the cells were generally packed close together, in places they were only loosely packed (Figure 7). There were areas in which there were stratified layers of flat cells on the outside of the capsule as in a mature capsule (Figure 9) but where small or irregularly shaped cells were present toward the inside of the capsule. In

other areas, the positions of the two layers were reversed (Figure 7), or all cell types were mixed instead of being segregated in regions.

The appearance of small round cells with densely staining nuclei as in Figure 8 was only transient. By 28 days of thiouracil feeding (not illustrated) and thereafter, small round cells were seen only occasionally, although small patches were sometimes found as late as 65 days. When they disappeared (Figures 4 through 6), the capsule was considerably narrow than when they were present but was much thicker than before the appearance of the round cells. At these late intervals, the cells in the capsule were largely flat, fibroblast-like cells with considerable space between the nuclei, suggesting that the cells either had a good deal of cytoplasm and/or matrix between the cells. There was some suggestion that the capsule was narrower at 100 days than at 65 days, but capsules were irregular in thickness and no effort was made to make a careful comparison.

Connective Tissue Partitions

Connective tissue partitions were noted primarily in thyroid glands from rats fed thiouracil. They were as thin as the capsule, in general, in normal controls (not clearly illustrated), but by 10 days many substantial partitions were observed penetrating from the capsule into the space between follicles (Figure 11), and the connective tissue partition response at this time was sometimes greater than that of the capsule. The partitions were somewhat more striking at 20 days (Figures 12 and 13) but they never became as thick as the capsule at this time. They were long and intersected with others and frequently were essentially without interruption. However, by 65 days (Figure 14) they were much less thick and appeared interrupted by blood vascular channels.

The cell complement in these partitions appeared to be largely fibroblasts. When cells with dense nuclei were observed in the capsule, they were also seen in the connective tissue partitions, although they were in smaller number and primarily in subcapsular regions (Figure 13). At later intervals, they disappeared from the connective tissue partitions just as they did from the capsule.

Blood Vessels and Lymphatics

Blood vessels were observed within the capsule and connective tissue partitions. Although some were veins of appreciable size (Figures 3, 6, and 9), they were usually tiny capillaries and were much smaller than the capillaries associated with nearby follicles. Lymphatics were observed in the connective tissue partitions occasionally (not illustrated). They were not abundant and will be described more fully elsewhere.

Regional Differences in the Capsule Response

There were regional differences in the connective tissue response. The capsule between the thyroid and parathyroid glands normally appears to be as thin as the thyroid capsule (Figure 15). However, when the rat was fed thiouracil and the thyroid capsule thickened, that part between the thyroid and parathyroid did not thicken (Figures 16 and 17). On the other hand, the rest of the capsule outside the parathyroid thickened, sometimes as much as the rest of the thyroid capsule, although the parathyroid itself did not exhibit a hyperplastic response. Some small blood vessels just outside the parathyroid did enlarge, however (not illustrated here).

Incorporated Neighboring Tissues

In addition to the fibroblasts and the associated extracellular materials, the capsule of the hyperplastic thyroid gland frequently was found to contain major veins, arteries, and nerves in the region near the trachea (Figure 18), striated muscle (Figure 19), and a lymph node (Figure 20) by 20 days. These were close to the surface of the normal thyroid and became incorporated into the capsule as it enlarged.

Discussion

The growth of the capsule during the development of thyroid hyperplasia is a very striking phenomenon. The percent increase in the volume of the capsule is several times more than the percent increase in functional epithelium.[3] Although an important part is clearly an increase in connective tissue, including fibroblasts, and extracellular materials, a small part of the increase in the capsule is by incorporation of neighboring nonthyroid tissue such as nerve, artery, veins, striated muscle, and, possibly, lymph node (Figures 18 through 20). The presence of striated muscle in the thyroid has been noted before,[4] but the present observations seem to be unrelated.

The increase in capsule thickness starts within a few days after the beginning of thiouracil feeding, and a major increase occurs between 11 and 20 days of thiouracil feeding. At intervals longer than 30 days, the thickness of the capsule appears to be stable; it may even decrease slightly. The major increase (during the period of 2 to 3 weeks of thiouracil feeding) is associated with the presence of cells with relatively dense nuclei, resembling lymphocytes and monocytes. Ultrastructural examination of these cells shows that they have the organelle complements of lymphocytes and monocytes.[5]

Vascular Response

The increase in capsule thickness is primarily a connective tissue response and not a vascular response. In this respect it differs from the response in the interior of the gland in which the thyroid volume change in rats fed thiouracil for long intervals is largely due to an increase in the volume of blood vessels. The blood vessels in the capsule are probably largely newly formed (many are seen as sprouts by electron microscopy [6]) and small (at least initially at 20 days). They are grossly different from those in the interior of the thyroid lobe which are largely if not entirely preformed and become very large during the development of hyperplasia.[7] The general vascular response within the connective tissue partitions is similar to that in the capsule and involves tiny capillaries despite the fact that the partition is embedded between follicles and their associated capillaries which enlarge enormously.

