Virchows Arch. path. Anat. 336, 99—114 (1962)

From the Department of Pathology, Banting Institute, University of Toronto, Toronto, Ontario, Canada (Head: Prof. A. C. RITCHIE)

Disturbances of Hydration of Cells of Rat Liver in Extrahepatic Cholestasis*

By

JAN W. STEINER, JOHN S. CARRUTHERS and S. ROBERT KALIFAT**

With 11 Figures in the Text

(Received June 26, 1962)

A number of our previous publications have referred to the focal or diffuse disturbances of hydration of biliary epithelial cells, endothelial cells or Kupffer cells which occur in the livers of rats after ligation of the common bile duct (CARRUTHERS and STEINER 1961; KALIFAT, CARRUTHERS and STEINER 1962; STEINER and CARRUTHERS 1962; STEINER, CARRUTHERS and KALIFAT 1962; STEINER, CARRUTHERS and KALIFAT 1962). As these studies progressed, it became apparent that all of these alterations might be the result of an injury by the same agent or agents.

It is the purpose of this paper to describe concisely these findings, and to suggest an explanation for their occurrence, on the basis of a study of the behaviour of colloidal mercuric sulphide particles injected retrogradely into the occluded common bile duct.

Materials and methods

Animals. White, male, Wistar rats, weighing approximately 250 gm, were maintained on Purina Laboratory Chow and water ad libitum.

Ligation of bile duct. The rats were anaesthetized with ether and the abdominal organs exposed by a mid-line incision. The common bile duct was divided between double ligatures of cotton.

Injections of colloidal mercuric sulphide. Ten rats, whose common bile duct had been ligated 7 days previously, were anaesthetized with ether, and given a retrograde intrabiliary injection of 2 ml of an 8% suspension of colloidal mercuric sulphide (HgS) (Hille & Co., Chicago, Illinois). The material was administered through a syringe and needle, but care was taken to exert minimal pressure on the plunger. The injection was completed within one minute, and a biopsy was taken less than 60 seconds later. The particles of colloidal HgS never exceeded 250 Å in diameter, and the smallest were 70 Å in diameter when measured in electron micrographs.

Sacrifice. A total of 20 rats were killed at selected times. Four were killed on the 1st day postoperatively, one on the 7th, 8th, 12th, and 13th days, two on the 14th, one on the

* Supported in part by a grant of the Medical Research Council of Canada and in part by a grant of the Atkinson Charitable Foundation, Toronto, Ontario.

** Interne des Hôpitaux de Paris and Research Fellow of the Canadian Arthritis and Rheumatism Society.

The authors wish to thank Prof. A. C. RITCHIE for his assistance with editing the manuscript. Thanks are also due to Miss BARBARA LAMBERT, Mrs. BARBARA SHIER, Mr. G. J. DOORNEWAARD for their excellent technical assistance, and to Mrs. JEAN CETKOVSKI for preparation of the article.

15th, 16th, 17th, and 20th days, two on the 21st and 25th days, and one on the 33rd and 39th days post-operatively. A further six rats, kept under the same conditions were killed from time to time as normal controls. All rats were killed between 9 and 10 a.m., to reduce as much as possible variations caused by diurnal cycles of feeding and other such factors. As soon as ether anaesthesia had been induced in the rats to be killed, the abdomen was opened and tissues taken from the right half of the median lobe of the liver.

Histologic techniques. Tissues for *light microscopy* were fixed in buffered formalin (pH 7.0) and embedded in paraffin. Sections were stained with haematoxylin and eosin and, whenever appropriate, with the periodic acid-Schiff method with or without prior salivary digestion.

Tissues for *electron microscopy* were fixed in Palade's buffered osmium tetroxide (pH 7.4) containing 0.25 M sucrose (CAUFIELD 1957). 2 ml of fixative was used for each sample. The fixative was stored at 4° C, and was maintained at this temperature for 90 minutes after the insertion of the tissue. Fixation was then continued for a further 30 minutes at room temperature. The tissues were dehydrated in a graded series of ethanol solutions, and embedded in epoxy resin (Epon 812) by the method of LUFT (1961). Sections were cut on a Porter-Blum ultramicrotome with glass knives and stained by flotation on a saturated aqueous solution of uranyl acetate at 60° C (WATSON 1958). The sections were picked up on Formvar-coated grids, and examined in an RCA-EMU 3 E electron microscope.

Results

All the changes to be described developed within 24 hours and persisted for the entire period of observation.

A. Changes in biliary epithelial cells

Examination of the dilated, newly-formed ductular channels, which arise after induction of extrahepatic cholestasis, revealed two changes indicative of a disturbance of the hydration of the biliary epithelial cells.

Firstly, many of the *microvilli* facing the ductular lumina were markedly oedematous, and were often pedunculated (Figs. 1 and 2). In some biliary epithelial cells, the entire luminal border was occupied by a single sessile microvillus which was enormously dilated (Fig. 2). In others, only a portion of the luminal border was taken up by such oedematous microvilli, the other microvilli being either relatively normal or lost (Fig. 1). The hyaloplasm of the oedematous microvilli was usually somewhat less electron-dense than that of the adjacent main perikaryonic mass of cytoplasm. On rare occasions, they were of equal electron opacity. The oedematous microvilli were devoid of organelles, except for occasional smooth-surfaced vesicles, which imparted to some a Swiss-cheese appearance. Near the base of oedematous microvilli, round profiles with a covering membrane of an intensity equal to that of the luminal limiting cell membranes were found. These were interpreted as residual tubular channels at points where adjacent microvilli fused during the phase of developing oedema (Fig. 2). The oedematous hyaloplasm of the microvillus was usually abruptly delineated from the subjacent cytoplasm of the cell (Figs. 1 and 2).

The second change suggestive of a disturbance in the hydration of the biliary epithelial cells was a *variation in the overall electron opacity of biliary epithelial cells*. Fig. 4 illustrates this point. The wedge-shaped cell in the left upper corner of the micrograph has a markedly more dense hyaloplasm than the other two cells seen in the picture. In the "dark" cell, the content of the cisternae of the endoplasmic reticulum is considerably less electron opaque than the surrounding

hyaloplasm, but in the "light" cells the ergastoplasmic content is equal in opacity to the hyaloplasm. It will be seen that the opacity of the "dark" cell is not due to a condensation of free ribosomes, since the cell in the lower half of the picture,

