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The Brush Cells of the Common Bile Duct of the Rat

Thin Section, Freeze-Fracture and Scanning Electron Microscopy

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Summary. Two different fixative procedures (immersion and perfusion) and four different fixative solutions were used in order to obtain the best preservation of the brush cells of the common bile duct of the rat. The results indicate that only perfusion fixation through the common bile duct is suitable, independent of the fixative solutions and their osmolarity.

Numerous brush cells were seen in the proximal and distal regions of the common bile duct. In these locations, they could be implicated in a registration and/or regulation of intraluminal pressure variations.

Key words: Brush cells – Common bile duct – Thin section – Freeze-fracture – Scanning electron microscopy

The brush cells (BC) are usually considered as infrequent or rare elements which can be detected among other cells in epithelia lining different hollow organs. However until now, a surprisingly high number of BC have been found in two locations: 1) the cardiac region of the rat stomach (Isomäki 1973); 2) the common bile duct of the rat (Luciano et al. 1977a). In the common bile duct, we observed that satisfactory preservation of the BC for morphological studies was particularly difficult, whereas the surrounding cells were well fixed. Consequently, we studied this organ with two purposes: 1) to find out the most suitable fixative solution and fixation procedure for the BC, and 2) to establish the reason(s) for their unusually high occurrence.

Materials and Methods

I. A group of young adult rats (from 150 to 200 g b.w., both sexes and feed ad libitum) were anesthetized with an intraperitoneal injection of Inactin (Byk Gulden, Konstanz; 0.1 ml of a 10% solution/100 g b.w.).

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Two fixation procedures were used: 1) *Immersion.* The duct was severed, immersed into one of the fixative solutions (see below), and minced under a dissection microscope. 2) *Perfusion through duct.* This was exposed and cannulated (gauge No. 17, or by an equivalent sized polyethylene catheter) close to the duodenal loop. The duct was opened as close as possible to the liver at the same time perfusion with the fixative solution was begun. A total of about 0.5 ml of the fixative solution was slowly injected through the lumen. The duct was then isolated, transferred into the same fixative solution, and minced into small rings.

The following fixative solutions were used:

a) 2% glutaraldehyde (ultrastructure grade, Polaron), 2% formaldehyde (prepared from paraformaldehyde, Fluka), 0.025% $CaCl_2$, in 0.1 M cacodylate buffer, pH = 7.3; 1080 mOsm;

b) 4% glutaraldehyde in 0.1 M cacodylate buffer, pH = 7.3; 650 mOsm;

c) 4% glutaraldehyde in 0.1 M s-collidine buffer (purified sym-collidine buffer, Polysciences Inc.), pH = 7.2; 550 mOsm;

d) 1% OsO_4 in phosphate buffer (Millonig 1961), pH = 7.3; 330 mOsm. This solution was used for OsO_4 primary fixations;

e) 2% OsO_4 in 0.1 M cacodylate buffer, pH = 7.3. This solution was used after primary fixation in solution *a* as well as solution *b*;

f) 1% OsO_4 in 0.1 M s-collidine buffer, pH = 7.2. This solution was used after primary fixation in solution *c*.

The fixation time was 120 min for primary fixation, and 90 min for secondary fixation (postfixation). The specimens were dehydrated in alcohols and embedded in epoxy resin.

Light Microscopy. 1–2 μm thick sections were cut with an LKB Ultratome 4800-III ultramicrotome and stained with an alkaline solution of toluidine blue.

Transmission Electron Microscopy. Thin sections were cut with the same ultramicrotome, collected on formvar-carbon membranes (Dowell 1964) and stained with uranyl acetate and lead citrate. *Freeze-fracture replicas* were prepared from specimens fixed in the fixative solution *a*. After glycerination (1 h in 30% glycerol solution), these specimens were oriented on specimen holders, frozen in liquid Freon 22 (monochlorodifluoromethane) at about −150° C, and transferred into a Balzers 360 M apparatus (Balzers AG, Liechtenstein) equipped with an electron beam gun. The specimens were fractured and replicated according to the technique of Moor and Mühlethaler (1963). The replicas (about 2.5 nm thick) were cleaned in hypochlorite bleach and chromic acid, repeatedly washed in distilled water and collected on formvar-carbon membranes.