Origin of Capsular Fibroblasts

There are several possible sources of the new fibroblasts in the growing capsule during the development of thyroid hyperplasia: 1) New fibroblasts could be derived from preexisting capsular fibroblasts by mitosis. Mitotic cells are seen only infrequently in the capsule, but it is clear from studies of nuclear labeling with tritiated thymidine that they must divide at an appreciable rate.[8] On the other hand, it is difficult to interpret these observations since there are a variety of cell types in the capsule and the mitotic cell types have not been identified. It is not clear to what extent mitosis of fibroblasts can account for capsular growth. b) Capsular fibroblasts could arise by adherence to the capsule of fibroblasts from neighboring loose connective tissue. In some areas the cells in the growing capsule are loosely packed; this might be a transition region. However, the cells do not have the shape of fibroblasts and have not been identified. In any case, such a mechanism of capsule growth by accretion might explain the lack of the capsule response between the thyroid and parathyroid glands since this region is not in direct contact with the loose connective tissue around the thyroid gland. c) Fibroblasts might originate by conversion of the mononuclear leukocytes in the capsule into fibroblasts. In the analogous case of wound healing, this was at one time considered the origin of the fibroblasts in healing wounds, although it may now be considered unlikely from the results of parabiotic studies by Ross, Everett, and Tyler.[9] No direct evidence is available on this for the thyroid.

Leukocytes in the Capsule

Lymphocytes and monocytes are found in the capsule during its period of most rapid growth. This is reminiscent of some other cases in which

there is the formation of a well-defined capsule. Such cases include the foreign body reaction to the subcutaneous implantation of a sheet of plastic [10,11] and the formation of a capsule around some tumors.[12] In some respects the phenomenon differs from the classic picture of inflammation. The leukocytes are in and near areas of rapid growth rather than of repair. The blood vessels are dilated in the subcapsular region nearby but not particularly in the capsule. There are no signs of thromboses or vascular stasis or edema. However, there may be platelet aggregation, adherence of lymphocytes and monocytes to blood vessel walls in or near the capsule, and, possibly, some microhemorrhage [2]; the peak in microhemorrhage in the thyroid preceded the presence of leukocytes in the capsule by a week or more, and almost all traces of extravasated erythrocytes were gone before sticking leukocytes were noted.

The source of the leukocytes in the capsule is uncertain. Some might have come from the blood within the gland as suggested by the adherent lymphocytes and monocytes mentioned above. However, the occasional high incidence of lymphocytes toward the outside of the capsule suggests that some may have migrated from outside the gland. A lymph node was generally present in or on the capsule and it might conceivably have served as a source of some cells.

A possible attractant for the monocytes is the newly formed collagen being laid down by fibroblasts in the capsule since solubilized collagen and some of its fragments have been reported to be chemoattractive for monocytes.[13] Chemoattractive factors in addition to collagen are probably in the growing connective tissue, however, as it appears that collagen and its fragments are not attractants for the lymphocytes that also accumulated in the capsule.[14]

The functional role played by the monocytes in the capsule is unclear. It is possible that they might not have any function and just accidentally be attracted by the same agent, ie, collagen, that attracts fibroblasts.[15] It is possible, however, that they are part of a mechanism for building up a capsule rapidly. In wound healing there is evidence for a local function of the monocytes since activated monocytes can produce a substance that stimulates growth in fibroblasts.[16] Further studies will be required to see if monocytes can serve a similar purpose in the capsule growth. For example, there is no information on whether the monocytes are activated in the capsule.