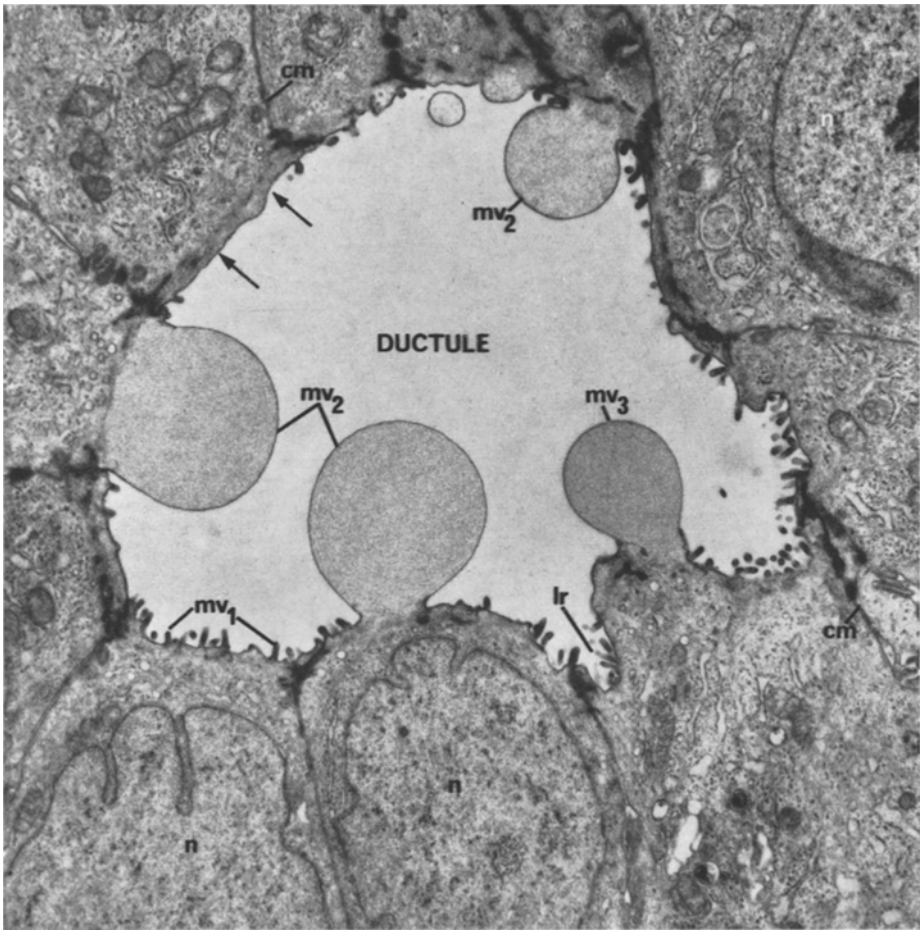

Fig. 1. A bile ductule is surrounded by numerous biliary epithelial cells. A luminal recess (*lr*) and loss of microvilli on one cell surface (arrows) indicate dilatation of the lumen. Normal microvilli (mv_1) are present on one cell surface, and on others some microvilli are oedematous. Their matrix is either lighter (mv_2) or equal to (mv_3) the density of the hyaloplasm of the parent cell (*c.m.* cell membrane, *n.* nucleus). 6240 ×

which contains many more free Palade granules than the other cells, remains "light". These changes in the overall electron density were found not only in cells which abutted on a lumen, but also in intercalated cells which did not reach a lumen in the plane of section.

B. Changes in endothelial cells

Disturbances in the hydration of the cytoplasm of endothelial cells lining blood capillaries and lymphatics were observed in focal areas in the granulation tissue which develops around the new ductules which proliferate in the rats

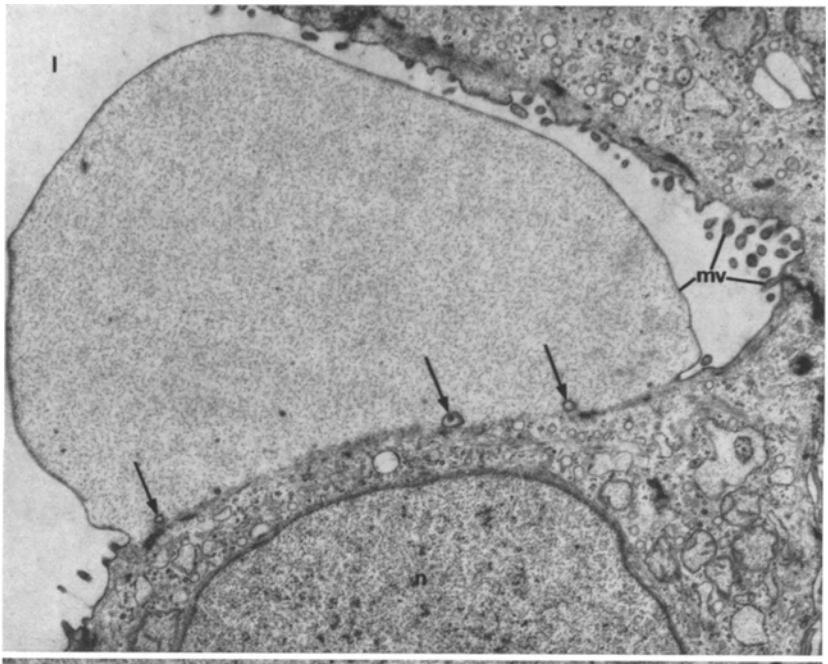

Fig. 2

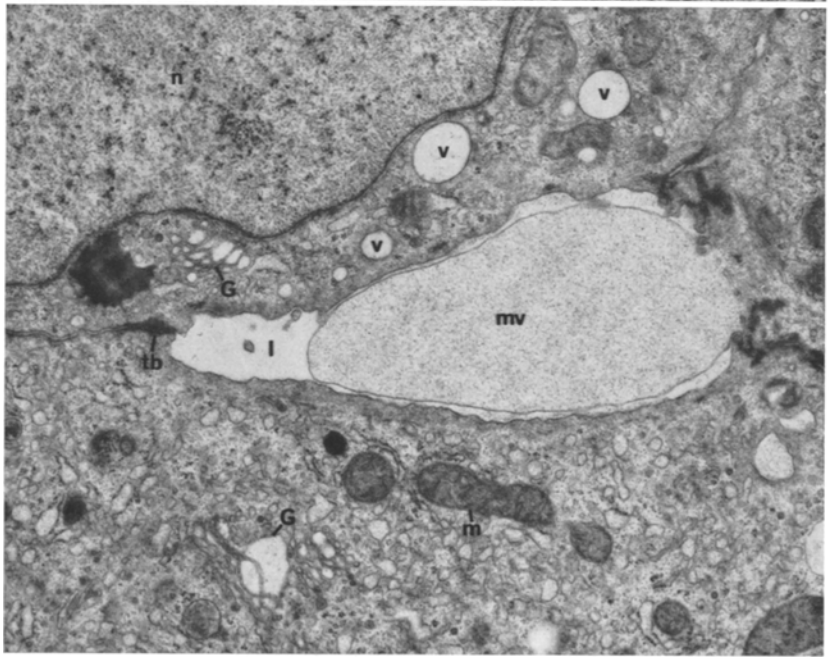

Fig. 3

Fig. 2. An oedematous microvillus is projecting into the lumen (*l*) of a ductule. Note the abrupt delineation between the matrix of the microvillus and the hyaloplasm of the subjacent cell. The round profiles at the base of the microvillus (arrows) are interpreted as residual tubules after fusion of microvilli during the process of developing oedema (*n*. nucleus; *mv* microvillus). 10260 ×

