THE DISSOLUTION OF FATTY CYSTS IN PRECIRRHOTIC AND CIRRHOTIC LIVERS OF CHOLINE-DEFICIENT RATS TREATED WITH LIPOTROPIC FACTORS *

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Much of the stainable lipid in livers which have become cirrhotic following prolonged and excessive accumulation of fat is not contained within single parenchymal cells but is enclosed by a number of cells (up to sixty) which together form pathologic fatty cysts. In paraffin sections the cysts are represented by spaces which may attain diameters five or six times that of a normal liver cell (comparative volumes would differ by more than one hundred times). The evidence for the existence of these cysts and a description of their pathogenesis have been published previously.[1-4] The cysts are concentrated in those regions of the hepatic lobule in which trabeculation develops in the cirrhotic liver. This arrangement is not merely a matter of chance. It has been shown that in cirrhosis associated with deficiencies of choline and its precursors, the trabeculation is largely the result of condensation of those reticulin fibers which supported the cells of the cyst walls. These cells (and the cysts) have atrophied and disappeared. As a part of this sequence the fat in the cysts leaves the liver.[3] This is the reflection, on a microscopic level, of the gross observation that the extents of cirrhosis and steatosis are frequently inversely related.[5]

The fat may leave the liver under these conditions by either the biliary or vascular systems; the evidence for this has been presented elsewhere.[3] Restoration of lipotropic factors to the diet of experimental animals at any stage before or during the development of cirrhosis produces a striking fall in the level of hepatic lipids.[6,7] In the cirrhotic liver much of the fat is within cysts. Does the fat leave the cysts of such animals during treatment with choline by the biliary or vascular routes, or are other mechanisms invoked? The data in this paper show that under the influence of choline some of the intracystic fat returns to the normal liver cells where it presumably is metabolized.

METHODS

Fifty Wistar albino rats (both male and female) which had received a hypolipotropic diet[3] for 8 to 12 months, were maintained on this same basal food-mixture with an added choline supplement (0.35 per

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cent choline chloride) for an additional period ranging from 3 days to 3 months. The animals were sacrificed under ether anesthesia and multiple blocks of liver tissue were fixed for 6 to 24 hours in Bouin's solution for paraffin sectioning, or in Baker's formol-calcium[8] for frozen sectioning. To reduce traumatic artefact to a minimum, the frozen sections were floated on a solution of 10 per cent formol-saline and transferred immediately to gelatinized glass slides, to which they were affixed by exposure to formalin vapor for at least 30 minutes. Thus at all stages of the staining procedure (Wilson technic[9]) the sections were adequately supported and the introduction of manipulative artefact was reduced to a minimum. Hematoxylin and eosin stains were used on all Bouin-fixed paraffin sections, and an assortment of special stains were applied to selected sections to demonstrate connective tissue, ceroid pigment, and iron deposits.

The Cytology of Fatty Cysts

Before proceeding to a description of the alterations in cysts after choline has been restored to the animals' diet, it is necessary to consider the cellular structure of the cyst wall in the choline-deficient rat. The lumen of a cyst is filled with fat and its wall is made up of a variable number (two to sixty) of simple epithelial cells. These are derived from parenchymal cells which have become greatly modified during the formation of the cyst. These cells, although simple, are adapted to their function in a highly interesting manner.

The Cyst Wall in Cross Section

When viewed on edge (Fig. 1), as they most frequently are in random sections, the cells in the wall of a cyst appear greatly flattened and even atrophic. The cytoplasm extends in both directions from the nucleus, as a thin, curving plate which joins that of neighboring cells in the wall. The junctions between cells are often difficult to see in these "edgewise" views. The nucleus appears to have stained so darkly that pyknosis is suggested (Fig. 1); but as will be seen when considering tangential sections of cysts, this may often be misleading. Similarly, the cytoplasm which in cross section appears almost devoid of internal structure, will be shown to contain the usual inclusions and organelles associated with functioning parenchyma.

The Cyst Wall in Tangential Section

When the microtome knife passes through the edge of a cyst tangentially, the two-dimensional picture in the resulting section differs strikingly from that seen in the more conventional cross-sectional view.

