PATHOGENESIS OF THE CIRRHOSIS PRODUCED BY CHOLINE DEFICIENCY

ESCAPE OF LIPID FROM FATTY HEPATIC CYSTS INTO THE BILIARY AND VASCULAR SYSTEMS *

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Pathologic fatty cysts † which form in the livers of rats fed choline-deficient diets have been described previously by one of us.[1] A series of changes was initiated by the rapid accumulation of fat within each parenchymal cell[2,3] (Figs. 1 and 2), particularly those in centrolobular regions of the livers. Within a period which varied with the age, weight, sex, and food-intake of the animals, some of the intracellular fat became extracellular (Figs. 3, 7, and 8). This occurred initially in centrolobular regions (Fig. 13) where the cytoplasm of each parenchymal cell had become distended by a large lipid spherule which displaced the nucleus to one side. Limiting membranes of contiguous cells, thus distended, became so flattened and stretched that they appeared as single tenuous septa compressed by adjacent globules of intracellular lipid. In the livers of young adult rats fed the low-choline diet for 6 to 8 weeks, there was microscopic evidence that many of these attenuated septa had ruptured. Repeated intercellular septal breaks of this nature (Figs. 4 to 6) culminated in the formation of cysts (up to 100 μ in diameter in paraffin sections) of a multicellular nature. Their unilocular lumina were completely filled with fat. Their walls consisted of a continuous, single layer of dedifferentiated hepatic cells which had each contributed the intracellular lipid they originally contained to the fat now lying within the cyst.

Most of these non-portal fatty cysts became replaced by fibrous tissue in the form of trabeculae which extended from one central vein to another. A well marked pattern of annular fibrosis was developed in this manner in the livers of young adult rats which had been fed the low-choline diet for periods of 6 to 7 months. Biochemical investigations have established[4] that the amount of fat in livers of comparable animals decreases as fibrosis increases. This is mirrored in miniature by the escape of lipid from fatty cysts which atrophy with

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† The name *lipodiastemata* was originally given these structures, but this has now been abandoned for the simpler and more descriptive term fatty cysts.

replacement fibrosis. The history of these cysts demonstrates the cirrhogenous rôle of excessive accumulation of hepatic lipid.

In the present communication, evidence will be presented to show that fat escapes from the cysts not only into the biliary tree, but also into the blood stream. The latter pathway of escape results in intermittent showers of minute fat emboli which have been demonstrated in the heart, lung, and kidney, where they have produced secondary pathologic lesions. Evidence obtained from a study of material taken at necropsy from alcoholic patients * suggests that a similar lesion may sometimes occur in human subjects. Factors responsible for rupture and atrophy of the cysts will be discussed.

Experimental Procedure

For this investigation, 240 weanling rats of the Wistar strain (both sexes) reared in our own colony were used. The rats (initial weights, 36 to 42 gm.) were housed in individual all-metal cages provided with a false bottom of coarse wire screen, and were fed diets low in choline and its precursors.

In the first group, 200 rats (equal distribution of sexes) received a diet with a protein mixture similar to that used by Copeland and Salmon.[5] This diet had the following percentage composition: peanut meal, 30; casein (fat-free, vitamin-free), 6; salts, 4 †; sucrose, 39; "vitamin powder," 1 ‡; beef fat, 15; corn oil, 5; cod-liver oil concentrate, 0.015 §; and alpha-tocopherol acetate, 0.010. The peanut meal (expeller-process) was extracted with ethanol once with 5 to 7 volumes of 50 per cent, then 3 to 5 volumes of 70 per cent, once with 3 volumes of 95 per cent, and finally washed with absolute ethanol.

In the second group, 40 male weanling rats were fed a synthetic diet of the following percentage composition: gelatin, 5; casein (fat-free, vitamin-free), 4; arachine, 4; zein, 3; fibrin, 2; salts, 5 †; cellu flour, 2; sucrose, 53.8; "vitamin powder," 1 ‡; beef fat, 15; corn oil, 5; cystine, 0.175; cod-liver oil concentrate, 0.015 §; and alpha-tocopherol acetate, 0.010.

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†The salt mixture[6] is a modification of the McCollum salt mixture 185.[7] The composition in gm. per 100 gm of salts is as follows: Ca lactate ($5H_2O$), 35.15; $CaCO_3$, 5.28; $Ca(H_2PO_4)_2 \cdot H_2O$, 14.60; K_2HPO_4, 6.45; $NaH_2PO_4 \cdot H_2O$, 18.76; NaCl, 9.34; $MgSO_4$ (anhydrous), 7.19; ferric citrate ($3H_2O$), 3.19; $MnSO_4 \cdot 2H_2O$, 0.33; $ZnSO_4 \cdot 7H_2O$, 0.035; $CuSO_4 \cdot 5H_2O$, 0.039; KI, 0.00039.

‡The "vitamin powder" contained thiamine hydrochloride, 0.5 gm.; riboflavin, 0.25 gm.; pyridoxine hydrochloride, 0.2 gm.; calcium pantothenate, 1 gm.; nicotinic acid, 1 gm.; folic acid, 0.05 gm.; 2-methyl-1-4-naphthoquinone, 0.1 gm.; para-aminobenzoic acid, 10 gm.; and inositol, 50 gm.; made up with finely powdered sucrose to 1,000 gm. Rats consuming 10 gm. of diet daily received 50 μg. of thiamine hydrochloride and corresponding amounts of the other vitamins.

§Cod-liver oil concentrate (Ayerst, McKenna & Harrison, Ltd., Montreal, Que.) contained 200,000 international units of vitamin A and 50,000 i.u. of vitamin D per gm.

Fresh diets were prepared about once a week. The major dry ingredients were mixed thoroughly in a Hobart food-mixer, and a small portion (about 500 gm.) of this mixture was spread in a thin layer on a large tray. The minor ingredients ("vitamin powder" or cystine) were ground individually in a mortar with small portions of the dry mixture, and sifted (40-mesh sieve) over the surface of the material in the tray. The cod-liver oil concentrate and alpha-tocopherol acetate were dissolved in petroleum ether and sprayed over the mixture. The contents of the tray were mixed by hand and then returned to the Hobart-mixer and blended thoroughly. The beef fat was melted and brought to a temperature of 130° to 140° C., the corn oil was added, and the hot fat was poured all at once into the mixer and blended until uniform. The diets were kept in tightly closed tinned cans in a refrigerator at about 4° C. until required for use. Weighed amounts of fresh food were placed each morning in galvanized iron food-containers, designed to minimize scattering, and fresh water was available at all times. The amount of food left over and any scattered material were weighed daily, from which data the individual food consumption could be calculated.

Since young rats are very susceptible to choline deficiency, minimum supplements of choline were given in the preliminary period in order to prevent death from hemorrhagic renal disease. Each animal received individual attention and the dosage of choline was gradually decreased according to weight, food consumption, and general appearance. The supplement of choline chloride was discontinued when it was believed that the rat was able to survive without it. Animals were killed under ether anesthesia at intervals ranging from 6 to 18 months on this dietary regimen. In many instances they were moribund or losing weight at the time of sacrifice.

The weights of all organs (except brain and pancreas) were recorded at necropsy. Representative portions of the viscera, brain, and diaphragm were fixed in either Bouin's or formol-saline solution. Paraffin sections of Bouin-fixed blocks were stained routinely with hematoxylin and eosin. Frozen sections of the tissue fixed in formol-saline solution were stained with oil red O, hematoxylin, and light green by Wilson's technic.[8] Special staining methods for glycogen, elastic tissue, connective tissue, hemosiderin, ceroid, cholesterol, and calcium deposition were applied to selected tissues as indicated in the text.

The Microanatomical Relations of Fatty Cysts

Evidence supporting the concept of fatty cysts in livers of choline-deficient rats has been presented in previous publications.[1,3] For an

understanding of how lipid may escape from these cysts, their relations to surrounding structures, particularly bile canaliculi and sinusoids, must be considered in some detail.

Between hepatic parenchymal cells lies either a canaliculus or a sinusoid (Fig. 1). Early in choline deficiency, many small fat droplets form in the cytoplasm of each cell. The droplets conglobate into one or two large intracellular spherules which distend each cell and displace its nucleus to one side. Limiting membranes of adjacent cells, so expanded, are stretched, thinned, and compressed (Figs. 7 and 8). If a bile canaliculus (Figs. 9 and 10) lies between two of these cellular membranes, it must either be flattened between them or pushed to one side. If the former occurs, the canalicular wall will be disrupted when the tenuous septum formed by the compressed membranes of the adjoining cells is torn by the pressure of the intracellular lipid conglobates. The lumina of the torn canaliculus and the newly formed cyst would then communicate. Examination of sections indicates that this rarely happens in the initial stages of cyst formation from single cells (first generation cysts) when, instead, the canaliculus is pushed to one side (Fig. 9) so that it is not torn when cellular fusion produces a small fatty cyst. If only single cells enter into formation of a cyst, sufficient displacement of a canaliculus to escape rupture need be only a few micra. In later stages (Figs. 4 and 5), when small cysts fuse to produce larger ones (of the third or fourth generation), a bile canaliculus caught between adjacent cysts must be displaced a relatively great distance to escape rupture. Under these latter conditions canaliculi frequently rupture as will be shown in a later section.

If a series of septal breaks between distended cells and later between cysts of increasing size is accompanied by displacement without rupture of any of the intervening canaliculi, a large fatty cyst is produced, on the surface of which run numerous biliary channels (Figs. 4 and 5). As the walls of both the cyst and the canaliculi are exceedingly thin, there are many potential sites of rupture through which fat within the cyst could then escape into the biliary tree. In serial sections, over 60 cells have been found in the walls of some of the larger cysts[1]; there are, therefore, a correspondingly large number of possible places for tears of the compressed membranes between the lumen of such a cyst and any of the numerous bile canaliculi coursing over its surface.

Just as bile canaliculi separate parenchymal cells, so do sinusoids. These also may become compressed between adjacent limiting membranes of cells distended by lipid conglobates (Figs. 4 and 12). Large cysts are surrounded by many sinusoids which have come to occupy their positions by the same principle of displacement by septal com-

pression as has been described for bile canaliculi. If a sinusoid is not displaced, it will be ruptured when the intercellular (or intercystic) septa are torn. The lumen of the sinusoid will then communicate with that of the cyst. If large cysts fuse to form still larger ones, intervening sinusoids must be displaced relatively great distances to escape rupture and this may not be possible. Rupture eventually occurs instead of displacement, and this will be demonstrated. One might suppose that sinusoids would not be torn as easily as delicate bile canaliculi. But walls of vessels become stretched and very tenuous in their course around large cysts (Figs. 11 and 12). Rupture under these circumstances appears to be likely and this is borne out by the histologic evidence to be presented.

Release of Fat from Hepatic Cysts into the Biliary Tree

Since cysts and bile canaliculi are so close together, there are many points where intracystic lipid is separated from canalicular lumina only by tenuous septa (Figs. 10 and 14). In frozen sections, stainable fat droplets can be demonstrated passing through breaks in the septa at these sites of weakness and entering canaliculi (Figs. 15 and 16). Frequently the lumina of these cysts are only partially filled with lipid (Fig. 15), which suggests that reflux of canalicular fluid into the cyst has replaced whatever lipid has escaped into the biliary tree.

It is necessary to consider the possibility that the ruptures illustrated might have been technical artifacts; fat droplets might have been swept by the microtome knife from cysts to other regions of the sections. This is not considered the case in the instances shown, and in others of a similar nature, on the following grounds. The plane of focus occupied by the droplets indicates that they are neither on top of, nor below, the section, and that they occupy positions precisely within the canalicular lumina. The diameters of the droplets (Fig. 15) are adapted rather exactly to the diameters of the canaliculi—the wider the passage, the greater the droplet-diameter and *vice versa.* Droplets are not scattered over the tissue and general artifacts of this nature are absent. In areas of the section (periportal, for example) where the parenchyma has undergone less fatty change, lipid droplets are found in canaliculi (Figs. 20 and 21), and technical displacement of fat would have had to be far-flung, indeed, for the droplets to have been carried into these areas otherwise relatively free of fatty change.

Further evidence of the true nature of these communications between cyst lumina and biliary canaliculi was obtained from specially prepared material. India ink under very low pressure was introduced into the biliary tree of rats which had been fed the basal diet for 10

months. Under ether anesthesia, a small plastic cannula was inserted into the main bile duct; plastic tubing connected the cannula to a small funnel. Filtered, diluted (ink to water; 1 to 2) India ink was poured into the funnel and allowed to flow into the bile duct under a pressure of not more than 15 cm. of water. Blocks of liver tissue subsequently were immersed in formol-calcium for fixation. This technic produced complete injection of main biliary ducts and immediately adjacent canaliculi, but those in the mid-lobular and centrolobular regions were not well filled. The same procedure was carried out using control animals of similar ages and weights, which had been fed the basal diet supplemented with 0.35 per cent of choline chloride (Fig. 22). In the cirrhotic rats (Fig. 23) many of the fatty cysts could be seen partly or completely filled with ink. In some instances a bile canaliculus, filled with the injection medium, could be followed to the cyst (Fig. 23). Frozen sections (Fig. 17) of these livers contained not only bile passages, but also cysts, filled with ink alone and sometimes with mixtures of ink and stainable fat.

Escape of lipid from fatty cysts into the biliary tree apparently has little ill effect on the animal compared with its escape into the vascular system. The cysts from which the lipid has drained into the biliary tree in some cases shrink and become replaced by the condensed reticulum which originally surrounded each of the cells in the walls of these cysts. The ultimate fate of these and of other cysts which become transformed into "new" bile ducts will be described in greater detail in a later section.