Fig. 3. Three hepatocytes form the lining of a distended bile canaliculus which has lost its complement of microvilli except for an oedematous one (*mv*) which projects from the hepatocyte at the right margin of the micrograph. The matrix of the microvillus is considerably lighter than the hyaloplasm of the cells (*G* Golgi apparatus; *m* mitochondrion; *n* nucleus; *tb* terminal bar; *v* vacuole). 11970 ×

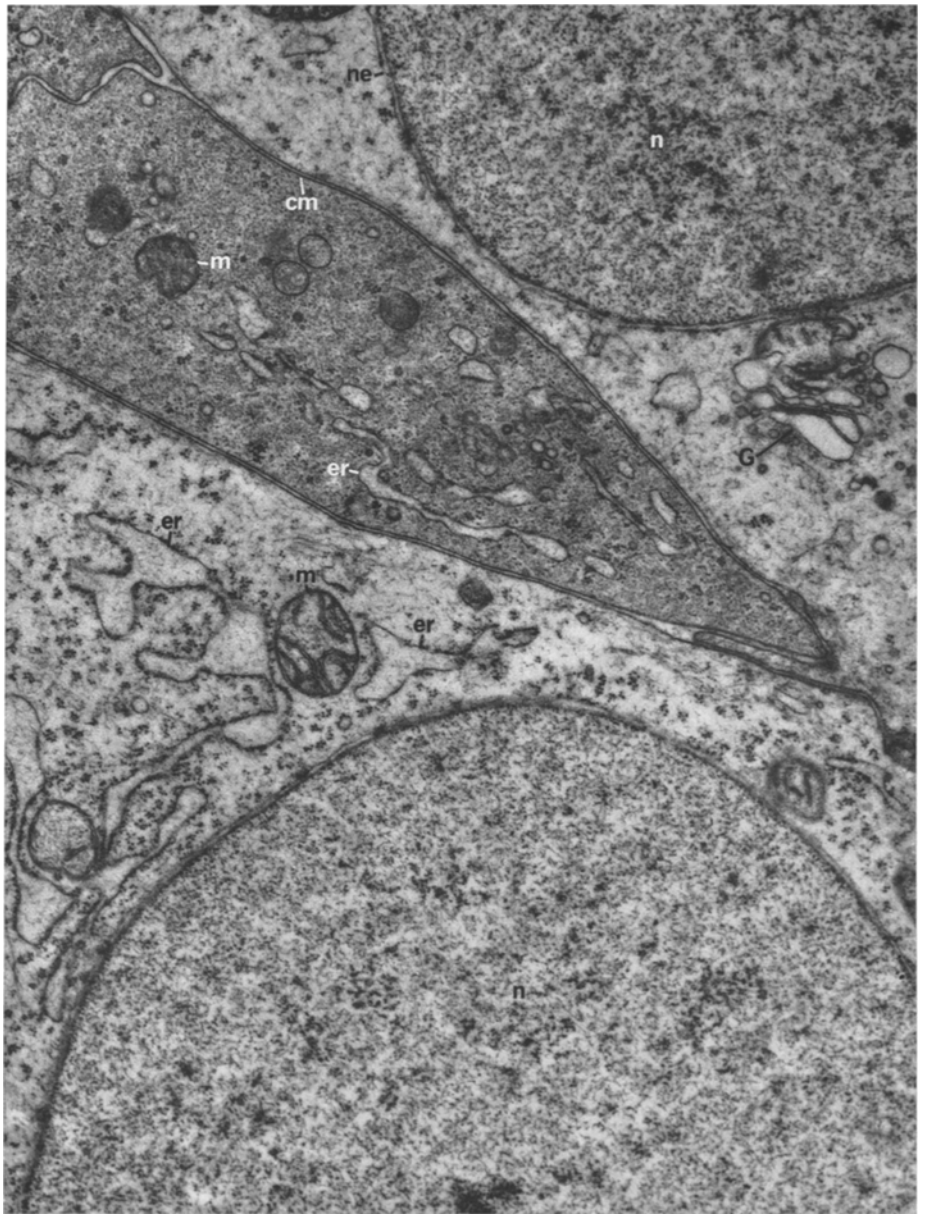

Fig. 4. Three biliary epithelial cells are seen. The one in the left upper corner is a "dark" cell. Its matrix is condensed and considerably more opaque than the contents of the ergastoplasmic sacs (*er*). (*m* mitochondria; *n* nucleus; *ne* nuclear envelope; *cm* cell membrane; *G* Golgi area). 17100 ×

with extrahepatic cholestasis. In larger channels of capillary calibre, the endothelium showed marked variations in electron opacity, as illustrated in Fig. 6. In smaller capillaries, these changes were frequently extreme, so that oedematous cells with a markedly transparent cytoplasm projected into the lumen, while