Tangential sections afford the observer an opportunity to study the cells of the cyst wall *en face*. It is then apparent (Figs. 2, 3, and 4) that these cells are compressed only in one dimension and that this is an adaptation in structure to meet their function of retaining fat within the cyst lumen. The nucleus, which on edge appeared rod-like and dark, *en face* is large and round (Fig. 2), and within its membrane may be easily discerned a large nucleolus, nucleolar satellites, and a network of chromatin. This indicates that neither is the cell atrophic nor the nucleus pyknotic. The cytoplasm in similar sections can be seen to surround the nucleus and extend out in every direction so that it resembles a disk. The junctions between neighboring cells may be easily visualized and these resemble the junctions between cells in any flattened type of epithelium. Within the cytoplasm, mitochondrial rodlets and other organelles may be visualized with the aid of suitable stains or phase microscopy. Stainable fat *within* the cytoplasm of the cells of cysts in choline-deficient rats is minimal or absent, although in frozen sections fat completely or nearly completely fills the lumen of most of the cysts (Fig. 11). These observations indicate that although these cells are specially adapted to form an epithelial lining which is remarkable for being only 1 or 2 μ in thickness, this is not necessarily associated with any regressive or atrophic change. This point is of importance for an understanding of the series of changes which occur in mural cells when choline is restored to the animals' diet. These will now be described.

Changes in Cysts When Choline Is Restored to the Diet

The alterations in cysts now to be considered are discussed under three headings (stainable fat in the walls of cysts, escape of lipid from cysts, and the final dissolution of cysts), but this is merely to facilitate description. Actually, all stages may frequently be seen in a single liver section and sometimes even in different regions of the same cyst.

Reappearance of Stainable Fat in the Cyst Wall

The cytoplasm of cells involved in cyst formation is initially laden with fat. When adjacent cells conjoin to form cysts, this intracellular fat escapes from within the cytoplasm due to rupture of the cell membrane. As noted earlier, little or no stainable fat is then present within the cytoplasm of the flattened mural cells. When choline is restored to the animals' diet, the first visible result is seen in frozen sections of the cysts, for stainable fat now appears within the cytoplasm (Figs. 5 and 11). This may be seen as early as 3 days after the animals have been placed on the supplemented diet. The cytoplasmic plates may not

be appreciably thicker than in the period of choline deficiency, but many small droplets of stainable fat are present throughout. This may give the entire cell a red hue so that it stands out clearly defined from nearby parenchymal cells which are free of visible fat (Figs. 5 and 6). This indicates that alterations of a special nature have been produced in the mural cells by the restoration of dietary choline. Essentially, this consists of a reversal of the phenomenon which occurs in the choline-deficient rat during formation of cysts; *i.e.*, the conversion of intracellular to extracellular fat. There is no proof that the fat which appears in the mural cells under the influence of choline is derived from that in the lumen of the cyst, but all morphologic evidence strongly suggests this. Proof could be obtained only by special investigations in which labelled fat was employed.

An interesting finding concomitant with the appearance of fat within mural cells is the frequent absence of stainable fat within the lumina of the corresponding cysts as seen in thin (4 or 5 μ) frozen sections. This is the exception, rather than the rule, in livers of choline-deficient animals. Either all the fat has left the cyst lumen after treatment of the animal with choline for only a few days or, more likely, the fat is held less firmly within the cyst and is readily lost during the sectioning and staining procedures. This is additional evidence to suggest that the intracellular fat in the mural cells of the cyst is derived from the lumen of the cyst.

In most instances, mural cells which contain visible fat possess a more rounded or hexagonal shape (even when sectioned tangentially) than those which are free of stainable lipid (Fig. 6). The fatty cells have lost their special, flattened shape. This may be due to a swelling effect associated with the entry of the fat into the cytoplasm. In any event, this leads to retraction and dissociation of adjoining cells (Fig. 7), so that their junctions are now more prominent and this may facilitate emptying of the cyst by more direct routes, to be considered later in this study.

It may well be asked how fat within a cyst lumen may pass through the limiting membranes of mural cells when choline is available. A process of hydrolysis or phosphorylation may be suggested. The problem is akin to that of how fat passes through the cell membrane of the epithelium covering an intestinal villus.