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Acknowledgments

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[*Illustrations follow*]

Figure 1—Control gland. A section showing the peripheral area of the thyroid gland. There is an absence of an obvious capsule (*CS*) at the surface of the gland. There are some loose strands of connective tissue. Thyroid follicles with distended lumens (*L*) appear normal. (×330)

Figure 2—Thyroid gland from a rat fed thiouracil for 10 days. There is a substantial capsule (*CS*) approximately five layers of flat cells in thickness. Follicle lumens (*L*) are very narrow, and capillary lumens (*CL*) are enlarged. (×330)

Figure 3—Rat fed thiouracil for 20 days. The capsule (*CS*) is grossly enlarged, and the outer part contains several enlarged venules (*V*) and some small capillaries (*SC*). The region between the capsule and the thyroid follicles is largely free of capillaries, although there is a narrow one at the left (*arrow*). (×220)

Figure 4—Rat fed thiouracil for 65 days. The capsule (*CS*) is narrower than at 20 days although much thicker than at 10 days. The cells are generally flat. Narrow capillaries (*S*) are present in the capsule but none is apparent in the region (*arrow*) between the follicle and the capsule. *L*, follicular lumen. (×330)

Figures 5 and 6—Rats fed thiouracil for 100 days. The capsule (*CS*) is irregular in width; the capsule (*CS*) in Figure 6 is narrower than that in Figure 5. There are small as well as large capillaries. There are capillaries in the thyroid follicle area that extend into the region between the capsule and the follicle. *CL*, capillary lumen; *L*, follicular lumen. (×330)

Figures 7 through 10—Four areas of capsule (*CS*) from a single thyroid lobe showing variation in capsule morphology in a rat fed thiouracil for 20 days. Outside of the gland is to the left. (×330)

Figure 7—An area of loosely packed cells that are toward the outside of the gland. The region toward the follicles has a very mixed population, including flat fibroblast-like cells. The *arrow* marks the boundary between the capsule and follicular epithelium.

Figure 8—An area containing a high population density of small cells with densely staining nuclei resembling lymphocytes (*arrow*). The inner region contains cells with larger and paler staining nuclei, some of which are flat.

Figure 9—An area in which the cells in the outer part of the capsule are flat and fibroblast-like. Some have flat dense nuclei and may be monocytes (*arrow*). The inner layer has many closely packed small round cells with relatively densely stained nuclei. *L* marks a distended follicular lumen.

Figure 10—An area with a very mixed population of cells including some small clusters of epithelial cells and small follicles. *L*, follicular lumen; *CL*, capillary lumen.

Figures 11 through 14—Comparison of the connective tissue partitions within the thyroid gland at different stages of the development of thyroid hyperplasia.

Figure 11—Rat fed thiouracil for 11 days. There are many connective tissue partitions (*arrows*) extending from the capsule (*CS*) into the gland. Capillaries recognizable from the enlarged empty lumens (*CL*) frequently border on the partitions. A few small capillaries (*SC*) are recognizable within the partitions. *F*, follicle. (×130)

Figure 12—Rat fed thiouracil for 20 days. A somewhat more prominent connective tissue partition (*arrows*) than in Figure 11, extending from a portion of a thicker capsule (*CS*) than in Figure 11. Small capillaries (*S*) can still be recognized in the partition. Note that the partitions are long and continuous. (×130)

Figure 13—Rat fed thiouracil for 20 days. Connective tissue between a large capillary or venule and a follicle with a large lumen (*L*). The region is just subjacent to the capsule at the *top* of the figure. There are many small cells with dense nuclei (*arrowheads*) adhering to the wall of the vessel and many in the connective tissue (*arrows*). The capsule, at this stage of stimulation, may also be heavily infiltered with lymphocyte-like cells, as in Figure 8.(×330)

Figure 14—Rat fed thiouracil for 65 days. Connective tissue partitions can still be found within the thyroid gland. They tend to be much less prominent than at shorter times of stimulation. They are narrower and sections of them are relatively short segments (with gaps between them [*arrows*]), partially covered by endothelial cells (*E*) and partially by follicular epithelium. Some follicles have distended lumens (*L*). There are small capillaries (*S*) still present in the partition and also somewhat larger capillaries (*C*), but these are much smaller than the general vascular spaces (*VS*). (×130)

Figures 15 through 17—Specificity of the capsule response. These figures show that capsule between the thyroid (*T*) and parathyroid (*P*) glands does not enlarge when the capsule enlarges around the outside of the combined thyroid-parathyroid glands.