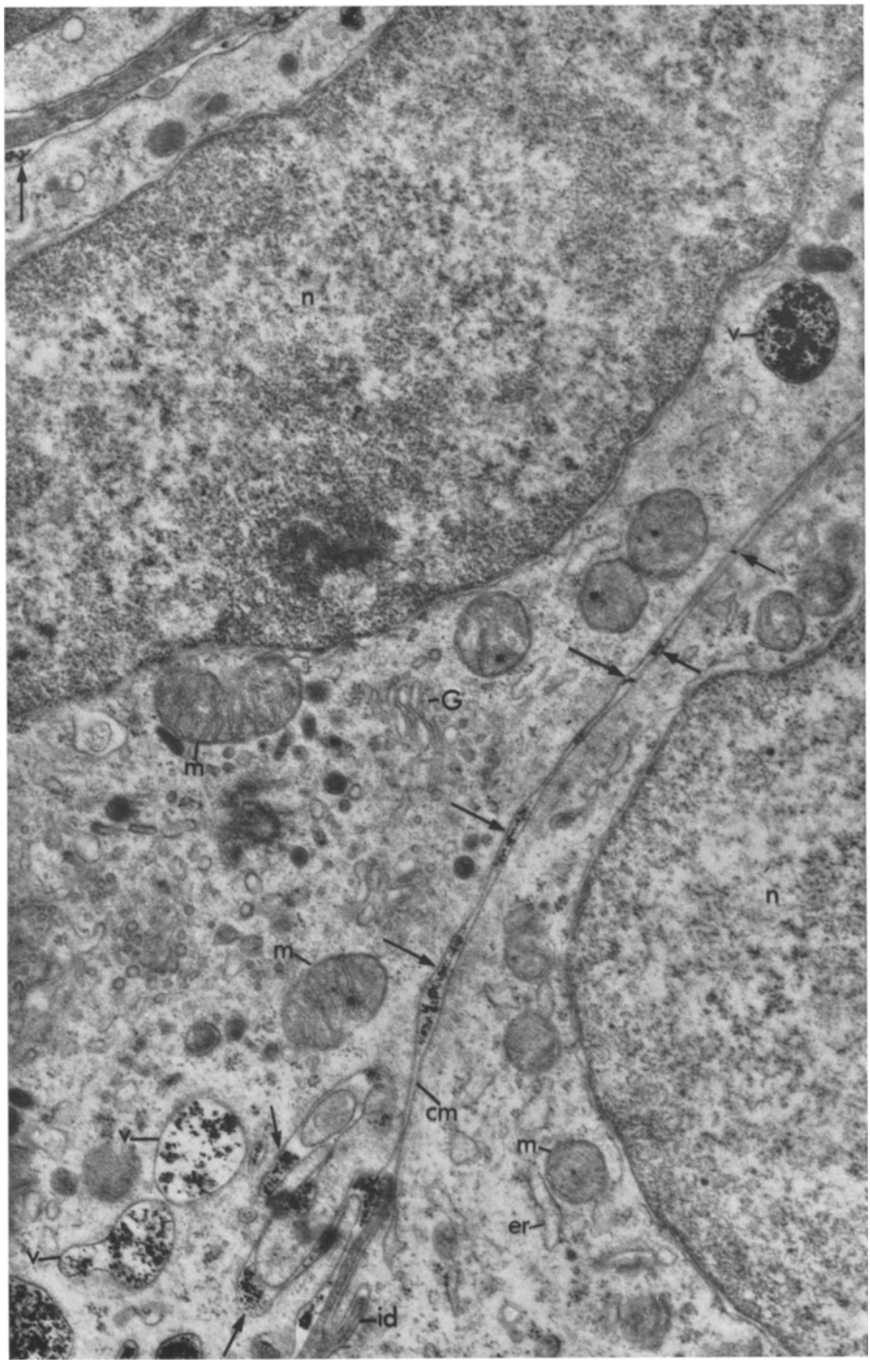

Fig. 5. Particles of colloidal mercuric sulphide (arrows) have penetrated from the lumina into the intercellular spaces between biliary epithelial cells. They are also present within intracytoplasmic vacuoles (*v*); (*m* mitochondria; *G* Golgi zone; *er* endoplasmic reticulum; *cm* cell membrane; *id* cellular interdigitations; *n* nucleus). 23 220 ×

"dark" cells appeared shrunken (Fig. 7). The shrinkage was never so severe as to lead to a disruption of the continuity of the capillary lining, but in many of the smaller vessels, the swollen endothelial cells narrowed the lumen, resulting in sludging of blood constituents.

C. Changes in Kupffer cells

Variations in the overall electron opacity of the main cytoplasmic mass of Kupffer cells and of their trabecular extensions were found in sinusoids adjacent to the granulation tissue, surrounding the proliferating ducts. The changes in Kupffer cells were similar to those of the capillary endothelial cells. Fig. 10 illustrates a portion of the main perikaryonic mass of cytoplasm in a Kupffer cell which is markedly oedematous. The opacity of the contents of the dilated ergastoplasmic cisternae has become identical with that of the surrounding hyaloplasm. It should be noted in Fig. 10 that, as was frequently the case, the microvilli of the adjacent hepatocyte remained unaltered. Occasionally, however, these microvilli also became oedematous and projected into or beyond the Space of Disse (STEINER, CARRUTHERS and KALIFAT, 1962).

D. Changes in microvilli of bile canaliculi

In the majority of bile canaliculi, the number of microvilli was reduced, and the canalicular lumen dilated. Occasional channels were found which were partly or almost totally filled by a swollen microvillus (Fig. 3). These oedematous masses sometimes occupied the entire lumen, sometimes only a part. They were usually sessile, and their markedly electron-lucent hyaloplasm, devoid of organelles, was clearly demarcated from the adjacent hyaloplasm of the parent liver cells.

E. Injections of colloidal mercuric sulphide

We have described elsewhere (KALIFAT, CARRUTHERS and STEINER, 1962) the distribution of colloidal mercuric sulphide injected retrogradely into the ligated common bile duct in rats. The particles lay singly or in small aggregates in virtually all the bile ductules and pre-ductules. They were also present, though less constantly, in the lumina of the biliary canaliculi. In addition, the particles penetrated into the intercellular spaces between adjacent ductular cells (Fig. 5). The particles tended to aggregate at the level of the basal portion of the biliary epithelial cells, where they were sometimes so numerous as to fill the intercellular spaces and the space between the cells and their basement membrane (Figs. 8 and 9). Particles were also found within peripheral cytoplasmic vacuoles in biliary epithelial cells (Fig. 5) and in the hyaloplasm of some, but by no means all, hepatocytes. Small numbers of particles penetrated the basement membrane, and were found in the ground substance of the connective tissue (Fig. 9), in the lumina of lymphatic or blood capillaries, and in vacuoles of macrophages. No free particles were seen in the lumina of sinusoids, but particles were found engulfed within deep indentations in the surface cell membranes of Kupffer cells (Fig. 11), or in vacuoles in the peripheral portion of their cytoplasm. The particles adhered not only to the surface of Kupffer cells which faced sinusoidal lumina,