Escape of Cyst Fat Through Biliary and Vascular Channels

In choline-deficient rats it has been established[3] that, following rupture, the fat in cysts may be removed through either biliary or vascular channels. Examination of microsections of livers of animals treated

with choline indicates that these pathways are still employed, in addition to the resorptive mechanism described in the preceding paragraphs. The conversion of mural cells from thin plates to a more normal polyhedral configuration seems to be accompanied by widening of the interstices between the cells in the wall (Fig. 7). Such interstices often may be continuous with the lumina of either canaliculi or sinusoids, as has previously been demonstrated.[3] Thus the escape of fat from the cyst lumen into neighboring portions of the biliary or vascular systems is facilitated.

It is difficult to assess the relative importance of the resorptive process in relation to the routes considered here. It would be of interest to know by which of these alternative pathways the greatest amount of fat is removed. Examination of sections from all animals revealed that in an individual cyst any one mechanism may predominate, but taken *in toto* it has been impossible to ascribe greater importance to a particular method.

Eventual Dissolution of Cysts

No matter how the lipid departs from the cysts, as soon as this process has neared completion the structure of the cysts begins to dissolve (Figs. 11 and 12). The rosette disposition of the mural cells persists for some time, but they no longer form a continuous lining membrane. The cells regain their individual character and revert to the characteristics of normal parenchyma. At this stage, when cut in any plane, they are rounded or polyhedral with centrally placed, spherical nuclei. This process is accompanied by diminution in the diameter of the lumen of the cyst, which is encroached upon by the surrounding cells. By these stages, each cell withdraws from its neighbors, takes up space from the lumen, and becomes lost in the cords or plates of hepatic parenchyma.

Occasionally, a variant in this sequence of events is encountered. The lumen of the cyst has become small, but the surrounding mural cells have not reverted to the appearance of normal liver cells (Figs. 9 and 10). Instead they have become crowded together around the contracted lumen so that many are included in single random sections. Were it not that all gradations in form can be observed (Fig. 9), one would not be likely to associate these cells with the usual mural cell. The nuclei are dark, pyknotic, and assume bizarre forms. The cytoplasm is scanty and stains more intensely than before. With the lower magnifications, these could easily be mistaken for small, round cells, but higher magnification reveals that they differ significantly from lymphocytes in respect to their nuclear shape and nuclear-cytoplasmic

ratio. It is possible that these represent mural cells which have undergone such extensive differentiation that they are unable to revert to the normal state when choline is restored to the diet.

Identification of Cysts and Their Various Stages in Routine Sections

There is considerable evidence that certain types of fatty cirrhosis in man, particularly that associated with excessive consumption of alcohol, are related to the cirrhosis in rats fed low-choline diets.[10] From our experimental observations it is apparent that the microscopic appearance of cysts in sections may afford the practical pathologist an indication whether the cirrhosis is progressing or regressing. It is therefore important to define the criteria by which cysts may be identified and their microscopic features interpreted.

The Identification of Cysts in Sections

It is not always apparent whether large fat vacuoles are contained within single cells or lie in small cysts. The limiting membranes between two adjoining cells each distended by a large spherule of fat may fuse into a single morphologic septum which becomes stretched and tenuous and is eventually ruptured. This results in the formation of a central pool of lipid which is now surrounded by the two conjoined parent cells. By definition,[1] this structure is now a small, two-celled, fatty cyst. Unless the microtome knife has passed through the greatest diameter of such a microcyst, it would be impossible from a single random section to distinguish it from a large intracellular fat vacuole. It is apparent, however, that the larger a cyst, the greater will be the number of cells in its wall. Identification of cysts thus becomes progressively easier as they become larger, for the number of mural nuclei in single sections becomes greater. These are the characteristics which are the most useful for the identification of cysts in routine paraffin sections.