Figures 15—Control gland. The section shows the narrow capsule (*CS*) at the outside of the thyroid and parathyroid glands and the narrow partition (*arrow*) between the thyroid (*T*) and parathyroid glands (*P*). (×130)

Figure 16—Rat fed thiouracil for 20 days. The capsule outside the glands (*CS*) is very much increased in thickness, whereas the partition between thyroid and parathyroid is barely perceptible at this magnification. (×130)

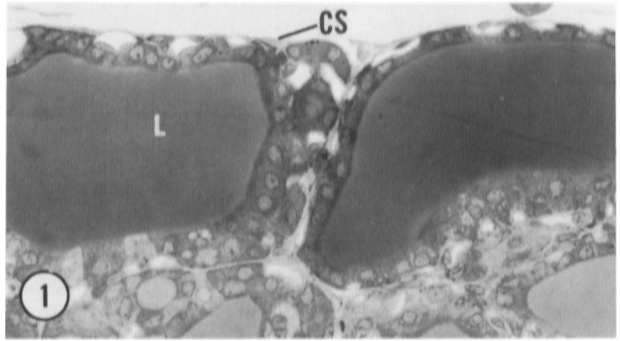
Figure 17—Detail from the lower end of the boundary between thyroid (*T*) and parathyroid (*P*) in Figure 16. Note the narrow partition (*arrow*) between the glands. (×330)

Figures 18 through 20—Incorporation of neighboring extrathyroidal tissue into the thyroid capsule. Rats fed thiouracil for 20 days.

Figure 18—Major nerve (*N*), artery (*A*), and vein (*V*) within the thyroid capsule (*CS*). (×130)

Figure 19—Section showing that a thin layer of flat cells (*arrow*) at the outer edge of the capsule (*CS*) surrounds both the capsule of the thyroid and a layer of striated muscle (*M*). (×330)