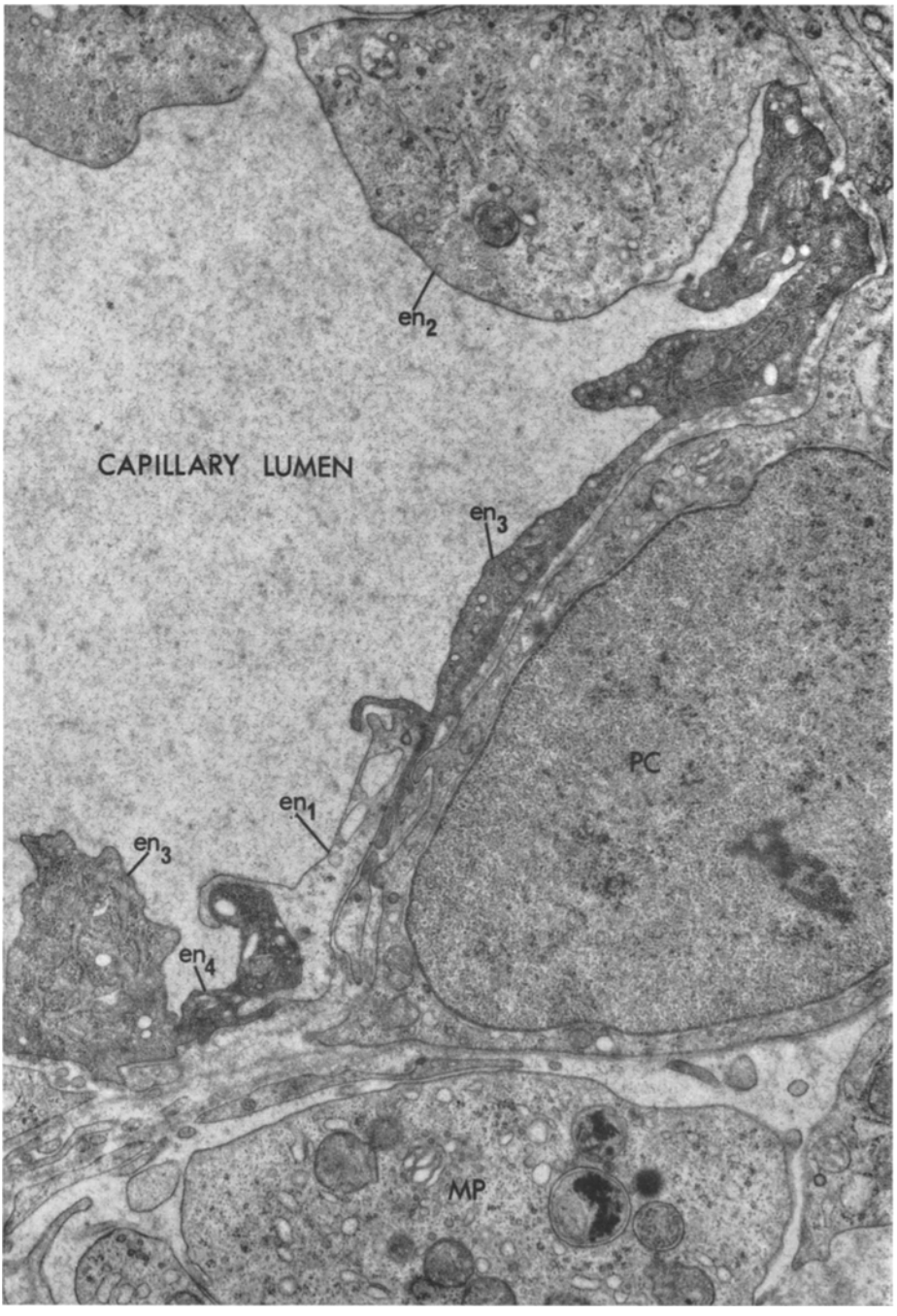

Fig. 6. Large capillary in the connective tissue around newly proliferated ductules. The endothelial cells show variations of electron opacity of their matrix. The very "light" cell is labelled en_1 and the darkest en_4. en_2 and en_3 show intermediate degrees of condensation of their cytoplasm. The pericapillary cell (*PC*) is normal. The basement membrane of the capillary cannot be seen clearly. A macrophage (*MP*) is present in the connective tissue. 14440 ×

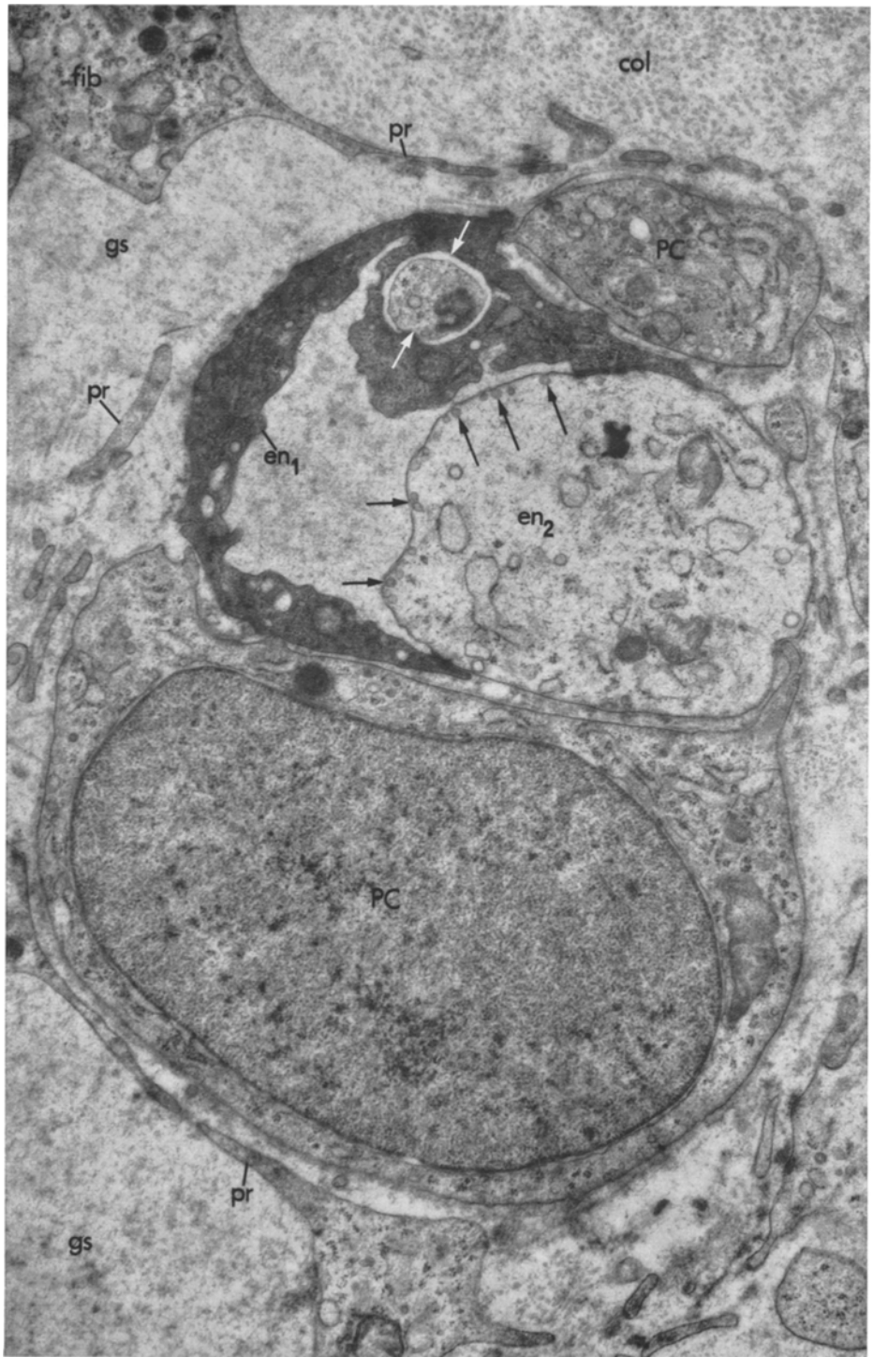

Fig. 7. Small capillary in the connective tissue around newly proliferated ductules. The endothelial cell (*en*) has a markedly condensed hyaloplasm. The other (en_2) is oedematous and projects into the lumen. Note the marked pinocytic activity on its luminal border (black arrows). The white arrows point to a process of a "light" cell which lies in a tunnel formed by the "dark" cell (en_1). The pericapillary cell (*PC*) shows no alteration (*pr* processes of fibroblasts; *gs* ground substance; *col* collagen). 15960 ×