Release of Fat from Hepatic Cysts into the Blood Stream

By examination of thin (4 to 5 μ) frozen sections of livers of choline-deficient rats, direct morphologic evidence has been obtained which indicates that lipid droplets from some of the fatty cysts may enter the circulating blood through tears in the membranes separating the lumina of cysts and sinusoids. (Indirect evidence that this occurs will be presented also in a subsequent portion of the paper.) It already has been shown that sinusoids may course over the surface of cysts and may be caught between walls of adjacent cysts (Fig. 12). Rupture at such potentially weak sites probably occurs by the same mechanisms already described for bile canaliculi. Fat droplets then enter the lumen of the sinusoids (and thus the systemic circulation) and erythrocytes and plasma replace whatever lipid has escaped from the lumen of the cyst (Figs. 6, 24, 25, and 26). Plugs of fat may completely block sinusoids (Figs. 19 and 28) or as small droplets may be intermixed with red cells (Fig. 18). Exceptionally, large centro-

lobular veins have been observed completely filled with stainable fat in which a few erythrocytes may be embedded (Fig. 29).

If there is only one tear in the cyst wall, the structure persists for some time as it slowly shrinks. Ruptures, however, may be multiple as shown in Figure 27, where tears have developed at two and possibly three sites on the wall of a single cyst. Cells of this cyst wall between the tears are contracted and degenerating. Erythrocytes have replaced the cyst and surrounded the released conglobate of lipid (which appears as a large vacuole in the paraffin section photographed). Multiple ruptures in the wall of a single cyst lead to complete dissolution of the structure, since it is apparent that if the endothelium of the sinusoids at the periphery of the photomicrograph shown in Figure 27 were repaired at the sites of ruptures, and if the degenerated cells were carried away with the circulation, no trace of the ruptured cyst would remain.

Release of fat from a cyst into a blood vessel results in a pathologic interchange of blood and lipid. The changes in erythrocytes isolated in ruptured cysts which persist are of interest and will be described in a subsequent part of this paper. The extravasation of these red cells probably has little effect on the general health of the animal, but the fat which enters the circulation may produce embolic lesions in other organs which may hasten its death.

Chronic Fat Embolism

Fat droplets which have escaped from cysts into hepatic sinusoids might conceivably produce pathogenic effects on any organ, but the evidence to be presented here is limited to heart, lung, and kidney in which lesions were readily apparent. In frozen sections of cardiac muscle of some of the cirrhotic rats, small, elongated fat emboli could be found in the capillaries (Fig. 30). These sometimes were associated with small foci of necrotic muscle cells surrounded by fibrous tissue in which calcium salts were deposited (Fig. 31). The lesions resembled small ischemic infarcts.

In frozen sections of lungs of many of the choline-deficient rats, lipid droplets were found in relatively large numbers lying in arterioles and capillaries (Fig. 33). In some instances, fat appeared to have escaped from septal capillaries into alveolar lumina in which macrophages with lipid droplets in their cytoplasm were present also. This suggested that fat which had entered the alveolar lumen from septal capillaries had been engulfed by these cells. They appeared to collect in the peribronchial lymphoid tissue, for in many of the experimental animals this region was found to be distended by large numbers of

lipid-containing macrophages (Fig. 34). Interference with bronchial lymphatic drainage in this manner could be a factor predisposing to the bronchiectasis and bronchopneumonia which developed in many of the rats.

The most striking demonstrations of the cumulative effects of small, intermittent showers of fat emboli derived from ruptured hepatic cysts were found in the kidneys. Lesions of this type were separate from, and additional to, changes which were secondary to the stainable fat which accumulates within the cells of proximal convoluted portions of nephrons as a direct result of dietary choline deficiency, both acute and chronic.[9-11] Embolic lipid was found within both interlobular and afferent glomerular arterioles (Fig. 35), as well as within the lumina of glomerular capillary loops (Figs. 36 and 37). Obstruction of the circulation in glomerular loops due to fat emboli could be demonstrated in kidneys of rats injected intravascularly with ink at the time of sacrifice (Fig. 38). Engorgement of capillary loops sometimes was found associated with their obstruction by fat emboli (Fig. 39). In corresponding paraffin sections there was evidence of focal necrosis and hyalinization of the glomerular tufts (Fig. 40). These lesions were like those described by Kimmelstiel and Wilson [12] in diabetic patients. It is possible that the material deposited in the hyalinized tufts in the rats was derived in part from arrested erythrocytes. Considerable amounts of pigment which reacted positively to Prussian blue tests (hemosiderin) were found in the lumina and walls of the tubules (Figs. 41 and 42). This material may have originated from products in the glomerular filtrate from red cells arrested in glomerular loops obstructed by fat emboli.

Stainable fat was found in the space of Bowman in many of the glomeruli in which fat was present in the capillary tufts (Fig. 37). This appeared to be analogous to the presence of lipid droplets in those alveolar lumina which were surrounded by septal capillaries containing fat, and the evidence suggested that in both the kidney and the lung, fat may pass from the capillary loops into the adjoining tissue space (alveolar or capsular). In both the proximal convoluted tubules and in the tubules of the medulla, particularly the latter, lipid casts were present in great numbers. In the medulla many had become calcified (Fig. 43).

Thus, focal myocardial lesions, possibly bronchiectasis and bronchopneumonia, focal necrosis of the glomerular tuft, renal hemosiderosis, and lipid obstruction of medullary tubules have been demonstrated in tissues of rats fed diets low in choline for periods of approximately 1 year or longer. The evidence strongly suggests that

the escape of fat from pathologic cysts into hepatic sinusoids and lodgment of the fat emboli in the various tissues was responsible for the lesions.

The Fate of Erythrocytes Which Enter Fatty Cysts

Blood in fatty cysts never appeared to have clotted (Figs. 25, 26, and 27), nor could Prussian blue-positive products of erythrocytic disintegration be demonstrated. But these livers contained abundant deposits of ceroid * in the regions where the hemorrhagic cysts had shrunk. Ceroid globules frequently resembled erythrocytes in size and shape, particularly if some allowance was made for effects of distortion and compression (Fig. 44). Red blood cells normally exhibit a strong affinity for light green (color index no. 670). In livers which contain large amounts of ceroid, clumped red cells sometimes were found in cyst remnants, which took up abnormally small amounts of this stain. In these same sections, granular débris in cysts was noted which, although faintly sudanophilic, was overcast with a pale green color (Fig. 45). These metachromatic deposits suggested transition forms between chlorophilic erythrocytes and sudanophilic ceroid. Further, it was found that in livers of a small series of cirrhotic rats in which ceroid deposition had been minimal,† hemosiderin (Prussian blue positive) pigment could be demonstrated in shrunken remnants of cysts (Fig. 49). This suggested that ceroid might result if red cells were immersed in liver lipid and, further, that this could prevent the usual liberation of reactive iron from degenerating erythrocytes. The hypothesis was supported by the results of tests performed not only *in vitro* but also *in vivo* as follows.

Heparinized red cells from choline-deficient rats were mixed with 10 volumes of cod-liver oil and incubated *in vitro* at 37°C. for 5 days. After centrifugation, the sediment was washed repeatedly with alcohol and ether. Histochemical examination demonstrated that clumps of red cells had been transformed into a substance which could not be distinguished from ceroid. Paraffin sections of fatty mesenteric tissue from rats sacrificed 3 weeks after it had been locally heparinized and crushed with hemostats also contained pigment histochemically identical

* Ceroid is the insoluble (in alcohol, ether, xylol) orange-brown pigment found in the fibrous trabeculae of the livers of choline-deficient rats.[14] It is fluorescent, acid-fast (Ziehl-Neelsen technic), sudanophilic, and stains with most basic dyes.

† Ceroid was absent in a series of 10 Wistar rats fed the basal (choline-deficient) diet supplemented with 10 mg. of alpha-tocopherol acetate per 10 gm. of food. The animals were sacrificed after a period of 6 months on this dietary regimen. This preventive action of alpha-tocopherol in relation to ceroid was first suggested by Victor and Pappenheimer.[15] The results in these 10 rats were not confirmed by a similar experiment carried out by György and Goldblatt.[16]

with ceroid. Details of these experimental procedures have been reported elsewhere.[13] The results support the hypothesis that ceroid pigment may be the product of a physicochemical reaction involving certain components of rat erythrocytes and liver lipid. Further investigations are being carried out using other tissues than blood and other lipids than cod-liver oil, and to assay the possible rôle of alpha-tocopherol in the reaction. The evidence obtained to date, however, seems to indicate clearly that erythrocytes which enter ruptured fatty cysts in livers of rats fed the basal diet are converted into ceroid, rather than into hemosiderin as commonly occurs in cases of interstitial hemorrhages under other conditions.

Small embolic masses of ceroid have been found in suitably stained paraffin sections of lung (Fig. 32) and kidney from rats in this series. This suggests that some of the lipid which enters the circulation from ruptured cysts may become converted into ceroid, or that ceroid is formed first in a ruptured cyst and is then washed into the general circulation. As ceroid was found in greatest amounts in the livers of choline-deficient rats, embolic ceroid in pulmonic and renal vessels mixed with embolic fat strongly supports the belief that these lipid emboli originate in the livers.

Factors Responsible for the Rupture of Cysts

The larger a cyst, the more numerous the cells of its wall and the bile canaliculi or blood sinusoids running over its surface, and thus the more likely that one or more of these vessels will be torn if the cyst is involved with others in further mergers. Again, the larger the cysts, the greater will be the distance of displacement required for a canaliculus or sinusoid to avoid being caught and torn between adjacent walls. Finally, even though intracystic pressure may be no greater within a large cyst than within a small one, the outward thrust on its wall will increase with its surface area and so favor rupture. The combination of the foregoing factors appears to have limited the size of cysts in the rat to a maximum of 80 to 100 μ in diameter (as seen in paraffin sections). Pathologic fatty cysts in livers of alcoholic persons have been noted which greatly exceed in size the largest observed in choline-deficient rats.

Occasionally in rats, unusual collections of very large cysts (Fig. 50) have been encountered. Some of these have been sufficiently large to be visible on gross examination. In frozen sections, these macrocysts contain small amounts of weakly sudanophilic material which appears to consist of an aqueous dispersion of lipid. Ink injected into either

bile ducts or vessels has never been found in macrocysts. This suggests to us that they completely lack communications with either the biliary or vascular trees. Lack of drainage may have been responsible for the gigantic proportions attained by these macrocysts.

Histogenesis of the Fibrous Trabeculae in Cirrhotic Livers of Choline-deficient Rats

The cytometaplastic changes which culminate in the formation of trabeculae in the final cirrhotic lesions seen in livers of choline-deficient rats are difficult to envision from an examination of only the final stage. Microscopy of every step in the pathogenesis of the cirrhosis has afforded much information concerning the histogenesis and cytogenesis involved. Actually only a relatively small fraction of the components of the so-called "fibrous" trabeculae gives the characteristic staining reactions of connective tissue (Figs. 52 and 53). These strands of reticulin and collagen are widely separated by interlacing channels lined by simple epithelial cells ("new" bile ducts) and aggregates of ceroid pigment in atrophic cyst remnants. The origin of the connective tissue in cirrhotic livers has long been a matter for speculation. The evidence presented in this paper strongly supports the view that in experimental dietary cirrhosis in rats, the fibrous tissue in the trabeculae represents a consolidation (without proliferation) of the reticular stroma. Reticulin in the form of single, fine strands originally surrounded each liver cell which became incorporated in the walls of cysts. Sixty or more cells have been demonstrated [1] in serial sections of the walls of single large cysts. With involution of a cyst, reticulin surrounding this number of cells is concentrated to a fraction of the volume of the mature cyst. This adequately explains the amount of collagen fibers found in the trabeculae, without the need for postulating stromal proliferation. When neighboring fatty liver cells fuse to form small cysts and these in turn conjoin to form larger ones, the reticulin supporting the original cells will be pushed to the outside of the cyst in the same manner as that by which bile canaliculi may be displaced (Figs. 4 and 5). Thus a mature cyst becomes surrounded by a delicate open meshwork of reticular fibers. With atrophy of the cysts, these fibers can be observed in sections condensed around the shrunken walls. Eventually the fibers clump together in a single mass which is compressed to only a fraction of the volume taken up by either the mature cyst or the cells from which it was originally formed. In this way the normal stromal content of a volume of tissue originally equal to as much as 0.01 cmm. becomes condensed into a space repre-

sented by only a few micra in sections. Periportal proliferation of parenchyma will compress the fibers into longitudinal and annular strands as seen in the final lesion.

Biochemical assays [17] of the amount of hepatic fibrous tissue in cirrhotic rats have indicated an increase in the stroma. This might seem to imply that the trabeculae had been formed, at least in part, by proliferation of connective tissue. But formation of new reticular fibers, which accompanies the compensatory proliferation of parenchyma in periportal nodules, could explain the increase in the total amount of hepatic stroma of cirrhotic livers, since the reticulin which surrounded atrophied or destroyed parenchyma in the centrolobular regions has at the same time persisted in the trabeculae. In experimental dietary cirrhosis in rats, there is no direct evidence that fibrous tissue proliferation occurs in the trabeculae, and the histologic observations strongly suggest that the connective tissue appears by stromal condensation only.