Figure 20—Section showing a portion of a lymph node (*LN*) embedded within the thyroid capsule (*CS*). Note the thin layer of cells from the capsule (*arrow*) peripheral to the lymph node. (×130)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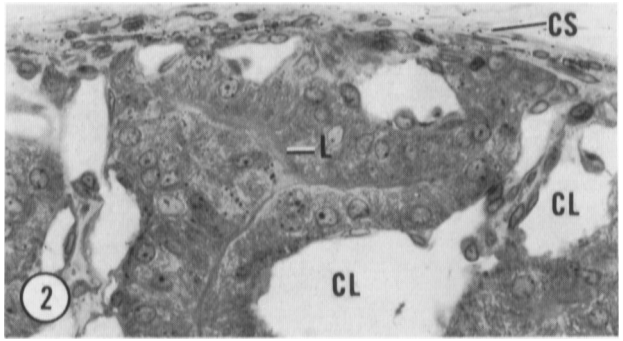
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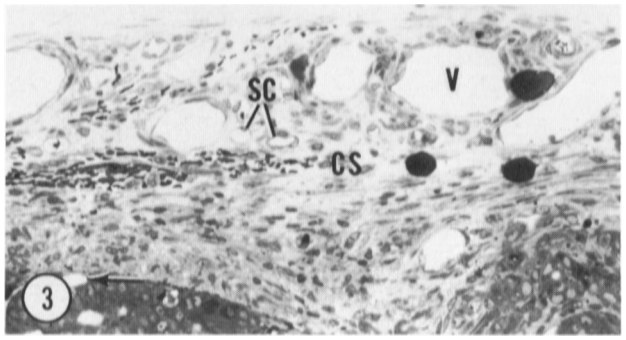
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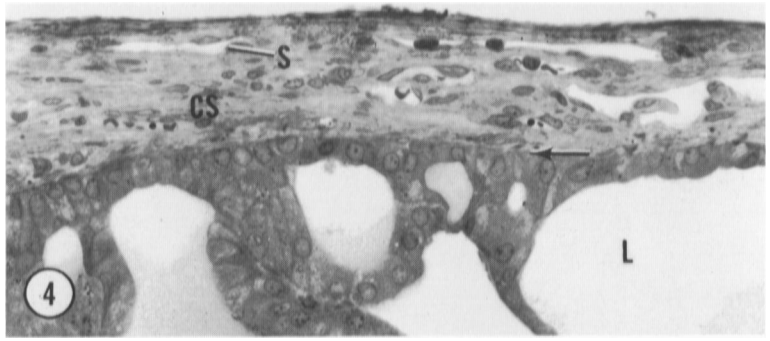