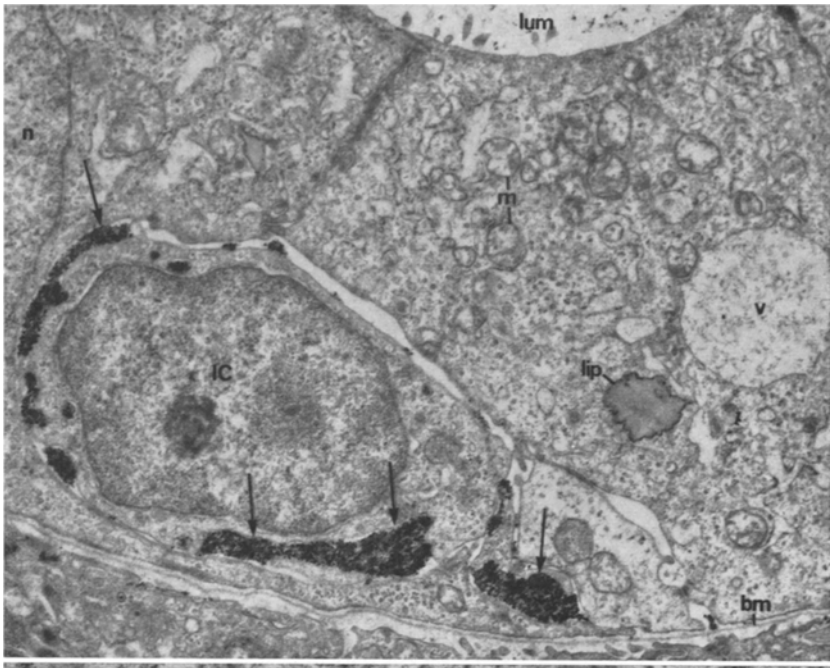

Fig. 8

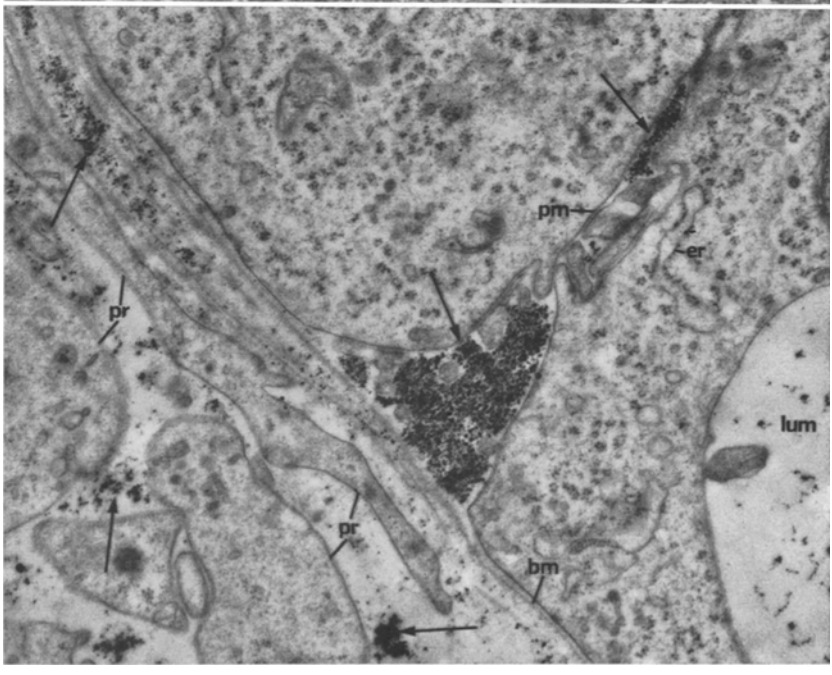

Fig. 9

Fig. 8. The lumen of a bile ductule (*lum*) is seen in the upper centre of the micrograph. A very few particles of colloidal mercuric sulphide are in the lumen. Many are aggregated (arrows) around an intercalated cell (*IC*) (*bm* basement membrane; *v* vacuole; *lip* lipid droplet; *m* mitochondria). 18050 ×

Fig. 9. A bile ductular lumen (*lum*) is seen at the right margin of the micrograph. It contains fairly numerous particles of colloidal mercuric sulphide. The particles (arrows) have penetrated into the intercellular spaces between ductular cells, traversed the basement membrane (*bm*) and find themselves in the ground substance around processes (*pr*) of fibroblasts (*pm* plasma membrane; *er* ergastoplasm). 18126 ×

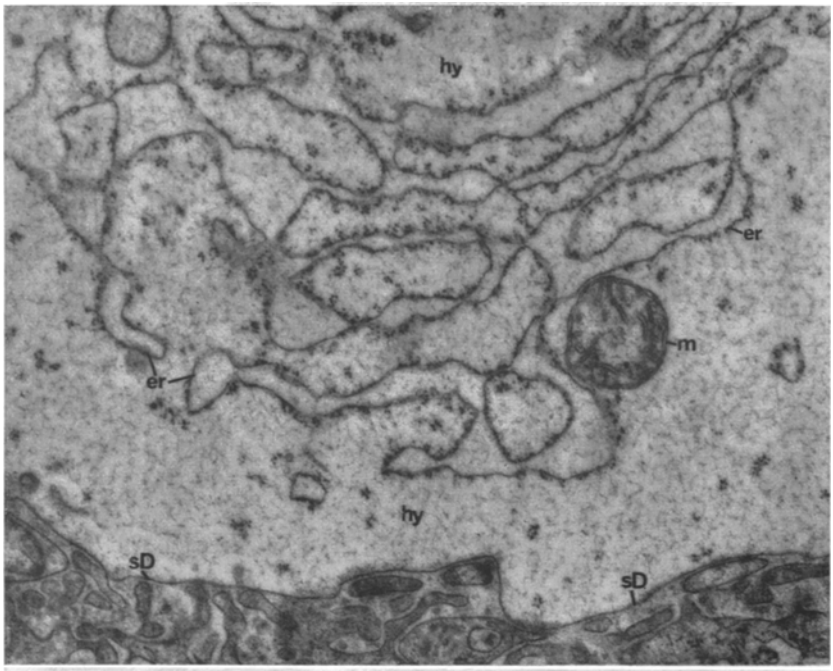

Fig. 10

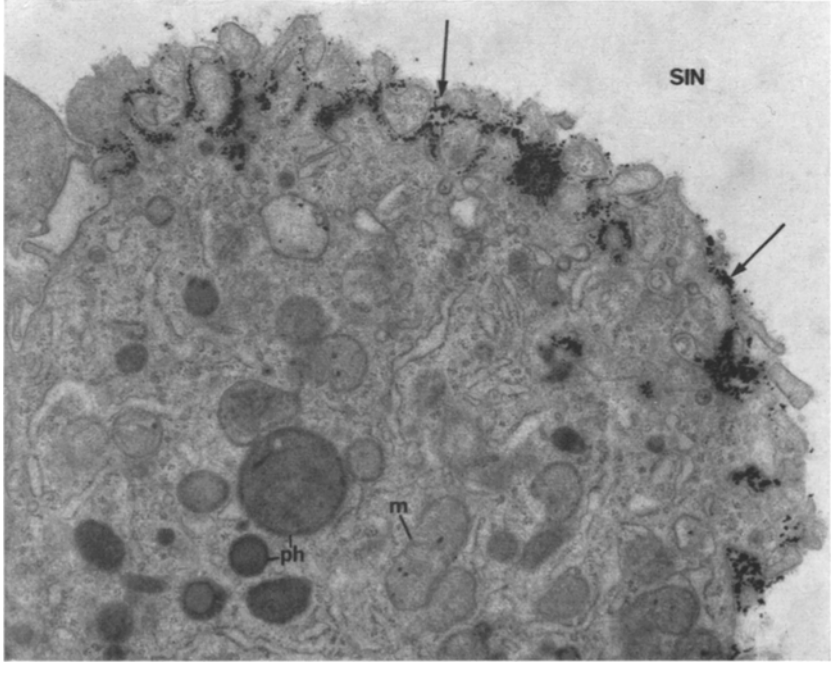

Fig. 11

Fig. 10. A Kupffer cell in the upper portion of the micrograph abuts on a Space of Disse (*sD*) at the lower margin. The microvilli in the space are of normal appearance. The hyaloplasm (*hy*) of the Kupffer cell is oedematous and equals in electron opacity the contents of the ergastoplasmic sacs (*er*). (*m* mitochondrion). 21600 ×