"New" bile channels are a prominent feature in the trabeculae, and these passages have been thought by many to arise by epithelial proliferation and budding. But it has already been shown [1] that cysts which have ruptured into bile canaliculi frequently take on the appearance of atypical bile ducts (Fig. 52). If communications are established between a cyst lumen and *several* adjacent bile canaliculi, a space is thus formed which is in continuity with the biliary tree and is lined by the atrophic, dedifferentiated hepatic cells which originally formed the cyst wall. The ruptured cyst has then assumed the morphologic criteria of a section of a true biliary passage. Cysts frequently form in chains (Figs. 11 and 12) which correspond to the original cord-like structure of the hepatic parenchyma. A number of such chains may eventually rupture into bile canaliculi, and thus long narrow spaces or channels lined by simple epithelium are formed. In sections of cirrhotic livers in which the bile duct had been injected with India ink, new bile passages formed in this manner are filled with the injected material (Figs. 54 and 55). Therefore, these ducts are not blind passages, but are continuous with the entire biliary tree. Thick, cleared specimens of livers which have been intravascularly injected with ink facilitate visualization of the relationships involved (Figs. 46 and 47). As liver lobules are arranged frequently in petal form around main branches of the portal vein (Fig. 46), there are several points at which centrolobular areas lie very close to main portal canals. At these regions it is possible to demonstrate links between the newly formed bile passages lined by epithelium and the

main branches of the bile duct (Fig. 48). Thus atrophic fatty cysts may become converted into functional bile passages without proliferation or budding of the epithelium lining the original bile ducts. This is in agreement with the fact that mitotic figures in epithelium of bile ducts have not been reported in cirrhosis of this type.

From the foregoing it is evident that it is unlikely that the "fibrous" trabeculae in livers of these cirrhotic rats arose by proliferation of new tissue elements. Instead, the trabeculae represent compressed and atrophied remnants of hepatic parenchyma and stroma which have been rendered functionless by the effects of prolonged and excessive accumulation of fat due to choline deficiency.

The Cirrhogenous Nature of Fatty Cysts

In many hundred sections of cirrhotic livers of choline-deficient rats, hepatic fibrosis has never been found in the absence of fatty cysts or their atrophic remnants; conversely, the greater the number of fatty cysts or their remnants, the greater has been the fibrosis. There is a constant demonstrable association at every stage in the pathogenesis of cirrhosis of this type with the formation and involution of fatty cysts. Every aspect of the anatomical lesions can be related directly or indirectly to abnormal accumulation of fat in the liver, which is, accordingly, the basic lesion responsible for all other changes. The latter must therefore be regarded as secondary to the fatty change. The sequence of events can be recapitulated briefly as follows.

Intracellular accumulation of lipid in the parenchyma leads to the formation of pathologic cysts filled with lipid which has become extracellular during this process. Fat escapes from nearly every cyst which attains a diameter (in paraffin sections) of 80 to 100 μ and enters either the biliary or vascular system. Release of fat from cysts leads to their shrinkage with accompanying condensation of the reticular stroma which supported the cells in their walls. Remnants of cysts which have ruptured into bile canaliculi persist as "new" epithelium-lined biliary passages. Red cells, masked as deposits of ceroid, may persist in cysts which have ruptured into sinusoids. By compensatory hypertrophy and hyperplasia, the periportal parenchyma replaces liver tissue lost in the formation and destruction of cysts in non-portal regions. Increase in the total amount of fibrous tissue in cirrhotic livers probably is due to the formation of new reticulin in the regions of periportal hypertrophy along with simultaneous persistence of condensed reticulin which surrounded cysts before they shrank or disappeared. Many of the cardiac, pulmonic, and renal lesions which

are found in rats fed hypolipotropic diets for many months may be regarded as manifestations of fat embolism of these organs from the liver. Thus all these pathologic processes are secondary to the basic lesion, *i.e.*, excess accumulation of lipid in the liver.

The evidence for the above sequence of events has been presented in detail and illustrated in photomicrographs. The chain of these pathologic changes in rats fed diets low in choline constitutes convincing evidence that accumulation of fat in the livers of these animals is the direct cause of the cirrhotic lesions. The available data indicate to us that there is nothing to justify the view that the fatty and fibrotic lesions are separate or independent manifestations. Indeed, the natural history of the cirrhotic lesions clearly shows that the fibrosis should be regarded merely as the morphologic end-product which inevitably results if a group of hepatic cells are increasingly overloaded with fat for a sufficient period. The extrusion of intracellular fat in an intracystic, extracellular form is the necessary and essential metaplastic link between the two lesions, fatty and fibrotic.

Intranuclear Fat Droplets

There are few available reports concerning the presence of stainable fat droplets within the nuclei of parenchymal cells of the liver. Hanser [18] made brief reference to this condition in cases of cirrhosis in man. During the actively progressing stages of cirrhosis, in an appreciable number of hepatic nuclei of almost every rat which had been fed the low-choline diet for a year or more, small droplets of stainable fat could be demonstrated. These intranuclear lipid aggregates resembled those found in the cytoplasm in that their number varied inversely with their size (Figs. 20 and 51). The largest droplets which have been encountered within nuclei are equal in size to a nucleolus. Droplets frequently have been found within nuclei which appear normal in every other regard. Histochemical tests of the intranuclear stainable fat indicate that it is probably a triglyceride. The significance of stainable fat within hepatic nuclei in experimental dietary cirrhosis of rats fed choline-deficient diets is not apparent as yet.

Fatty Cysts in Livers of Human Subjects

Frozen sections of livers, kidneys, and lungs from a necropsy series of 30 cases of alcoholism * have been examined. Cysts have been identified (Figs. 56 and 57) in every case in which fatty cirrhosis has

* This material was supplied through the courtesy of Professor William Boyd, Department of Pathology, University of Toronto.

been encountered in an active stage of the disease. Small amounts of embolic fat within the vessels of both lungs and kidneys (Fig. 58) have been detected in a number of cases. These findings suggest that a sequence of pathologic changes which are similar to those described for choline-deficient rats may occur in the cirrhosis associated with an excessive intake of alcohol by human subjects. This problem is now under investigation in the Department of Pathology, University of Toronto.

It has been established [19,20] that choline, restored to the diet of animals which have been fed a hypolipotropic diet for *short* periods, rapidly mobilizes the hepatic lipid which has accumulated during the time of dietary deficiency. Due to the brevity of the periods of choline deficiency in these experiments, it is likely that the liver fat would have been stored chiefly in intracellular form. Investigations in progress * indicate that extracellular (intracystic) fat resulting from longer periods of dietary deficiency of choline also may be mobilized when choline is restored to the diet, but that this occurs much more slowly than when lipids are intracellular. The data already at hand indicate that the clinical pathologist should distinguish between intracellular and extracellular forms of hepatic lipids, as the significance of these two types may be quite different.

Perhaps substances other than fat, such as glycogen, water, and even protein, may not always be stored within single cells under conditions of abnormal accumulation. Is some of the accumulated glycogen found in von Gierke's disease intracystic rather than merely intracellular? It is conceivable also that the principle of pathologic cyst formation as an extension of excessive intracellular storage of a substance may apply to organs other than the liver.

Microscopic Diagnosis of Fatty Cysts

Sometimes it may be difficult to determine whether stainable fat is actually extracellular (intracystic) or intracellular. Small droplets within the cytoplasm are easily recognized as intracellular, and, at the other extreme, the fat in very large cysts (Fig. 9) may be identified with equal ease as extracellular. Fat in shrunken cysts is surrounded by several nuclei of the cyst wall in a single section (Figs. 26 and 55) and the resultant multinucleate nature of the cyst is apparent, thus facilitating diagnosis; but in a tangential section of a small cyst (com-

* These experiments are being conducted by Professor E. A. Sellers, Department of Physiology, University of Toronto, and by one of us (W. S. H.). The results will be reported in detail elsewhere.

posed of only 4 to 8 cells) extracellular fat may appear indistinguishable from a large intracellular conglobate of fat. In such cases, several serial sections may be necessary to determine if the cytoplasm of only one cell contains the fat, or if several cells have joined to form a small cyst in which the fat is held. Serial sections will seldom be necessary, because, whenever extracellular fat is present, cysts will almost always be found in every stage of formation and shrinkage. Thus large cysts and involuting cysts (as indicated above) will indicate clearly that some of the fat is extracellular, and the determination of the exact distribution of the lipid in doubtful regions will ordinarily not be of importance as long as it has been recognized that fat of both types is present.

Summary

In a previous communication,[1] the structure, formation, and involution of pathologic fatty cysts (lipodiastemata) in the livers of rats fed a diet low in choline were reported. The manner in which lipid escapes from fatty cysts is described in the present paper.

Cysts may rupture into either a bile canaliculus or hepatic sinusoid. In the first instance the fat released from the cyst escapes into the biliary tree and may be demonstrated within the lumina of bile canaliculi and ducts. In the second case, fat droplets enter the vascular system.

Droplets of stainable fat were found as emboli in the vessels of the heart, lungs, and kidneys.

The series of morphologic changes undergone by red blood cells found in ruptured fatty cysts suggests that the erythrocytes may become closely bound to ceroid so that they are often indistinguishable from other deposits of this pigment.

The trabeculation in experimental dietary cirrhosis represents a condensation rather than a proliferation of the pre-existing stroma. The bile passages in the trabeculae may be derived to a large extent from cysts which have ruptured into biliary canaliculi.

Brief reference is made to the presence of fatty cysts in human subjects. Small fat emboli in the glomerular loops of the kidney of man are illustrated as found in cases of alcoholism.

An attempt has been made to reconstruct the sequence of events which leads to the development of the typical histologic lesions of cirrhotic livers of rats fed choline-deficient diets. Accumulation of excess fat within cysts in the liver appears to be the primary etiologic factor which is responsible in turn for the secondary fibrosis and nodular hyperplasia.

The microscopic identification of large fatty cysts in ordinary paraffin sections is possible if the cysts are of sufficient size, so that a single cut includes several of the nuclei in the cyst wall. In a random section of a shrunken cyst, many more of the nuclei in its wall are usually included, thus facilitating its identification.

Mrs. Kathleen Henderson assisted with the preparation of photomicrographs. We are indebted to Mrs. Louise Gordon for the execution of the diagrams in Figures 1 to 6. We also wish to thank Miss Maria Wishart, Director of the School of Medical Art, University of Toronto, for her generous interest in planning these illustrations.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 145

FIG. 1. Three-dimensional diagram to show relation of liver cells to bile canaliculi and sinusoids. A group of eight cells in the upper portion of the figure lies next a sinusoid. The bile canaliculi, here represented as small, dark tubes, course around and between the parenchymal cells. The lower group of eight cells are somewhat swollen by small droplets of intracellular lipid shown as light areas on the right.

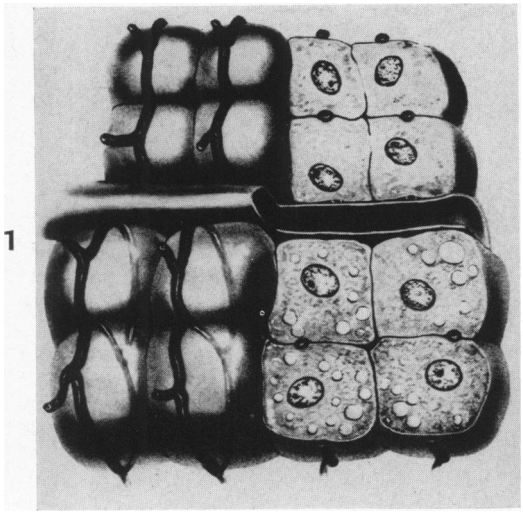
FIG. 2. Three-dimensional diagram to show the appearance of liver cells after they have been distended by large intracellular conglobates which have displaced the nuclei to one side. The transected cells in the upper portion are shown filled with the fat which has been omitted in the lower four transected cells so that their interiors may be revealed.

FIG. 3. Three-dimensional diagram to illustrate the formation of first generation fatty cysts. Ruptures in the thin, compressed membranes between adjacent, fat-filled cells are permitting the coalescence of lipid from the four neighboring cells. It may be noted that the bile canaliculi have been shown displaced to one side of the sites of the rupture so that these have escaped rupture. For comparison with Figure 2.

FIG. 4. Three-dimensional diagram to illustrate the appearance of the newly formed cysts. Each has been formed from a group of four cells, the nuclei of which can be seen in the upper two shown as transected. The displaced bile canaliculi, which thus escaped rupture at the time of cyst formation, now course over the surfaces of the cyst but do not communicate with their lumina at this stage.

FIG. 5. Three-dimensional representation of rupture of a cyst into bile canaliculi (above) and a sinusoid (below). These cysts have formed each by coalescence of two of the smaller cysts shown in Figure 4. In the lower cyst, bile canaliculi were not displaced at the time of the rupture of the membrane separating the smaller cysts (just beginning in Figure 4) and so were torn at the same time. As shown by the arrows, the lumina of the large cyst now communicate at several points with the ruptured canaliculi. A similar process has produced a communication between the sinusoid and the lumen of the upper cyst so that blood is entering the cyst and fat is entering the sinusoid.

FIG. 6. Three-dimensional representation of a large cyst which has established communications with two sinusoids by a process similar to that illustrated in Figure 5. Blood is entering the cyst by the sinusoid at the left, and blood and fat are leaving by the one at the right. Eventual collapse of such a cyst, when all lipid has been washed out, with atrophy of the epithelial wall will leave little trace of its former presence.