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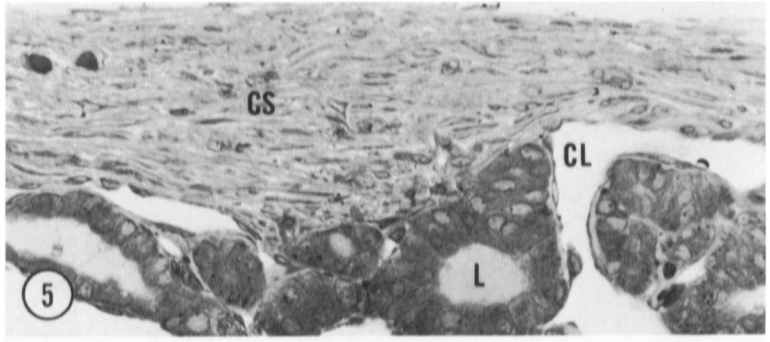
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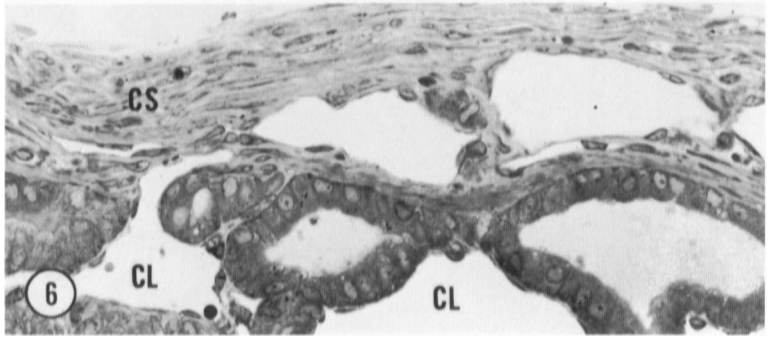
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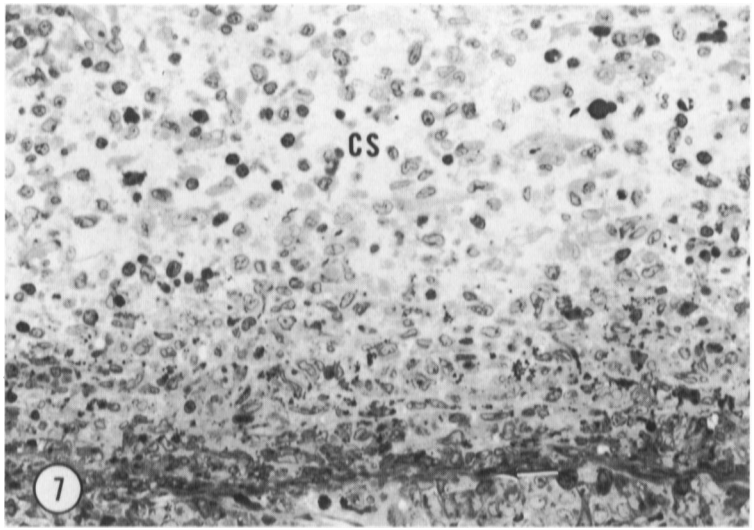
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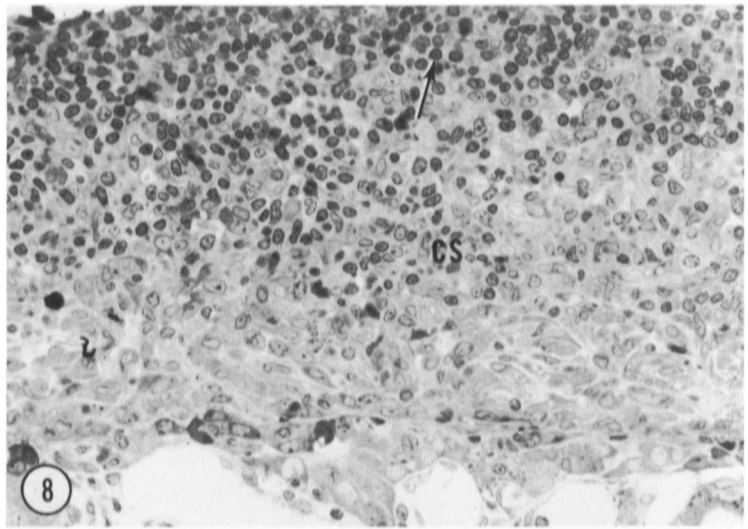
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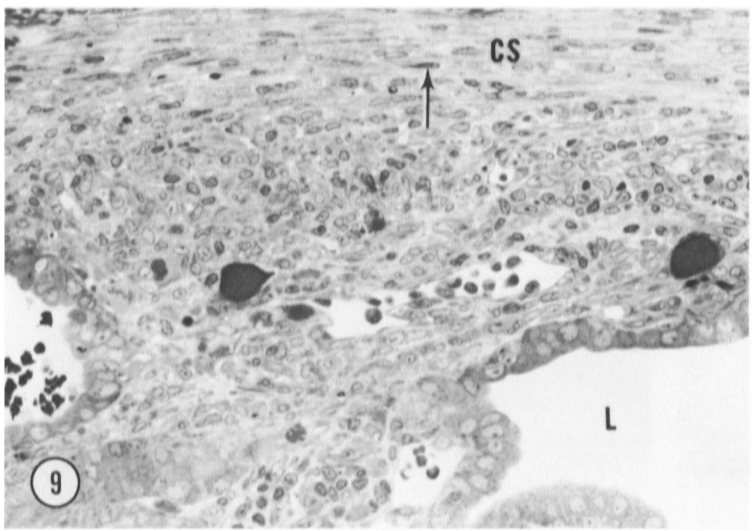
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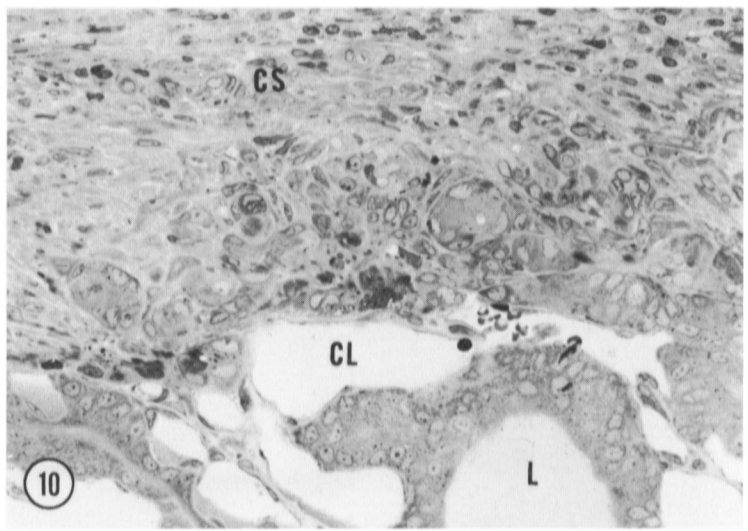