Fig. 11. The surface of a Kupffer cell facing the sinusoidal lumen (*SIN*) shows numerous deep indentations, in which particles of colloidal mercuric sulphide (arrows) are entrapped. A few have penetrated somewhat further into the cytoplasm and entered small vacuoles (*ph* phagosome; *m* mitochondrion). 14580 ×

but also to the surfaces which abutted on the space of Disse. They were found in Kupffer cells both in locations where the hyaloplasm of adjacent hepatocytes contained mercuric sulphide particles, and where they did not. No evidence was found to suggest that the injection disrupted the bile conduction channels.

Discussion

Disturbances of cell hydration develop in the liver of rats within 24 hours of ligation of the common bile duct and persist for at least 39 days. The microvilli of the ductular and parenchymal cells, which are normally in contact with bile constituents in lumina of bile ductules and bile canaliculi, become oedematous. In addition, cells, or parts of cells which are probably not normally in contact with bile, undergo alterations indicative of an overall change in the hydration of their hyaloplasm. This affects intercalated biliary epithelial cells (which do not reach a lumen in a given plane of section), endothelial cells lining blood or lymphatic capillaries in the granulation tissue surrounding newly formed ductules, and Kupffer cells which line sinusoids near the newly formed granulation tissue. The affected cells may either shrink, and become "dark", or may swell so that their electron opacity becomes reduced.

We think it likely that all the above alterations are caused by the same pathogenic agent or agents, and that this agent is derived from bile. More specifically, it may be that the bile acids or bile salts, the sodium salts of fatty acids, long-chain fatty acids or lecithins might be implicated in the development of the injury, perhaps acting by virtue of their detergent properties; their ability to reduce the surface tension of water and its interfacial tensions against oils; their emulsifying action for insoluble fatty liquids in water; or their activity as wetting agents. Their known ability to denature proteins and to lyse cell membranes may further contribute to their pathogenic potential (CAMERON and HOU 1962). Though it seems probable that unusually prolonged contact between these normal bile constituents and the various kinds of cell affected, could adequately account for the lesions described, we cannot dismiss the possibility, that abnormal pathogenic bile constituents may be generated during extrahepatic cholestasis, and may be responsible for the injuries found.

We have searched for routes by which such toxic substances could come in contact with *all* the affected cells. It is apparent that the luminal microvillous borders of ductular and parenchymal cells are in contact with them. The somewhat crude method of the retrograde intrabiliary injections of colloidal particles of mercury, and the equally unsatisfactory technique using thorotrast, are the only ones at present available (HAMPTON 1958, 1961) to show possible routes by which such substances might escape from the biliary tree and so reach the other types of cell affected. We can only assume that the findings arrived at by such procedures can be extrapolated to cover the behaviour of bile constituents, since the particle size (smallest diameter 70 Å) is likely to be larger than that of any of these chemical compounds.

The colloidal particles injected into the common bile duct of rats seven days after ligation, became arrested in canaliculi, as would be expected. However, they were also found in the hyaloplasm of some, but by no means all, the hepatocytes. The particles reach the sinusoids within two minutes of the retrograde

injection, and we suggest that the route they take is at least in part across the cell and that toxic bile constituents could pass by a similar route to come in contact with the Kupffer cells of sinusoids. It is also possible that some particles escape from canaliculi into sinusoids in areas of frank necrosis of the parenchyma.

It was more difficult to explain the injury to the endothelial cells of the capillaries. We were hesitant to accept the possibility of a retrograde diffusion of toxic agents from sinusoids to capillaries. However, our present study shows, that particles of mercuric sulphide rapidly gain access to the intercellular spaces between ductular cells, and that they find themselves within membrane-enclosed vacuoles in such cells with equal alacrity. We may therefore, assert, that at least under pathologic conditions, the ductular cells lining intrahepatic biliary channels behave as do the biliary epithelial cells lining the normal gall bladder. These were shown by HAYWARD (1962) to take up thorotrast particles which had penetrated into their intercellular spaces. We have also noted, that the particles of mercuric sulphide aggregate between the biliary epithelial cells and their basement membranes. It seems likely that the basement membrane presents an obstacle to their passage into the connective tissue. The direction of movement of particles cannot be proved from electron micrographs. Nevertheless, the aggregation of mercuric sulphide on the luminal side of the ductular cell basement membrane would seem to justify the deduction that they migrate from ductules outward into the connective tissue, rather than in the opposite direction. A few particles do seem to penetrate the basement membrane barrier, and are then found in the ground substance of the connective tissue, and in the lumina of the capillaries. If bile constituents escape by the same route, they could reach the intercalated biliary epithelial cells and the endothelial cells of the vascular channels around them.

This study may also provide some clarification of the much disputed problem of the causation of obstructive jaundice. EPPINGER (1937), suggested that rupture of bile canaliculi permits the escape of bile constituents from the obstructed biliary system. HANZON (1952, 1958) to some extent confirmed this theory, since he showed that uranin, administered intravenously after obstruction of the common bile duct, was secreted into canaliculi and regurgitated between adjacent hepatocytes to reach again the sinusoids. However, HANZON (1952), also noted the presence of intensely fluorescing, apparently intact parenchymal cells, and he suggested that a second, more common, route of escape of bile was *through* the hepatocytes rather than *between* them.

If rupture is defined as a mechanical breach of the smallest bile conduction pathways, electron microscopic investigations, with one exception (ROUILLER 1956), have failed to reveal any such breach, even with cases showing marked jaundice (CARRUTHERS and STEINER 1962; GOLDFISCHER, ARIAS, ESSNER and NOVIKOFF 1962; HAMPTON 1961; KALIFAT, CARRUTHERS and STEINER 1962; STEINER and CARRUTHERS 1961). There can be, however, no denying the possibility that such breaches might develop at the site of the focal necroses of hepatocytes which develop in cases of extrahepatic obstruction. However, even this possibility is by no means fully acceptable, since, as pointed out by CAMERON and HOU (1962), jaundice may not develop even though there is extensive necrosis throughout the liver.

The present work supports rather Hanzon's (1952) second suggestion. Colloidal particles were identified electron microscopically, in the hyaloplasm of some, but by no means all, hepatocytes and probably escaped thence into the sinusoids (KALIFAT, CARRUTHERS and STEINER, 1962).