Identification of Stages of Regression in Cysts

The cardinal feature of cysts which are regressing and disappearing is the presence of appreciable amounts of stainable fat within the cytoplasm of the cells forming their walls. This serves to emphasize the importance of utilizing frozen sections stained for fat in examining material taken from livers of this type. In paraffin sections, vacuolation of the cytoplasm of the mural cells can sometimes be discerned, but unless it is known that the vacuoles contain lipid, this finding cannot be relied upon as an index for resorption of fat contained within

cysts. The best indication that can be found in paraffin sections to suggest that fatty and cirrhotic lesions are arrested, is the presence of parenchymal cells arranged in rosettes, as shown in the cyst in Figure 12. Such configurations afford definite evidence that cysts are breaking up and their constituent cells reverting to a normal parenchymal pattern. It is suggested that these features, when present in specimens for biopsy from the fatty or cirrhotic livers of chronic alcoholic patients, could be interpreted as strong presumptive evidence that the progress of the pathologic lesions has been arrested.

Conclusions

Treatment of choline-deficient precirrhotic or cirrhotic rats with lipotropic factors for periods up to 3 months brings about dissolution and eventual disappearance of nearly all pathologic fatty cysts encountered in this condition.

After choline is restored to the diet of such animals, intracellular lipid can be demonstrated within the cytoplasm in the walls of the fatty cysts. This is associated with a decrease in the lipid found within the lumina of the cysts.

Fat may also leave the cysts through the biliary or vascular route in a manner similar to that previously found to occur in choline-deficient rats not treated with lipotropic agents.

After the lumina of fatty cysts have been emptied by any of the mechanisms described, the cells in the cyst wall re-assume the appearance of normal parenchyma and, after passing through a stage in which they exhibit rosettes, re-form in the usual cord or plate pattern. This is associated with an encroachment on the lumen of the cyst so that it is eventually obliterated. An abnormal variant of this procedure may occur in which the cytoplasm is scanty and the nuclei pyknotic.

If cysts are observed in any of the stages of disappearance, this may indicate that the fatty and cirrhotic lesions are actually regressing.

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DESCRIPTION OF PLATES

All photomicrographs are of livers of choline-deficient rats treated with lipotropic factors for varying periods of time (with the exception of Fig. 1). Magnifications throughout are × 800, unless otherwise stated.

PLATE 63

FIG. 1. A cross section of a cyst, which fills the center of the field, has included four mural cells which are seen on edge. Of note are the thinness of their cytoplasmic plates and the basophilia of the nuclei. Paraffin section, hematoxylin and eosin stain.

FIG. 2. The plane in which this cyst has been sectioned is tangential to its greatest circumference. The four nuclei at the top of the field are seen *en face*. Within the nuclei, nucleoli and a chromatin network are clearly visible. The cytoplasm is distributed almost equally around the nuclei. If these cells had been sectioned in a plane comparable to that in which the mural cells of Figure 1 were cut, their appearance would have been similar.

FIGS. 3 and 4. The same tangential section of a fatty cyst is seen in each photomicrograph. At the upper pole, the nucleus (in cross section) is thin and basophilic. The nuclei at the lower pole are seen almost full-face, and are surrounded with cytoplasm. The focus in Figure 4 is deeper than in Figure 3, and greater roundness (cupping) of the nuclei is apparent.