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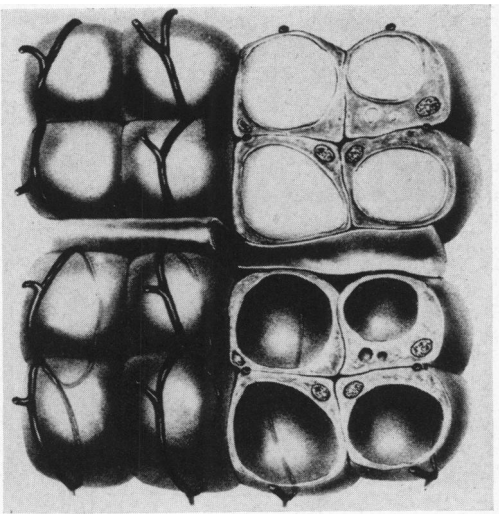

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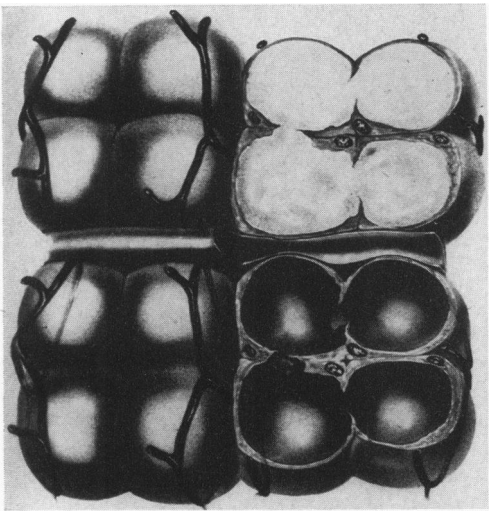

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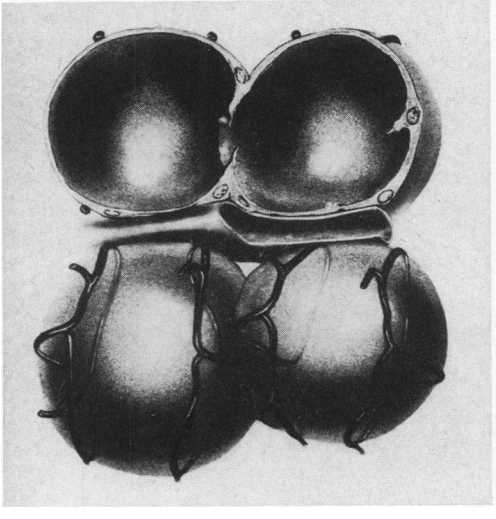

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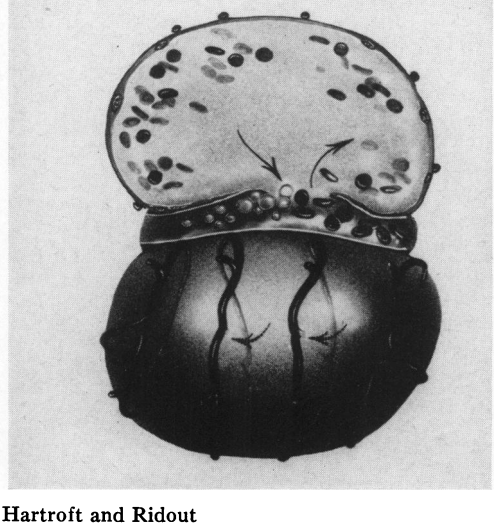

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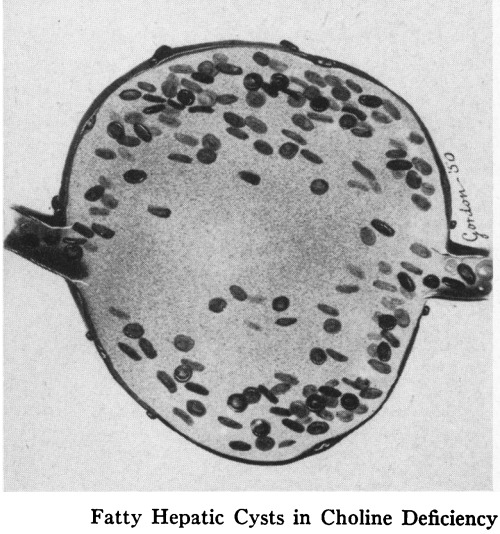

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Fatty Hepatic Cysts in Choline Deficiency

The photomicrographs shown in Figures 7 to 55 inclusive are made from sections from rats fed the basal choline-deficient diet for periods varying from 10 to 16 months (with the exceptions of Fig. 22 from a control animal fed a choline-supplemented diet, and of Fig. 26 from a rat fed the basal diet supplemented with tocopherol).

Plate 146

Fig. 7. Liver. Four pairs of fatty cysts are shown in the field either in the process of fusing to form larger ones, or at the stage just preceding fusion. Frozen section, Wilson's stain.[8] × 300.

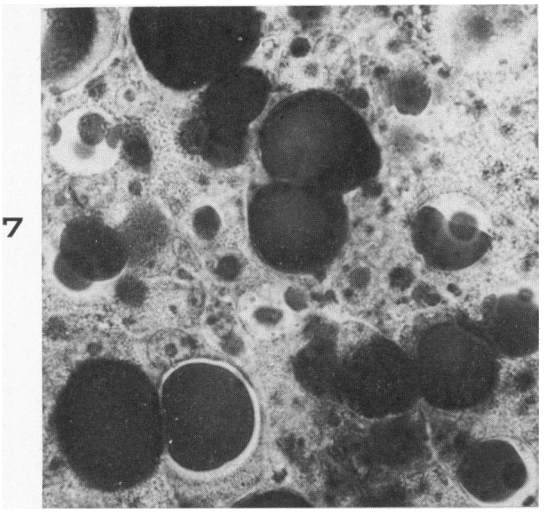
Fig. 8. Liver. Oil immersion view of fatty cyst just after the membrane between two smaller ones has ruptured so that a single larger one is being formed. The hourglass shape of newly formed or newly enlarged cysts is characteristic. Of note are the surrounding nuclei in the cyst wall. Frozen section, Wilson's stain. × 500.

Fig. 9. Liver. Two bile canaliculi, indicated by arrows, are shown in relation to two large fatty cysts. These canaliculi (which are actually continuous, although not in this section) lead up to the compressed intercystic membrane. The canaliculus has been displaced out of the plane of sectioning by compression of the intercystic membrane. Rupture of the membrane in the plane of the section would not tear this canaliculus which is out of the way of injury. Paraffin section, hematoxylin and eosin stain; photographed with the phase-contrast microscope. × 500.

Fig. 10. Liver. The bile canaliculi are filled with ink injected into the main bile duct at the time the animal was sacrificed. The canaliculus (black) at the left of the field runs over the surface of two cysts, but not between them. That to the right of the center of the field runs between two cysts in the thinned membrane separating them. If this membrane should rupture so that a larger cyst is formed from the two it separates, the canaliculus would be torn if it was not first displaced. Tearing of an intramembranous bile duct in this manner produces a communication between the lumen of the newly formed cyst and the biliary tree so that fat may be released from the cyst into the biliary system. Paraffin section, hematoxylin and eosin stain. × 500.

Fig. 11. Liver. A sinusoid, partly filled with red cells, is shown coursing along the surface of three large cysts which have formed a chain. As this sinusoid is not caught between any of the intercystic partitions, it is not likely to be torn in any fusions between the three cysts in the field. This may be compared with the sinusoid shown in Figure 12. Paraffin section, hematoxylin and eosin stain; photographed with the phase-contrast microscope. × 500.

Fig. 12. Liver. Between the largest cyst in the field and that on the right, a sinusoid partly filled with compressed red cells passes between the adjacent walls of the fatty cysts. If the sinusoid remains in this position and if the intercystic partition is ruptured, it can be seen that a communication between the lumen of the newly formed larger cyst and that of the torn sinusoid would result. Paraffin section, hematoxylin and eosin stain. × 500.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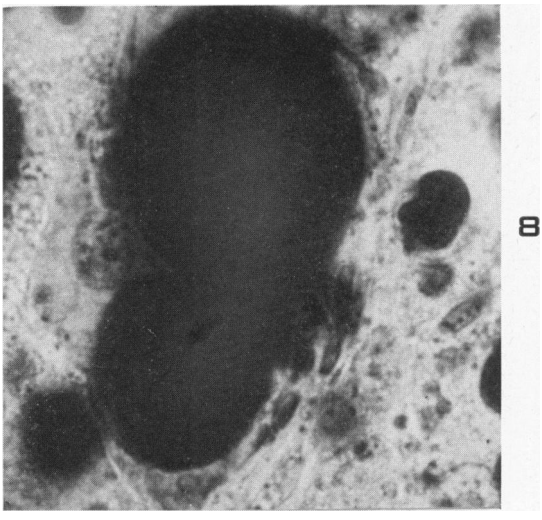
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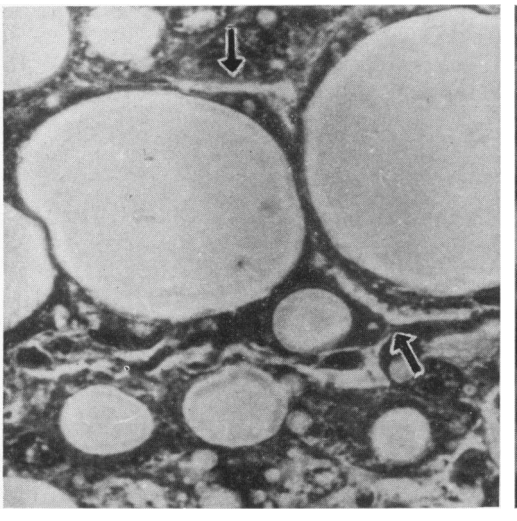
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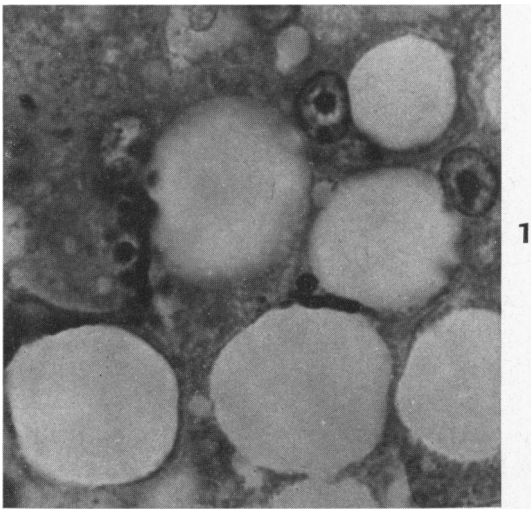
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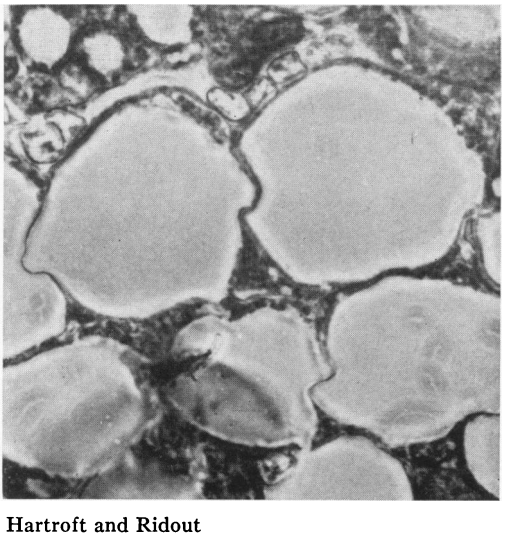
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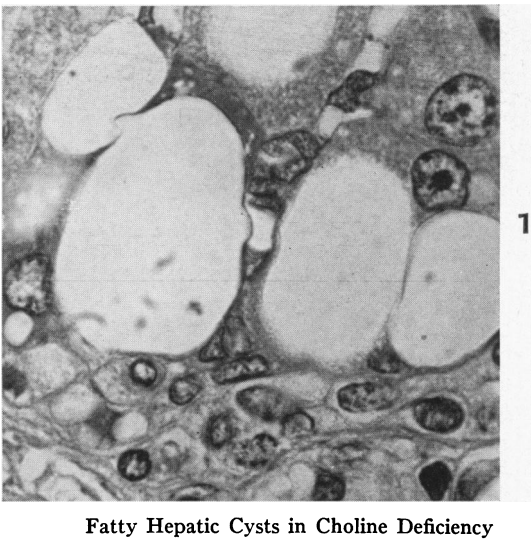
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PLATE 147

FIG. 13. Liver. The annular, non-portal distribution of fatty cysts is shown in this low-power photograph. The portal area (ink-filled vessels) occupies the center of the field. Central veins at the periphery are linked together by a wide band of fatty cysts filled with extracellular lipid (black). Small amounts of intracellular fat (small black dots) are present in the periportal, non-cystic parenchyma. Frozen section, Wilson's stain. × 200.

FIG. 14. Bile canaliculi (filled with black ink) are shown entering the field at the right and communicating with the lumen of the cyst in the center, which is filled with fat (gray). The ink has entered the lumen of the cyst but is confined to a small space between the contained fat and the cyst wall. Frozen section of liver of rat injected with ink through the bile duct at the time of sacrifice. Wilson's stain. × 1000.

FIG. 15. Liver. A large cyst fills the center of the field. It is only partly filled with lipid, which clings to the inner sides of the cyst wall in the form of a crescentic rim. In the lower right quadrant of the cyst wall, a free communication exists between the lumen of the cyst and that of a bile canaliculus. Droplets of fat can be seen breaking from the tip of the crescentic rim of lipid and entering the bile canaliculus. The fat which has left the cyst in this manner presumably has been replaced with refluxed fluid from the bile canaliculus. Frozen section, Wilson's stain. × 650.

FIG. 16. Liver. The field contains a large cyst only partly filled with fat. At the upper right quadrant of the cyst wall and at the lower and central point (between 5 and 6 o'clock), small droplets of fat may be seen leaving the cyst through small bile canaliculi. Frozen section, Wilson's stain. × 900.

FIG. 17. Liver. The field shows a bile channel in the fibrous trabecula of a cirrhotic liver. The main bile passage (entering field at lower left) is filled with ink (black in photograph). This passage communicates with an irregular cyst (center) partly filled with black ink and lipid (gray). Frozen section, Wilson's stain. × 900.