POPPER and SCHAFFNER (1957) have also provided evidence suggesting that Eppinger's theory was no longer tenable. They also suggested that "regurgitation may take place elsewhere, perhaps from the ductules." This hypothesis was in accord with Aschoff's original view that ductules were the "heel of Achilles" of the biliary system. Our present study confirms that this alternative route of escape of bile constituents does probably exist as well.

Thus, on present evidence, we may conclude that at least two and possibly three routes are available for the escape of bile constituents from the confines of the obstructed biliary pathways. The fundamental mechanism appears to be diffusion across natural barriers rather than rupture of channels. Little can be said at this time about the relative importance of these routes of escape of bile in the evolution of the clinical picture of obstructive jaundice. Our study of the behaviour of colloidal particles in the obstructed biliary tree was carried out one week after ligation of the common bile duct. It may be, that the sites of leakage vary from time to time during the course of the development of the hepatic lesions. Further investigation is therefore needed to establish the routes of bile leakage at various times after obstruction, and to examine possible differences in this phenomenon in various animal species.

Summary

Livers of rats were examined during a period of 39 days after ligation of the common bile duct. A widespread disturbance of cell hydration was noted. There was oedema of some of the microvilli of biliary epithelial cells in ductules, and of some of the microvilli of parenchymal liver cells. In other cases, a condensation or oedema affected the hyaloplasm of entire biliary epithelial, endothelial or Kupffer cells.

Retrograde intrabiliary injections of colloidal mercuric sulphide showed that these particles came in contact not only with cell surfaces lining biliary lumina, but that they also penetrated between biliary epithelial cells, and traversed their basement membranes to reach the lumina of capillaries and sinusoids. The particles also were found within biliary ductular cells and hepatocytes. These observations led us to suggest that bile constituents with detergent and protein denaturing properties may escape by these routes, and so cause the alterations in hydration. It has also been suggested that the mode of regurgitation of bile in extrahepatic cholestasis is by diffusion across natural barriers rather than by rupture of the bile conduction pathways.

Störungen der Hydration der Zellen der Rattenleber bei extrahepatisch bedingter Cholostase

Zusammenfassung

Die Leber von Ratten wurde bis 39 Tage nach einer Unterbindung des Gallenganges elektronenmikroskopisch untersucht. Dabei konnte eine ausgesprochene

Veränderung des Wassergehaltes der Zellen beobachtet werden. Einige der Mikrovilli der Gallengangsepithelien und manche Mikrovilli der Leberzellen waren ödematös geschwollen; in anderen Fällen hatte Kondensierung oder Ödem das Hyaloplasma einer ganzen Gallengangsepithelzelle, Endothelzelle oder Kupfferschen Sternzelle erfaßt.

Retrograde Injektion von kolloidalem Quecksilbersulfid in den Gallengang zeigte, daß diese Partikel nicht nur mit der Oberfläche der die Gänge auskleidenden Epithelzellen in Berührung kommen, sie dringen vielmehr auch zwischen die Epithelzellen ein, durchqueren die Basalmembran, um schließlich die Lichtung der Capillaren und der Sinusoide zu erreichen. Auch in den Zellen der Ductuli und den Leberzellen selbst wurden Partikel gefunden. Auf Grund dieser Beobachtungen erscheint die Annahme berechtigt, daß Gallenbestandteile mit der Fähigkeit, detergent zu wirken und Eiweiß zu denaturieren, auf diesem Wege austreten und so die Veränderungen im Wassergehalt der Zellen verursachen könnten. Dies würde mit der Anschauung übereinstimmen, nach der die Galle bei extrahepatisch bedingter Cholostase eher infolge einer Diffusion durch die natürlichen Barrieren, als infolge einer Ruptur aus den Gängen austrete.

References

Cameron, G. R., and P. C. Hou: Biliary cirrhosis. Pathological monograph No. 1. Eds. Sir Roy Cameron and G. P. Wright. Edinburgh: Oliver and Boyd 1962.

Carruthers, J. S., and J. W. Steiner: Studies on the fine structure of proliferated bile ductules. I. Changes of cytoarchitecture of biliary epithelial cells. Canad. med. Ass. J. **85**, 1223—1236 (1961).

— — Studies on the fine structure of the terminal branches of the biliary tree. III. Parenchymal cell cohesion and intracellular bilec analiculi. Arch. Path. **74**, 117—126 (1962).

Caufield, J. B.: Effects of varying the vehicle of OsO_4 fixation. J. biophys. biochem. Cytol. **3**, 827—830 (1957).

Eppinger, H.: Die Leberkrankheiten, Allgemeine und spezielle Pathologie und Therapie der Leber. Wien: Springer 1937.

Goldfischer, S., I. M. Arias, E. Essner and A. B. Novikoff: Cytochemical and electron microscopic studies of rat liver with reduced capacity to transport conjugated bilirubin. J. exp. Med. **115**, 467—474 (1962).

Hampton, J. C.: Electron microscopic study of extrahepatic biliary obstruction in the mouse. Lab. Invest. **10**, 502—513 (1961).

— An electron microscope study of the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the common bile duct. Acta anat. (Basel) **32**, 262—291 (1958).

Hanzon, V.: Liver cell secretion under normal and pathologic conditions studied by fluorescence microscopy on living rats. Acta physiol. scand. **28**, Suppl. 101, 1—268 (1952).

— Dye secretion and dye uptake by the liver. In Liver Function. Ed. R. W. Brauer. American Institute of Biological Sciences, Publication No 4, Washington, D. C., 1958.

Hayward, A. F.: Electron microscopic observations on absorption in the epithelium of the guinea pig gall bladder. Z. Zellforsch. **56**, 197—202 (1962).

Kalifat, S. R., J. S. Carruthers and J. W. Steiner: La fine structure des canalicules biliaires du foie de rat dans la cholostase extrahepatique. Z. Zellforsch. **57**, 607—626 (1962).

Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409—414 (1961).

Popper, H., and F. Schaffner: Liver: Structure and function. New York: McGraw-Hill 1957.

Rouiller, C.: Les canalicules biliaires; étude au microscope électronique. Acta anat. (Basel) **26**, 94—109 (1956).

STEINER, J. W., and J. S. CARRUTHERS: Studies on the fine structure of the terminal branches of the biliary tree. II. Observations of pathologically altered bile canaliculi. Amer. J. Path. **39**, 41—63 (1961).

— — Experimental extrahepatic biliary obstruction. Some aspects of the fine structural changes of bile ductules and pre-ductules (ducts of Hering). Amer. J. Path. **40**, 253—270 (1962).

— — and S. R. KALIFAT: The ductular cell reaction of rat liver in extrahepatic cholestasis. I. Proliferated biliary epithelial cells. Exp. Mol. Path. **1**, 162—185 (1962).

— — — Vascular alterations in the liver of rats with extrahepatic biliary obstruction. Exp. Mol. Path. (in press).

— — — Observations on the fine structure of rat liver cells in extrahepatic cholestasis. Z. Zellforsch. **58**, 141—159 (1962).

WATSON, M. L.: Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol. **4**, 475—478 (1958).

Professor Dr. JAN W. STEINER,
Banting Institute, 100 College Street, Toronto 2, Ontario, Canada.