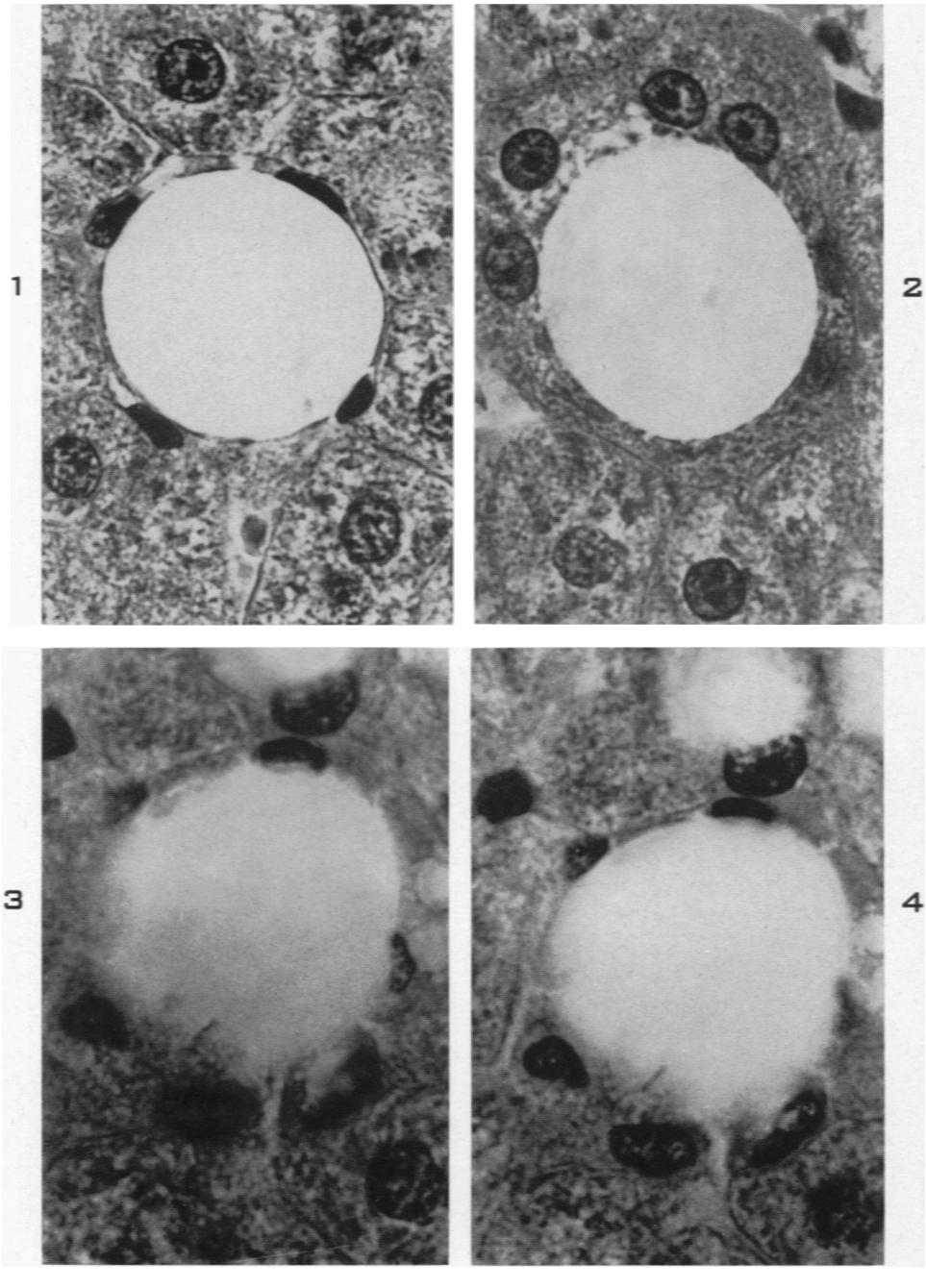

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PLATE 64

FIG. 5. A cross section of a fatty cyst 8 days after restoration of choline to the diet. The intense staining of the cytoplasm in the mural cells is due to myriads of fat droplets. Of note also are the thickening of these cells and the absence of stainable fat within the lumen of the cyst. Orange red O stain.

FIG. 6. A cross section after 12 days, a later stage of the same process. The mural cells (outlined by dark-staining fat droplets) have regained the characteristics of parenchymal cells. Little or no fat remains in the lumen. Orange red O stain.

FIG. 7. The mural cells again resemble parenchymal cells. Although they contain fat, they have become dissociated in the wall of the cyst and canaliculi can be seen between them. Orange red O stain.

FIG. 8. Fat is seen in the sinusoids in proximity to, and in connection with, a fatty cyst at the right. Orange red O stain.