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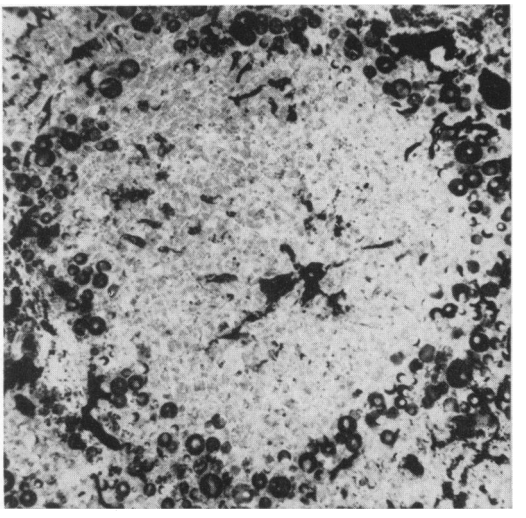

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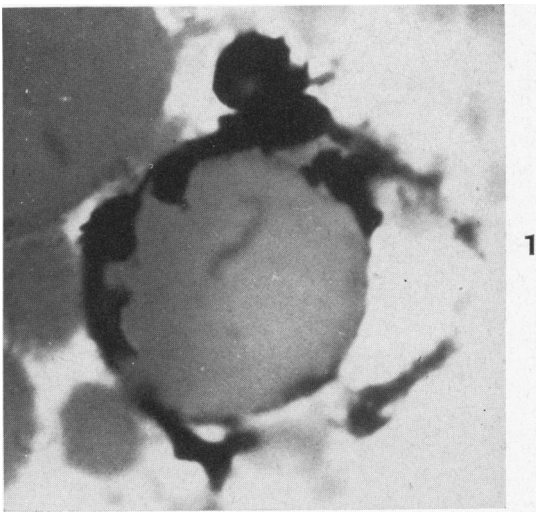

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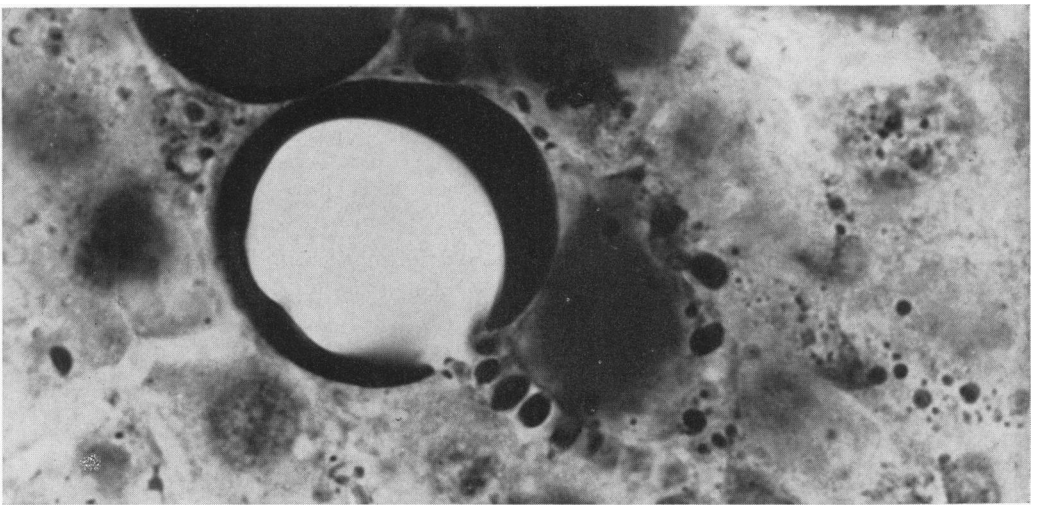

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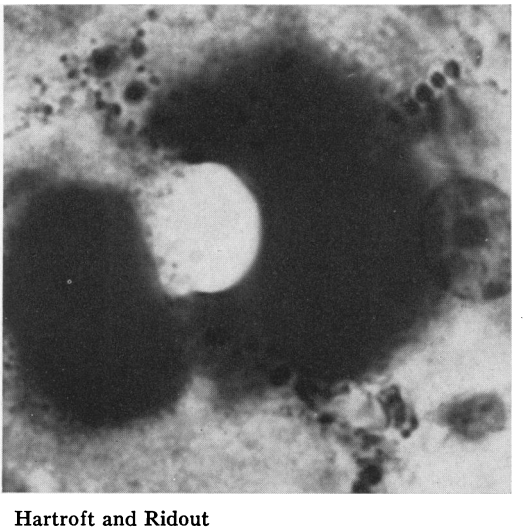

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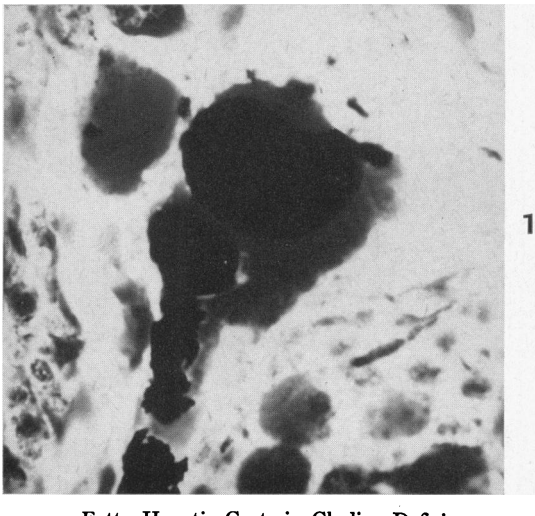

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PLATE 148

FIG. 18. Liver. A large retort-shaped cyst is seen in the upper portion of the field. From the cyst, droplets of fat (dark gray or black) may be seen entering the sinusoid which leads through the center of the field toward the bottom of the photograph. A few red cells in the sinusoid appear as pale, shadowy ellipsoids. Frozen section, Wilson's stain. × 700.

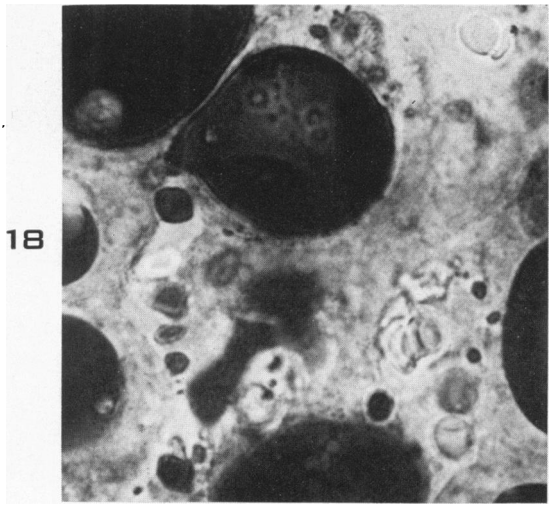
FIG. 19. Liver. A sinusoid cut in longitudinal section passes from the lower right corner of the field to the upper left where it is plugged by a large cylindric mass of fat (black). Two red blood cells (pale gray) can be seen at the lower right edge of the lipid plug. Frozen section, Wilson's stain. × 800.

FIG. 20. The bile canaliculi in the right and lower portions of the field contain small chains of fat droplets (black) in this periportal region of a liver lobule. Three of the nuclei in the field contain small droplets of intranuclear fat. Frozen section, Wilson's stain. × 800.

FIG. 21. Nearly every bile canaliculus in the field contains small droplets or plugs of fat (black) in this periportal region of the liver. Frozen section, Wilson's stain. × 800.

FIG. 22. The normal pattern of the biliary tree seen after injection of India ink under low pressure into the bile duct. Thick (100 μ) slice of liver cleared in benzyl benzoate. × 25 and 100 (inset).

FIG. 23. Many ink-filled fatty cysts may be seen at the ends of injected canaliculi along the edges of the fibrous trabeculae in this cirrhotic liver. Inset shows this in detail for one cyst. Preparation and magnifications as for Figure 22.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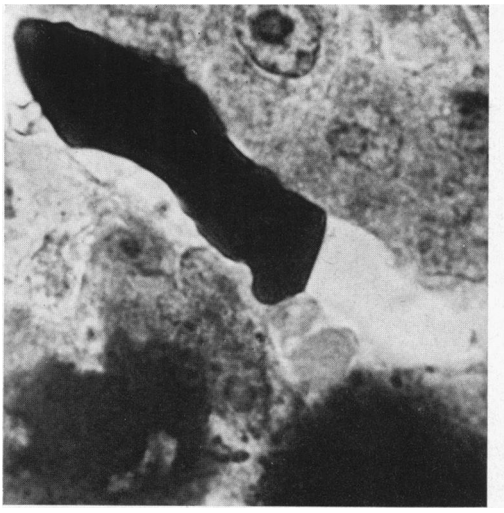
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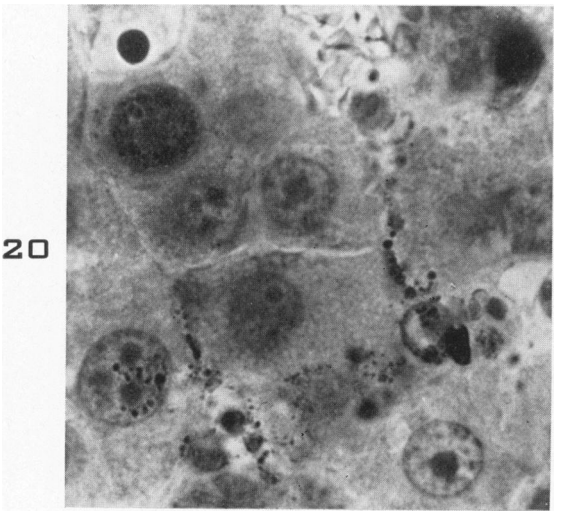
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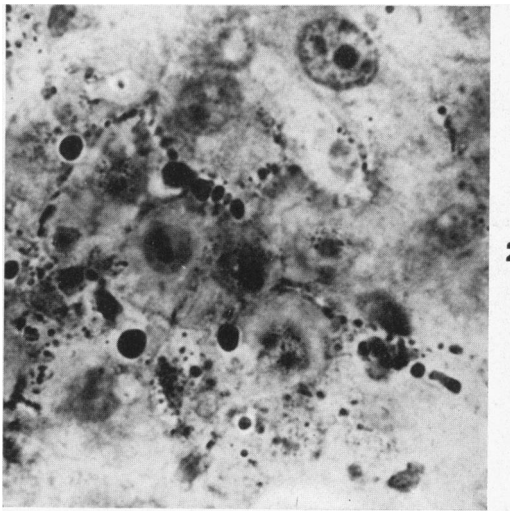
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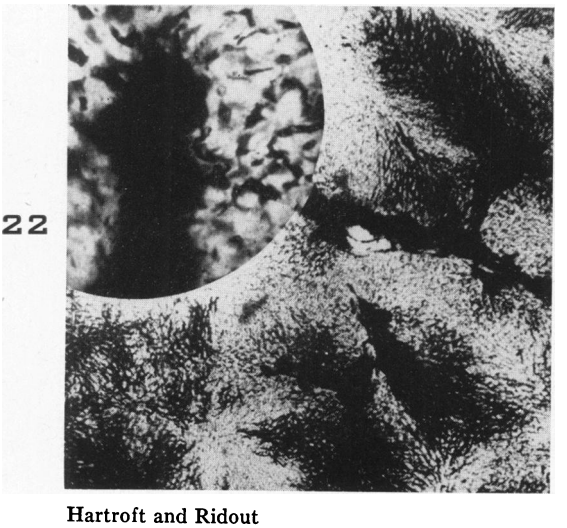
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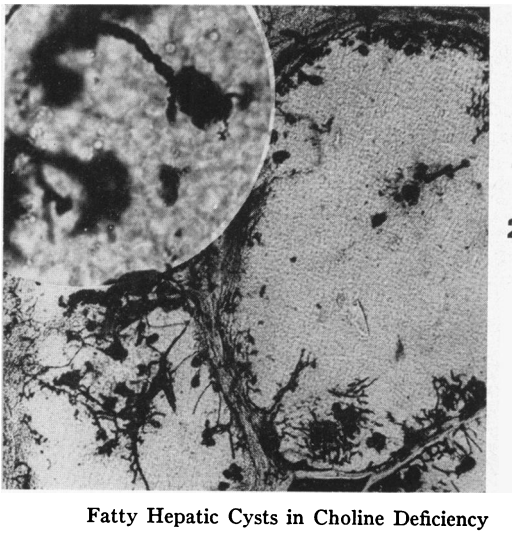
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PLATE 149

FIG. 24. Liver. At the top is a large branch of the hepatic vein partly filled with a crescentic mass of fat (black) and red cells (mottled gray). Leading from this vein a tortuous sinusoid, also containing both red cells and small fat droplets (black), can be traced to the bottom of the field where a ruptured cyst partly filled with erythrocytes is seen. Frozen section, Wilson's stain. × 200.

FIG. 25. The ruptured cyst shown at the bottom of Figure 24 is enlarged in this illustration. The crescentic mass of fat (black) is leaving the cyst and entering the sinusoid leading from the cyst to the bottom of the field. Red cells (gray) are entering from the sinusoid and replacing the fat. Same preparation as for Figure 24. × 800.

FIG. 26. A large cyst is shown partly filled with red blood cells. The cyst above this and slightly to the right contains four nuclei in its wall. Other cysts in varying stages of involution are shown at the top. The rat from which this section of liver was prepared received the basal diet plus a supplement of alpha-tocopherol. Paraffin section, hematoxylin and eosin stain. × 500.