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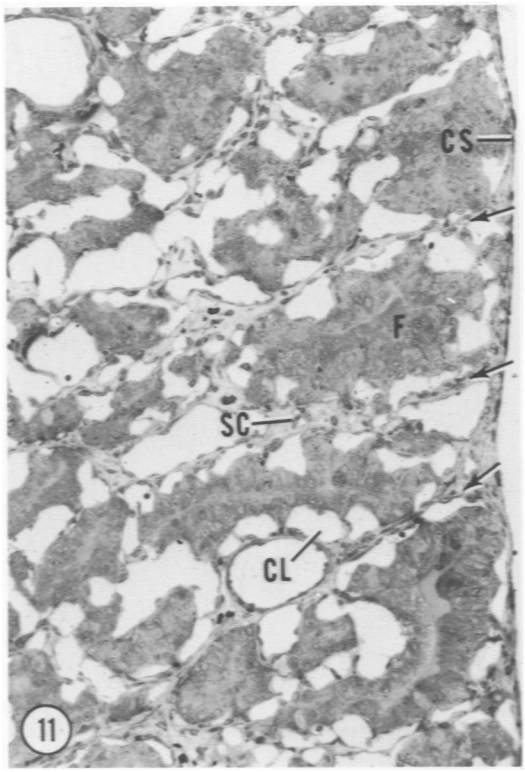
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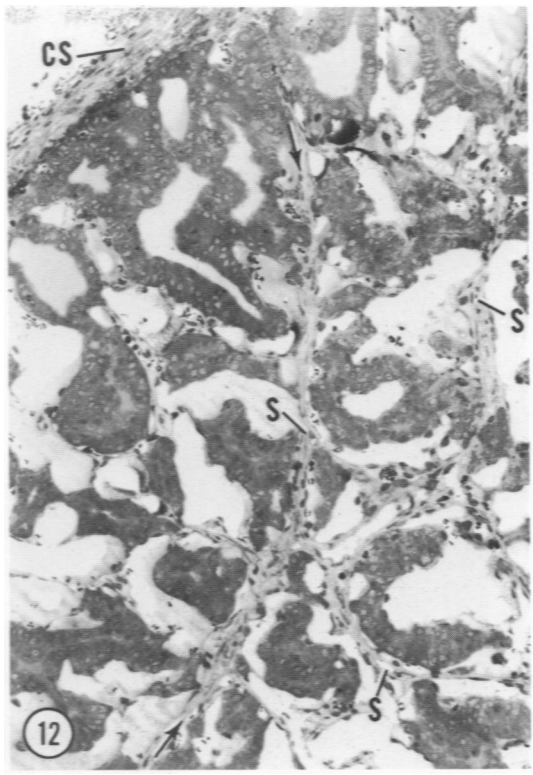
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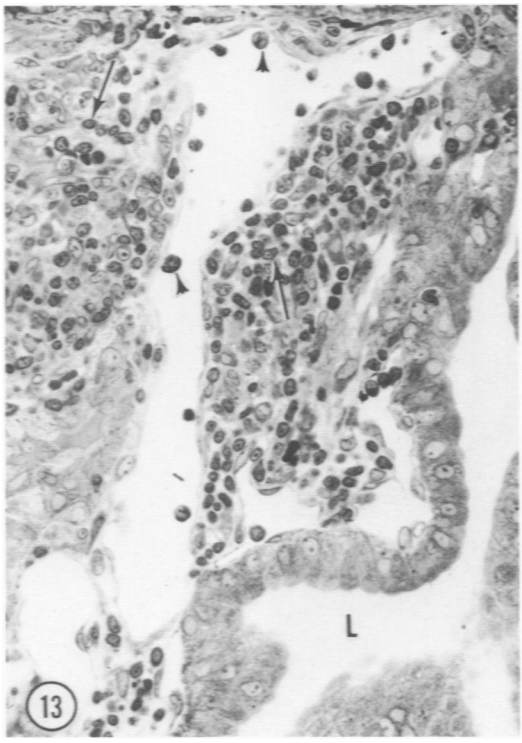
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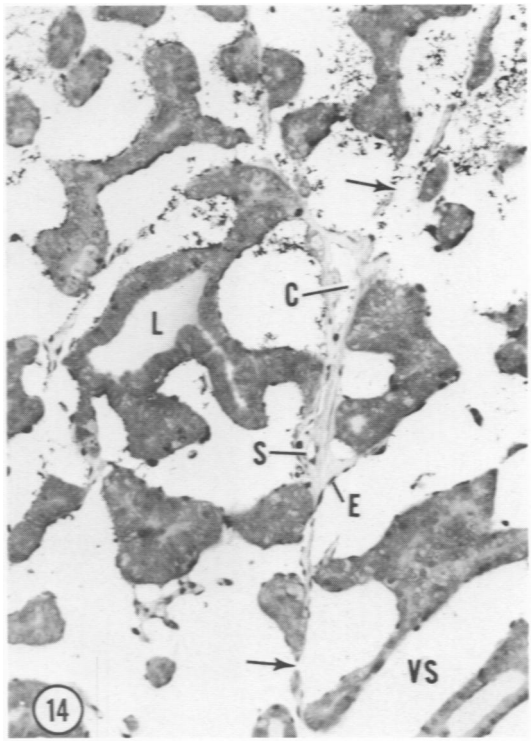
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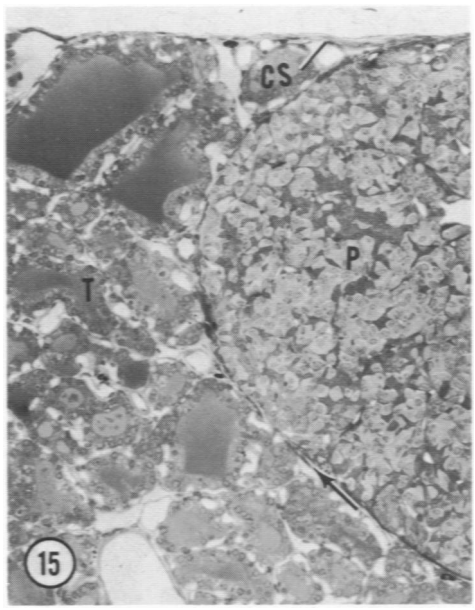