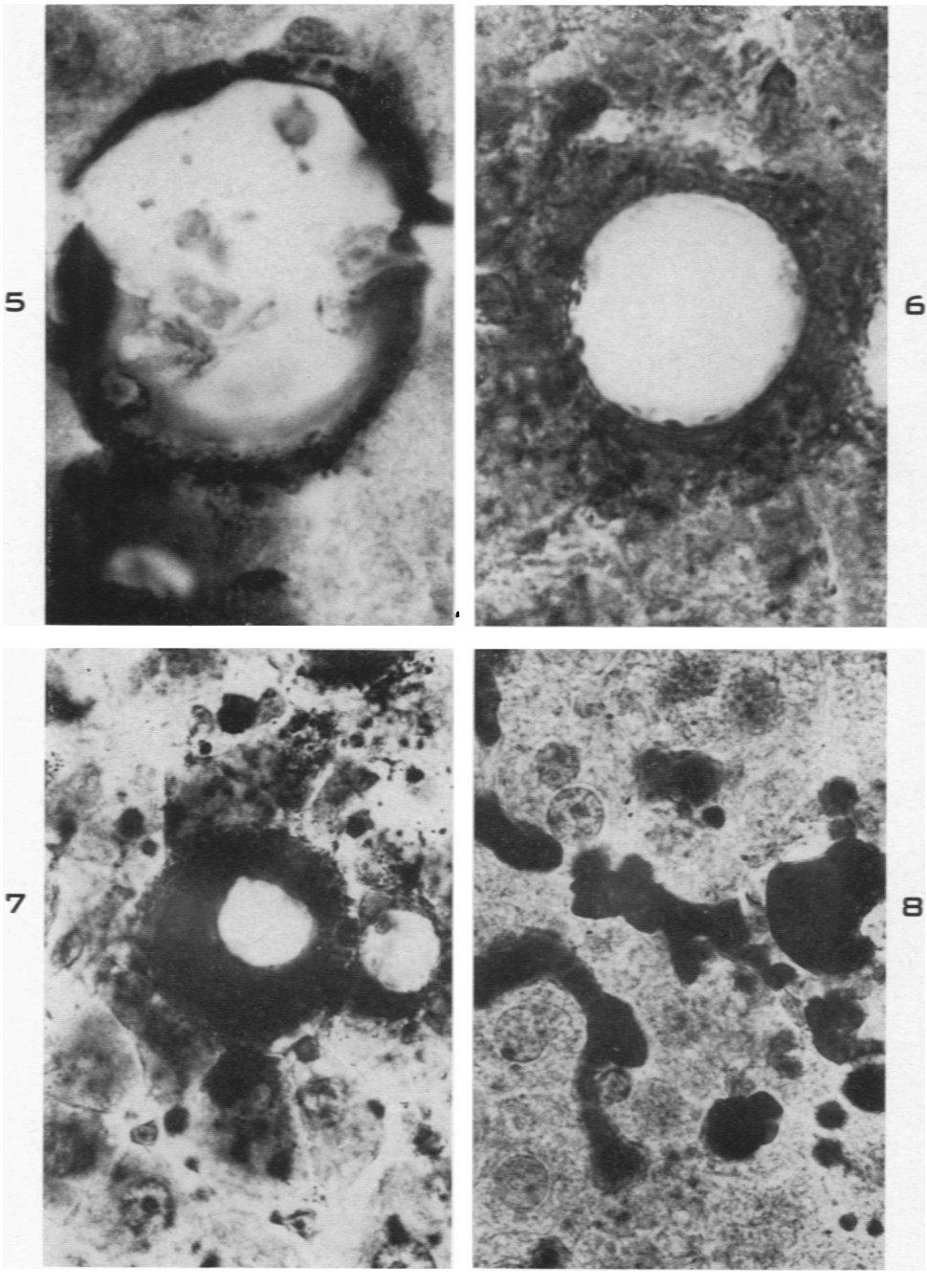

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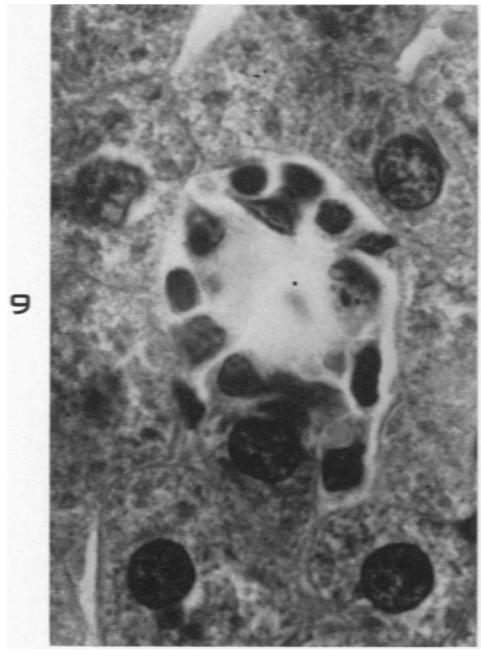
PLATE 65