FIG. 27. Liver. The center of the field is occupied by a ruptured fatty cyst which communicates with sinusoids at the upper right, upper left, and lower midportions of the field. The cells of the cyst wall have become dissociated and are atrophic. Red blood cells have entered the remains of the cyst, separating the cells of the wall and surrounding the fat vacuole. Paraffin section, hematoxylin and eosin stain; photographed with the phase contrast microscope. × 600.

FIG. 28. Liver. The fatty cyst in the upper right corner of the field communicates with the sinusoid which runs toward the lower left. Fat may be seen leaving the cyst and entering the sinusoid in a single large mass (black). At the upper right corner, the cyst may be seen to communicate with another sinusoid at the edge of the field. The crescentic form of the lipid which remains in the cyst is characteristic of fat in cysts partly emptied of their lipid content. Frozen section, Wilson's stain. × 700.

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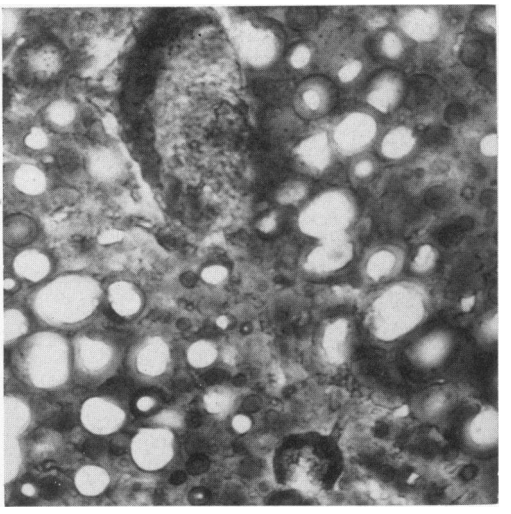

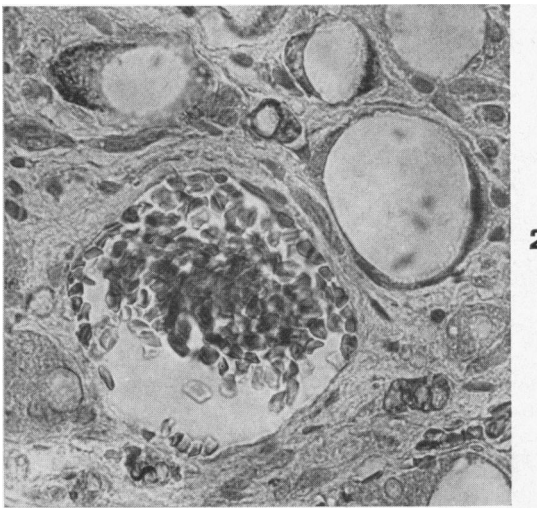

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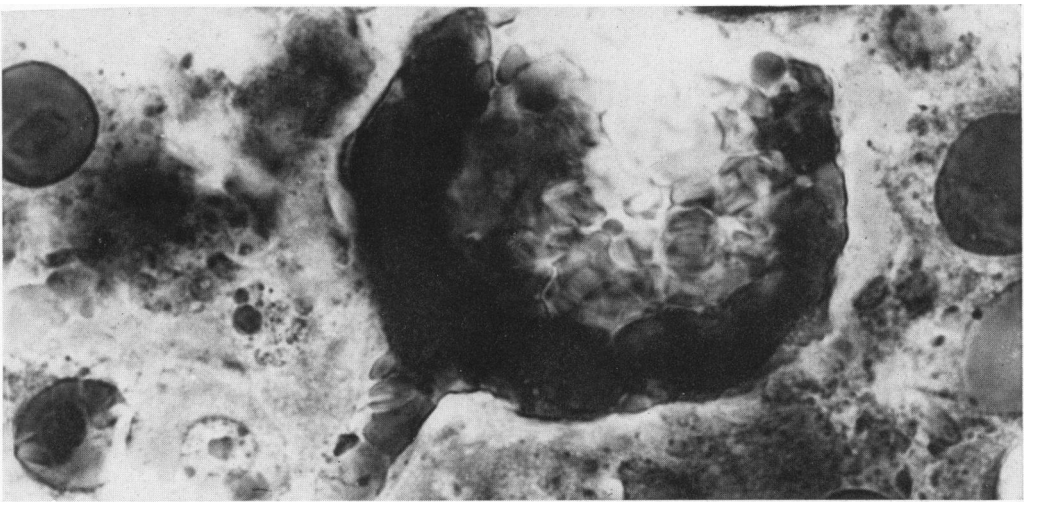

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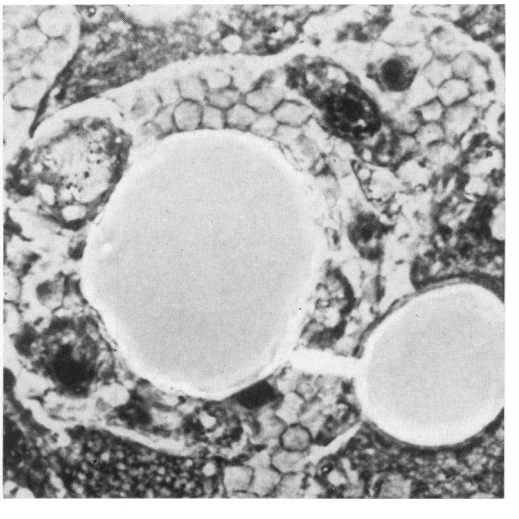

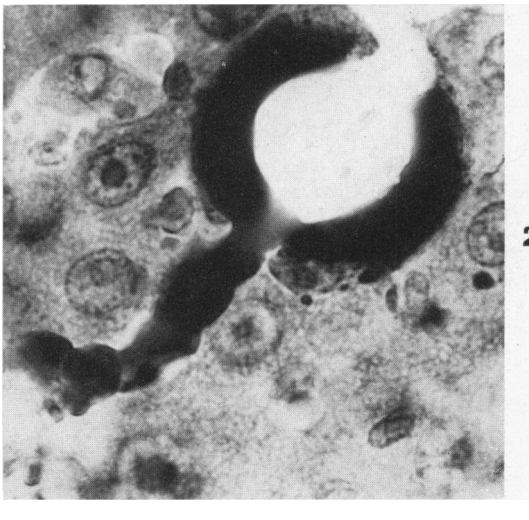

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PLATE 150

FIG. 29. A large branch of the hepatic vein in a trabecula of a cirrhotic liver. The lumen of the vein is completely filled with a mass of fat (black) in which a few red cells are clumped (upper portion of lumen of vein). Frozen section, Wilson's stain. × 750.

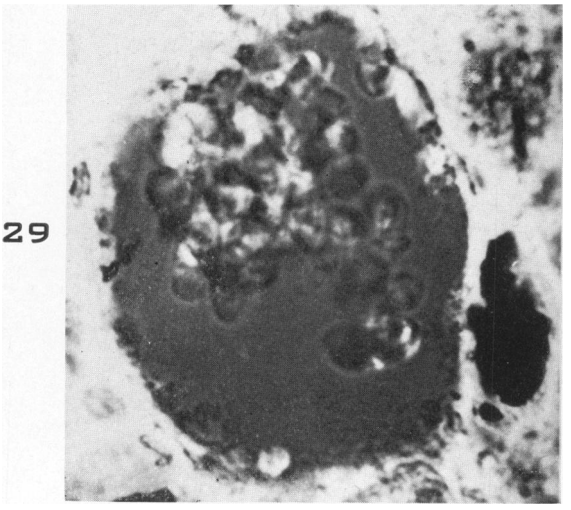
FIG. 30. A small capillary may be seen running between strands of cardiac muscle from the lower right to the upper left corners of the field. Several small fat emboli (black) may be seen in the capillary; the largest in the center of the field is elliptical. Frozen section, Wilson's stain. × 800.

FIG. 31. Heart. An area of focal necrosis composed of degenerating cardiac muscle and fibrous tissue is seen in the central and left portions of the field. The dark areas are masses of calcium salts deposited in the region. Frozen section, Wilson's stain. × 300.

FIG. 32. Lung. In the center of the field is a transected septal capillary in the wall of an alveolus. The lumen of the capillary is filled with a solid plug of ceroid (black). Paraffin section, oil red O and hematoxylin stains. × 800.

FIG. 33. Lung. The arteries and arterioles in the low-power view of this region of the lung contain numerous embolic droplets of fat (black). A representative area enlarged in the inset (upper left) shows a septal capillary, sectioned longitudinally, in which three or four fat droplets (black) can be seen. Some of the fat appears to be passing through the capillary wall into the lumen of the adjoining alveolus and entering pulmonic macrophages. Frozen section, Wilson's stain. × 100 and 400 (inset).

FIG. 34. Lung. Masses of swollen, lipid-filled macrophages (dark) have engorged the lymphoid tissue surrounding a bronchiole present just below and outside the field illustrated. A representative macrophage is shown in the inset (upper left) under higher magnification. The cytoplasm of this cell is filled with innumerable lipid droplets (black). Frozen section, Wilson's stain. × 100 and 500 (inset).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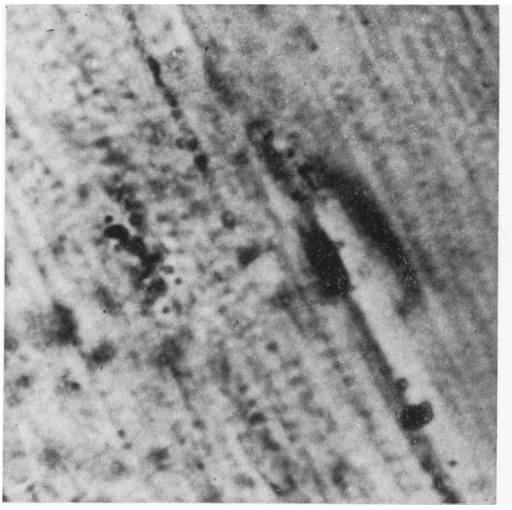
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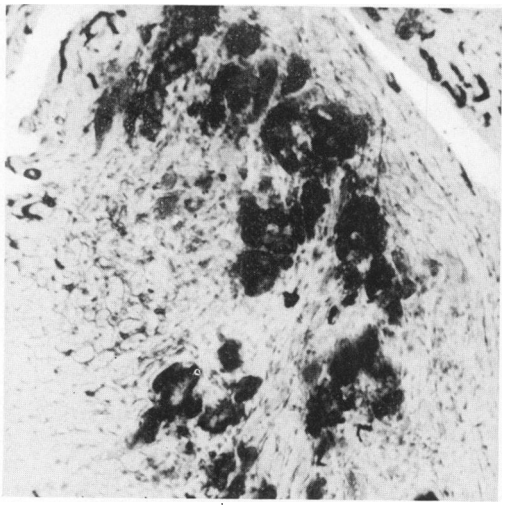
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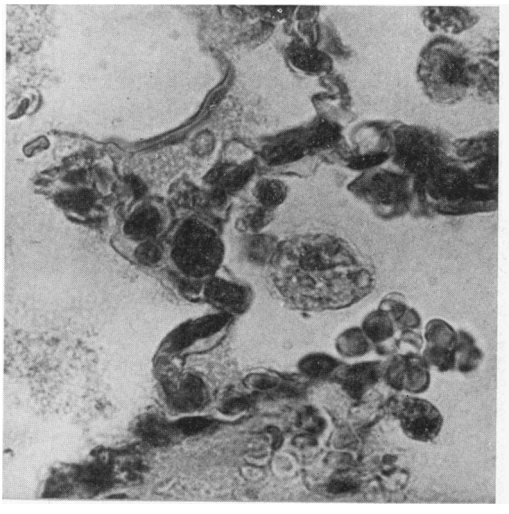
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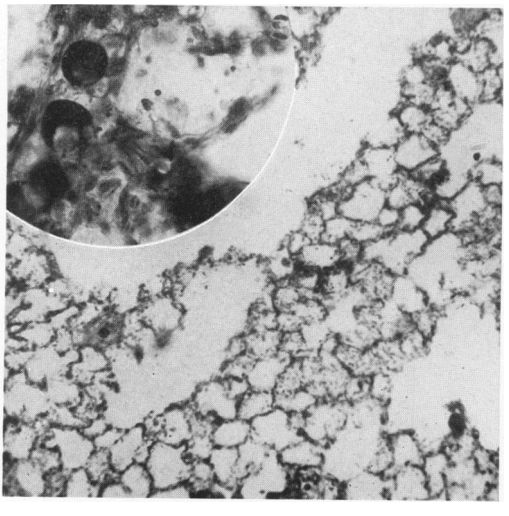
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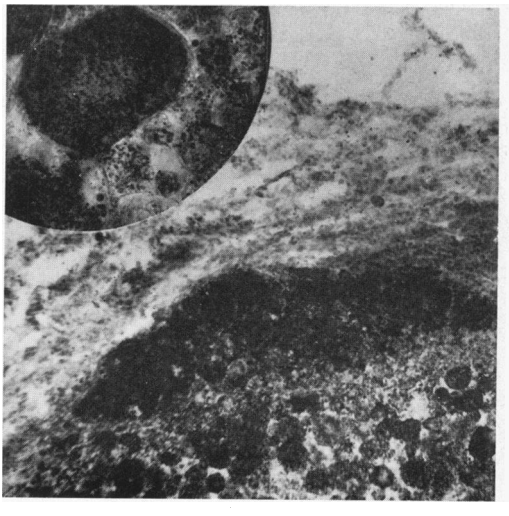
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PLATE 151

FIG. 35. Kidney. Masses of fat (black) mixed with red blood cells (gray) are present in the interlobular arteriole (cut longitudinally in the lower portion of the field) and in the capillary loops of the glomerulus. Frozen section, Wilson's stain. × 400.