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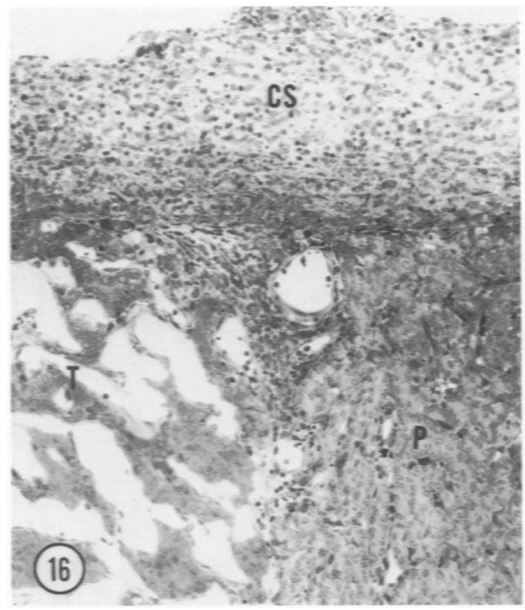
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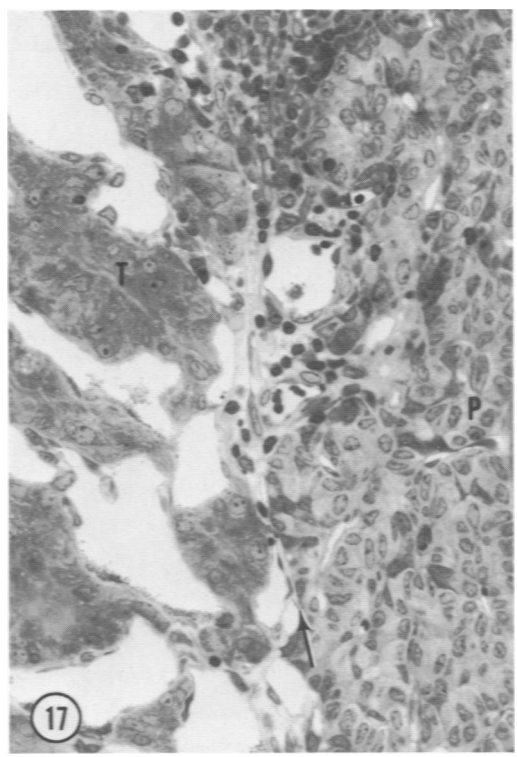
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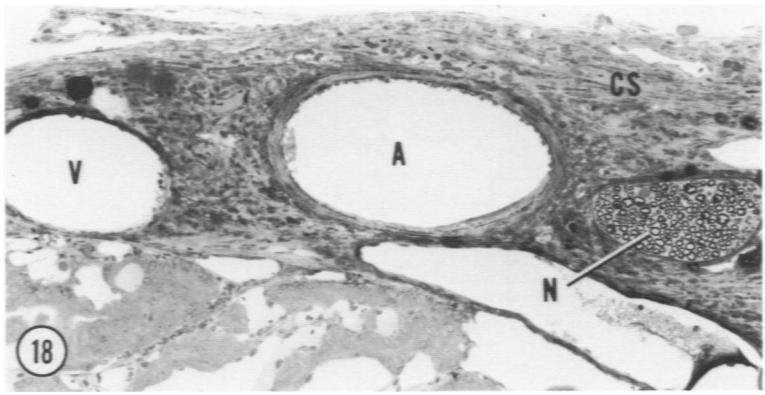
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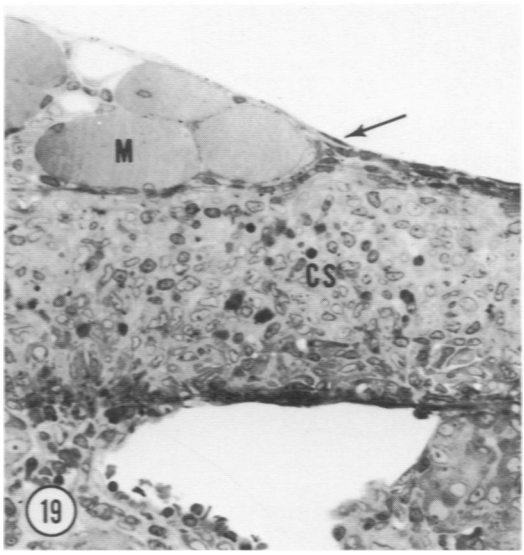
18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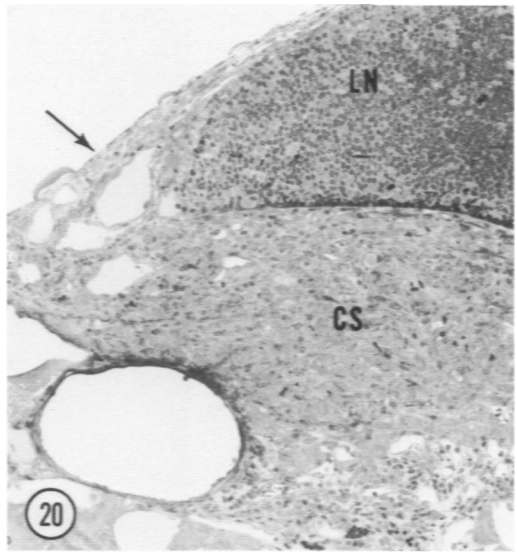
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