FIGS. 9 and 10. Collections of abnormal mural cells occasionally seen as a variant in the later stages of the process of dissolution. In both figures some cells resemble the parenchymal type while numerous transitional forms, many of which show signs of cellular degeneration, are present also.

FIG. 11. Cross section of a fatty cyst. Fat from the cyst lumen is bulging into the interstices between mural cells. The cytoplasm of these cells contains fat droplets, and is distributed around nuclei which appear larger and more nearly circular than previously (*cf.* Fig. 1). Orange red O stain. × 1000.

FIG. 12. A rosette of large, round nuclei, each surrounded with cytoplasm containing fat droplets, encroaches upon a clear area which apparently is all that remains of a fatty cyst. Orange red O stain.



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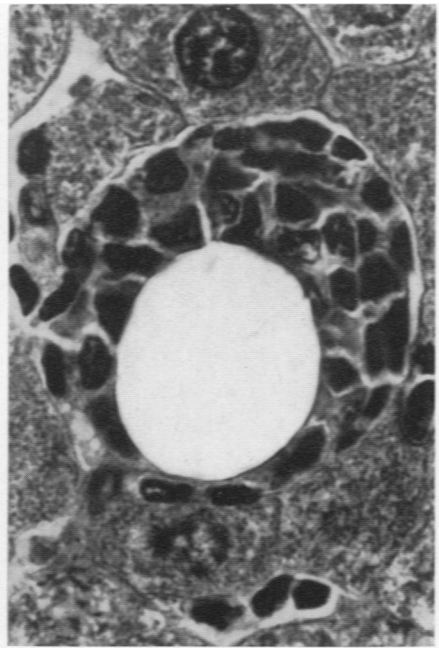

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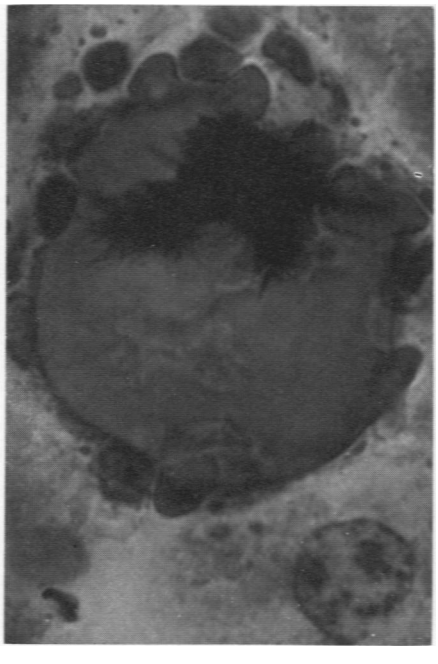

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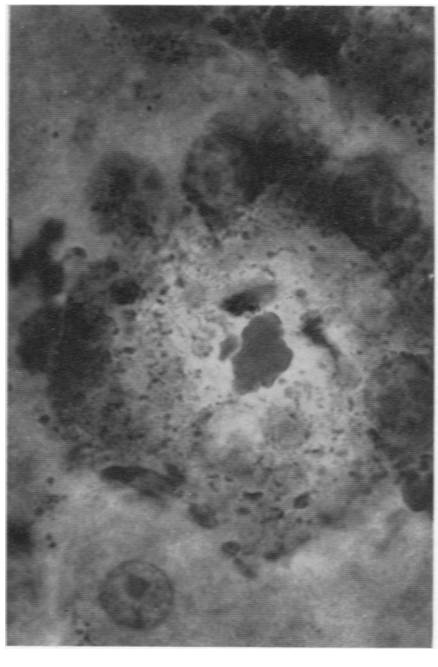

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