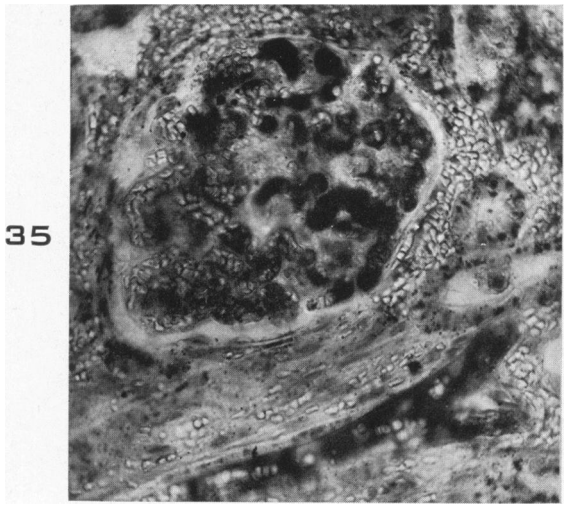
FIG. 36. Kidney. Nearly all of the capillary loops of this glomerulus are distended and plugged with masses of fat (black). Frozen section, Wilson's stain. × 500.

FIG. 37. Kidney. A single capillary loop which has been transected is shown in the central portion of the field. An endothelial cell is shown at one side of the capillary lumen which is completely filled with embolic fat (black). The space of Bowman (lower portion of field) contains a few small lipid droplets. Frozen section, Wilson's stain. × 900.

FIG. 38. Kidney. The animal from which the section illustrated was prepared was injected intravascularly with India ink at the time of sacrifice. In the glomerulus shown, most of the capillary loops are filled with the injection mass (black) but those in the upper right quadrant of the structure have not received the ink for they are blocked by small droplets (gray to dark gray) of fat. Frozen section, Wilson's stain. × 400.

FIG. 39. Kidney. Several of the capillary loops of the glomerulus which fills the field are blocked by embolic plugs of fat (black). The capillary loops in the lower and central portion of the glomerulus are congested and distended with arrested erythrocytes which appear clumped and irregular in outline. These red cells are in an early stage of disintegration. Frozen section, Wilson's stain. × 550.

FIG. 40. Kidney. In this paraffin section from the kidney illustrated in Figure 39, focal hyaline masses are present in and between some of the glomerular capillaries (right of center). This lesion is not unlike that described by Kimmelstiel and Wilson.[12] The close morphologic relationship of this lesion to that illustrated in Figure 39 suggests that they may be different stages of the same pathologic process. Paraffin section, hematoxylin and eosin stain. × 550.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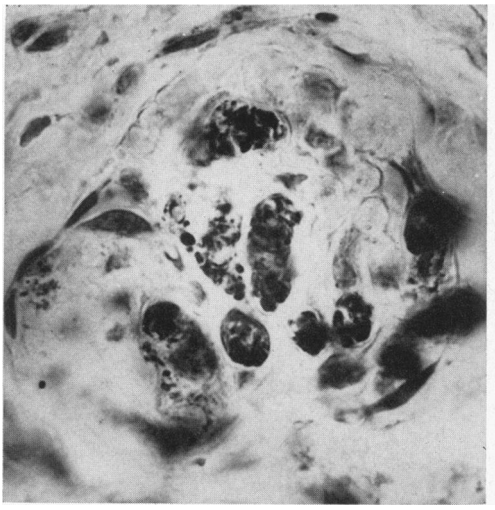
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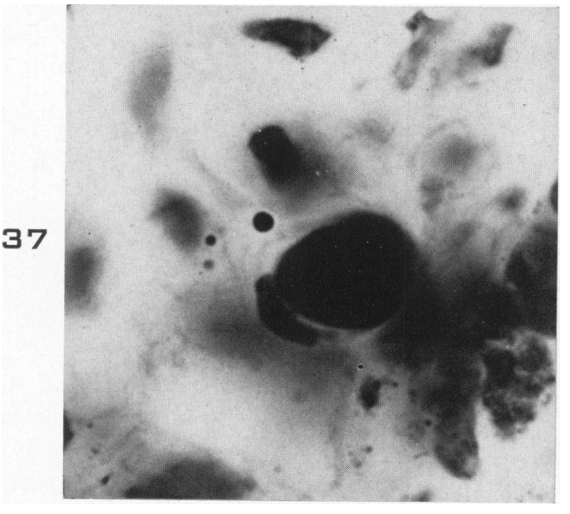
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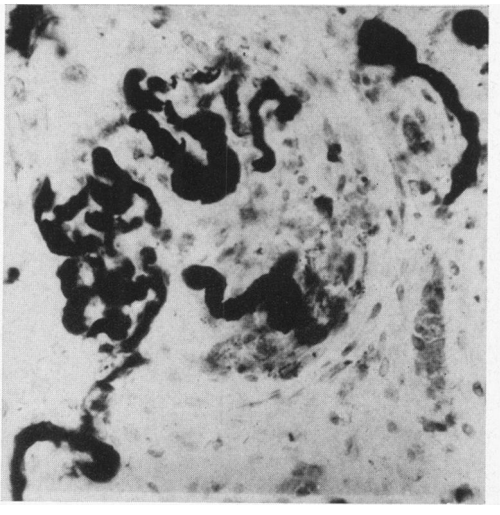
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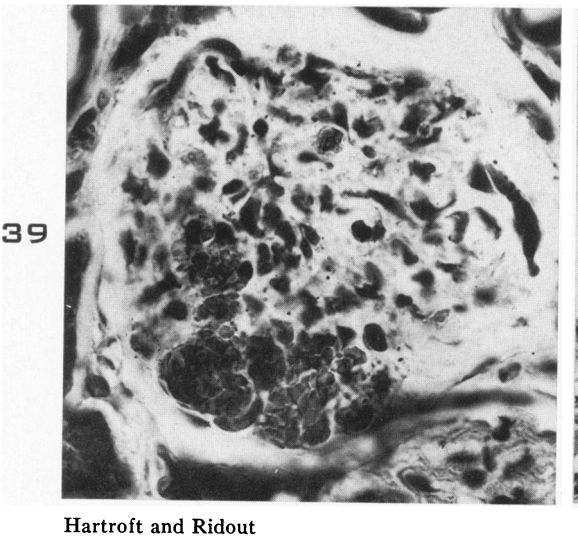
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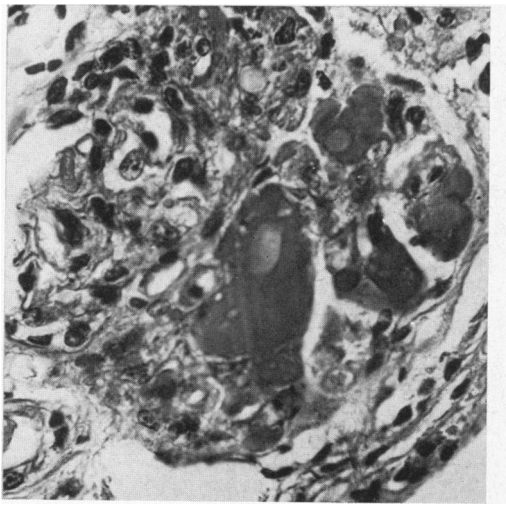
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PLATE 152

FIG. 41. Kidney. Hemosiderin casts and hemosiderin deposits in the walls of renal tubules. Paraffin section, Prussian blue stain. × 350.

FIG. 42. Granules of hemosiderin pigment in the cytoplasm of cells lining the proximal convoluted tubules. Preparation as for Figure 41.

FIG. 43. Kidney. The medullary tubules are filled with innumerable casts of intensely sudanophilic lipid (black). The inset under higher magnification shows some of these casts undergoing calcification. Frozen section, Wilson's stain. × 100 and 500 (inset).

FIG. 44. Liver. This field illustrates stages in the formation of ceroid pigment in ruptured cysts (liver). The cyst shown in the lower left of the field contains recognizable red cells. That sectioned tangentially in the upper portion of the field shows red cells which are breaking up and losing their outlines. The cyst shown at the lower right contains a few red cells, still recognizable as such, plus globules of ceroid. The ceroid globules are of the same order of size as the altered red cells. Paraffin section, stained to demonstrate ceroid, using Wilson's stain. × 700.

FIG. 45. Liver. Further stages in the formation of ceroid. Several ruptured cysts in various stages of involution are shown in the field. They are filled with erythrocytes, or erythrocytic débris, in the process of becoming sudanophilic (ceroid-like). The dark areas in the field were stained shades of red with oil red O, and the lighter areas with light green. Note how the material in the shrunken cysts merges almost imperceptibly from the one shade to the other. Frozen section, Wilson's stain. × 500.

FIG. 46. The lobular pattern of a rat's liver in the early stages of dietary (choline-deficiency) cirrhosis. The vessels were injected with India ink when the animal was sacrificed. The relatively clear areas represent early central and non-portal fibrosis. A large branch of a portal vein occupies the center of the field. There is a petal-like arrangement of lobules around this portal area. Thick (100 μ) slice of liver tissue photographed after clearing. × 30.

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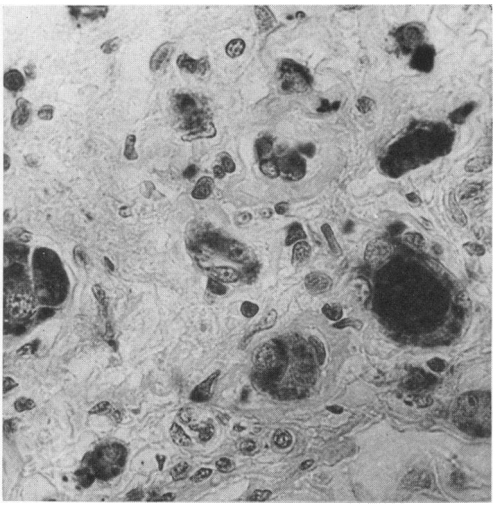

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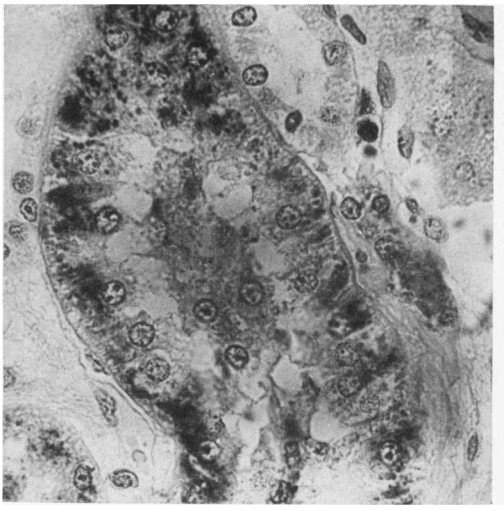

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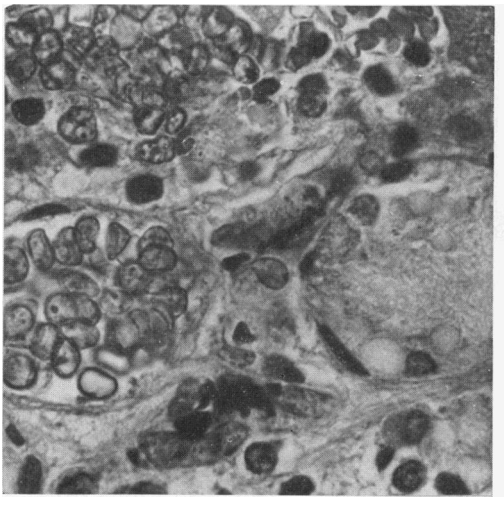

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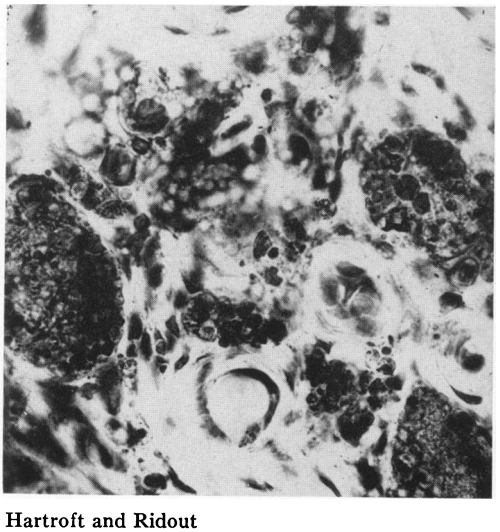

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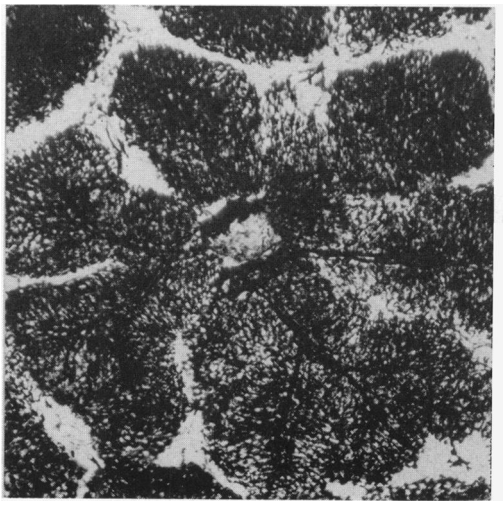

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PLATE 153

FIG. 47. Higher magnification of a portion of the field shown in Figure 46. The lower right portion contains the portal vein. The infolding centrolobular areas closely approach the portal area at several regions, where only small amounts of parenchyma (indicated by the injected sinusoids) separate portal and central areas. Preparation as for Figure 46. × 150.

FIG. 48. Liver. A fibrous trabecula is shown at the left. Bile passages in this area may be seen communicating with portal biliary ducts. It is probable that at such regions "new" bile passages, formed from chains of involuting cysts which have ruptured into bile canaliculi, communicate with main branches of the bile duct. Paraffin section, reticulin stain (silver). × 200.

FIG. 49. Liver. Hemosiderin deposits in and around shrunken cysts in the fibrous trabeculae of cirrhotic liver. The rat from which this preparation was made received a supplement of tocopherol. Paraffin section, Prussian blue stain. × 350.

FIG. 50. Liver. "Macrocysts" in the liver of a choline-deficient rat. The lining of these cysts, although much stretched and thinned, resembles that of the usual fatty cysts found in these livers. Groups of these cysts can sometimes be seen with the naked eye. Paraffin section, hematoxylin and eosin stain. × 80.

FIG. 51. Liver. The nucleus above and in the central portion of the field contains many droplets and small rods of stainable fat (black) surrounding the nucleolus. The nucleus below this also contains stainable fat in small masses (black), but differs from the upper nucleus in that it is undergoing degenerative changes. Frozen section, Wilson's stain. × 1000.

FIG. 52. Liver. The appearance of "fibrous" trabeculae in the liver of a choline-deficient rat. The amount of connective tissue in this type of preparation appears deceptively great. For comparison with Figure 53. Paraffin section, hematoxylin and eosin stain. × 550.

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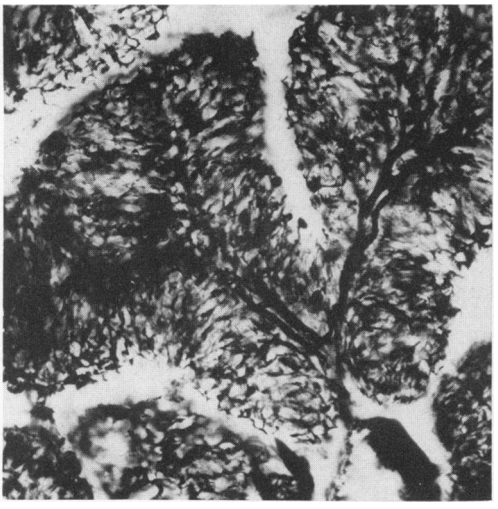

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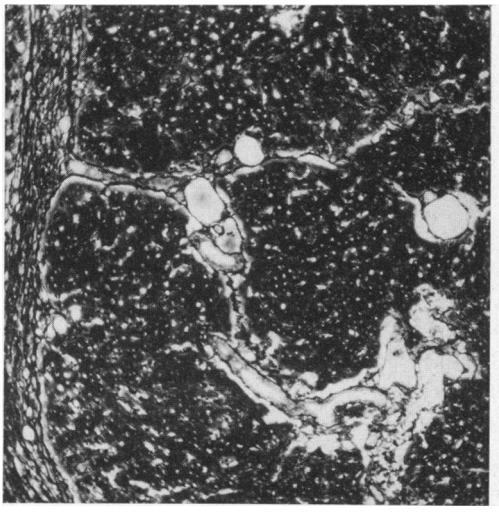

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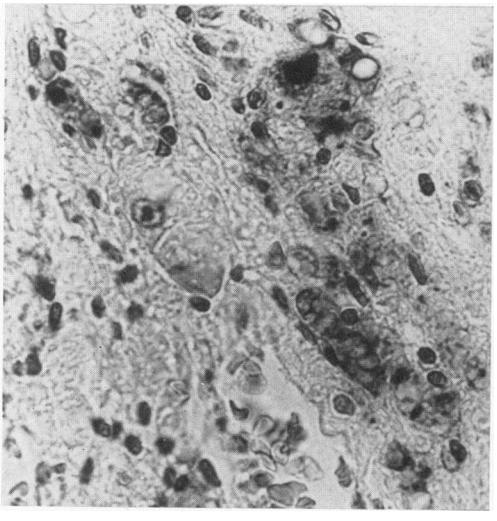

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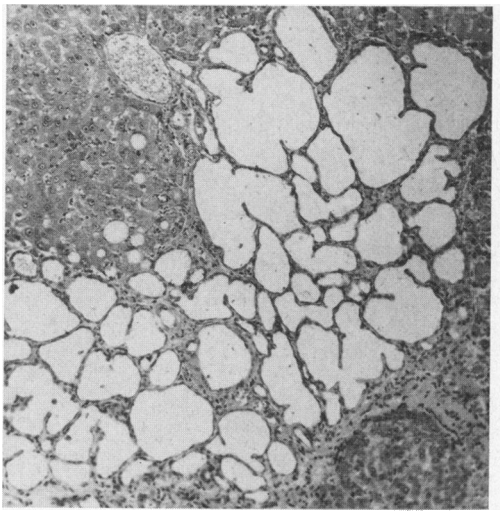

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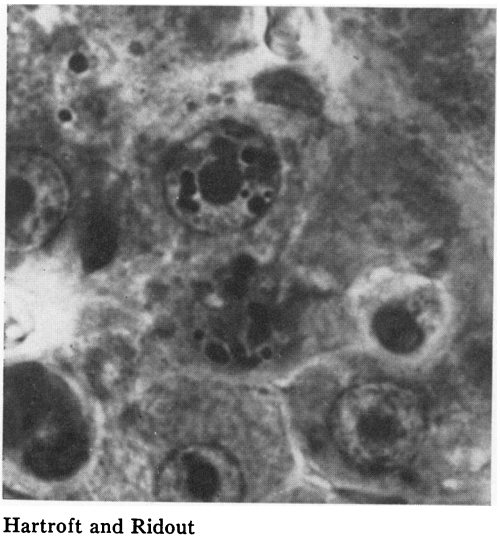

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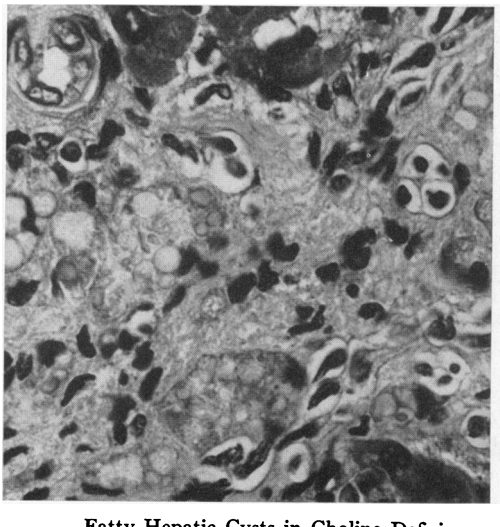

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PLATE 154

FIG. 53. Liver. A region similar to that illustrated in Figure 52, but stained for connective tissue. The only materials which take the connective tissue stain are a few fibrils scattered throughout the field and compressed at the right of the band. The remainder of the trabecula is composed of "new" bile ducts, and shrunken cyst remnants partly filled with globules of ceroid. Paraffin section, azocarmine, aniline blue and orange-G stains. × 550.

FIG. 54. Liver. In the fibrotic region shown, a network of bile passages filled with ink injected through the main bile duct can be seen. Their relations to cysts filled either with fat (gray) or ink (black) may be noted. Surrounding this network of biliary vessels, the parenchyma has been completely replaced by fibrous tissue and cyst remnants. Frozen section, Wilson's stain. × 380.

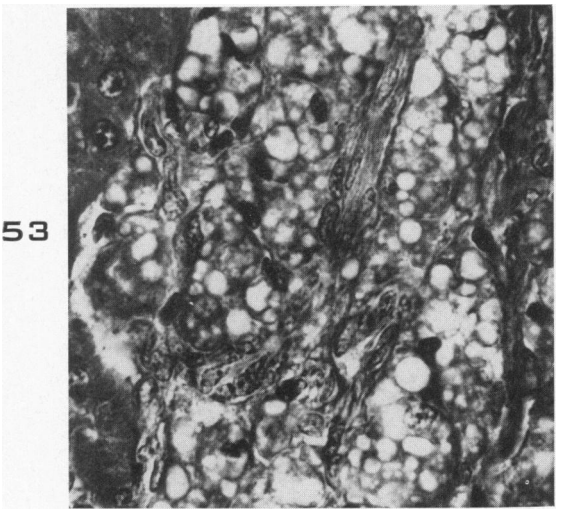
FIG. 55. Liver. "New" bile passages filled with ink, which had been injected into the main bile ducts, are seen coursing through a trabecula. Shrunken cysts, some filled with ceroid globules, lie between the injected ducts. There is a simple epithelial lining surrounding the ink. Paraffin section, hematoxylin and eosin stain. × 450.

FIGS. 56 to 58 inclusive are photomicrographs of sections of tissues obtained at necropsy from cases of alcoholism in man. The preparations illustrated are all frozen sections stained by Wilson's technic.

FIG. 56. Liver. Two fatty cysts in the process of fusing to form a single, even larger structure. Fat appears black. × 400.

FIG. 57. A single, giant fatty cyst in the liver of an alcoholic patient. At the left (at approximately 10 o'clock on the periphery of the cyst) a small extrusion of fat may be seen between two of the cells forming the wall of the cyst. This fat appears to be entering a small bile canaliculus between the two cells, the lumen of which communicates with that of the cyst. × 400.

FIG. 58. Glomerulus in kidney of alcoholic patient. The dark cylindric mass in the glomerulus represents a capillary loop which has been completely plugged with embolic fat. × 250.



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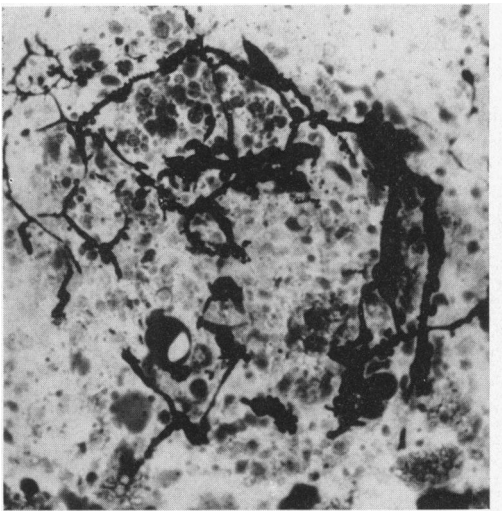

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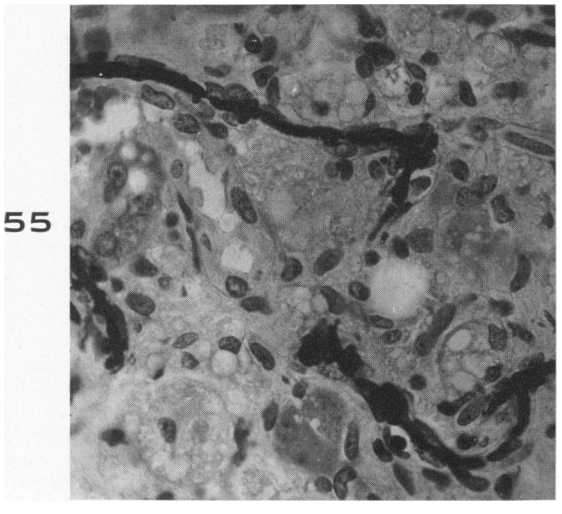

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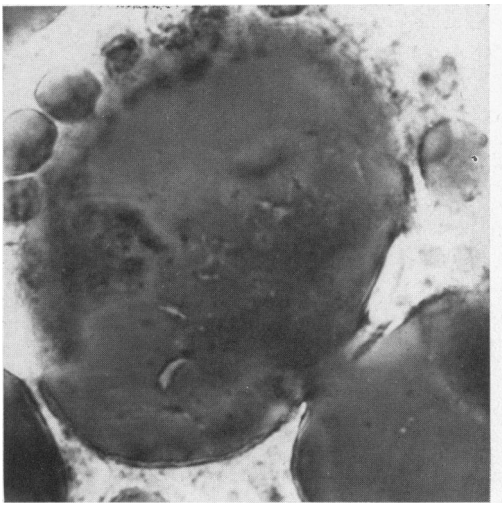

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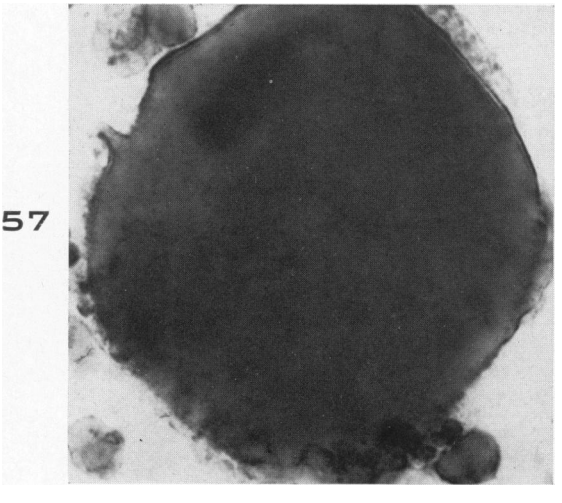

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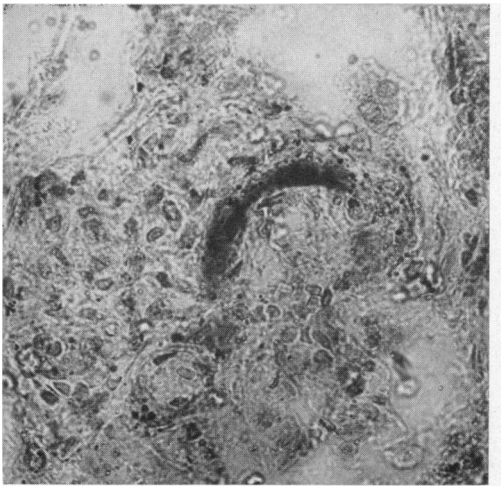

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