Thin sections and replicas were examined using a Siemens Elmiskop 101 electron microscope.

Scanning Electron Microscopy. Specimens fixed both by immersion and perfusion were used. After dehydration in a graded series of ethanol, they were transferred in isoamylacetate and dried at the critical point in a Sorvall apparatus. The dried samples were mounted on metal stubs using conducting silver paint and coated with gold in a sputter device (Hummer I, Technics Inc., Virginia, USA). All specimens were examined and photographed using a Cambridge Stereoscan 600 or a Philips 500 electron microscope.

II. Another group of rats, male, weighing from 300 to 350 g, was used to determine osmolarity and pH of the bile. For this purpose a polyethylene catheter (about 3 cm long and 1.2 mm in diameter) was inserted into the lumen of the common bile duct through a small incision in the wall of the distal region. The bile was allowed to flow for 1 or 2 h into a collecting bag tied in place. During each experiment, the animal was heated with a lamp and the exposed body parts were maintained wet with a 0.9 NaCl solution. At the beginning and in the course of the experiment drops of bile were collected on sample discs and their osmolarity was measured with a 5130 B Vapor Pressure Osmometer (Wescor Inc.). All values ranged around 300 mOsm. At the end of each experiment, the value of the osmolarity of the total collected bile was confirmed with a Wide-Range Osmometer (Advanced Instruments Inc.) and showed values of 303 – 308 mOsm. The pH of bile measured with indicator papers gave a value of 7.5 – 8. The collected bile was 0.6 – 0.7 ml/h, i.e., 14–16 ml/24 h for rats of 300 – 350 body weight, in agreement with previous studies (McMaster 1922; Schmidt and Ivy 1937). The bile of the rat had a yellow color and, as reported by McMaster (1922), did not change during the experiment nor in the following 24 h.

Results

I. Immersion Fixation

A) Light Microscopy. After fixation by immersion in aldehydes and postfixation in OsO_4 (solutions *a* and *d*), the mucosa of the common bile duct of the rat showed a simple columnar epithelium and formed infoldings of variable number and depth. Everywhere in this epithelium, light cells were seen displaying a spindle- or flask-like shape and with a tuft of microvilli protruding into the organ cavity (Fig. 1a). As previously demonstrated in comparative light and scanning electron microscopy investigations (Luciano et al. 1977a), these light cells corresponded to the BC. Light microscopy revealed also that the BC were more numerous in both the proximal (towards the liver) and the distal regions of the common bile duct than in the middle region. Fig. 1a shows a cross section of the common bile duct in its distal region where 1 BC is seen to every 3 principal cells.

B) Transmission Electron Microscopy. 1. *Thin sections.* The BC of specimens prefixed by immersion in the aldehyde solutions *a* or *b* and postfixed in solution *e* usually showed poorly preserved structures (Fig. 2a – c). Only occasionally a BC showed intact apical microvilli. In this case the fine structure of the whole cell was also satisfactorily preserved. In most of the BC, however, the apical part was profoundly altered. Large blebs occurred here instead of microvilli (Fig. 2b), or the plasma membrane was completely missing. Cytoplasmic filaments and microtubules which otherwise characterize this region of the intact BC, were also absent or disorganized, the mitochondria were apically displaced and largely swollen as those described by Ruska (1961) after influence of tensioactive substances. Whereas the BC were difficult to detect on account of their alterations, the principal cells, the goblet cells and the endocrine cells as well as the connective tissue cells of the lamina propria, were satisfactorily preserved.

After immersion fixation with the OsO_4 solution *d*, the BC (especially on the bottom of the mucosal infoldings) showed morphological characteristics which were comparable with those previously described after using the same fixative solution (Luciano 1972). The filament bundles which are axially located within the microvilli, and the microtubules of the cell apical region were not seen (Fig. 3a, b). However, numerous filaments were present within the cytoplasm beneath the apical region (Fig. 3a).

2. In freeze-fracture *replicas* of specimens fixed in the solution *a*, alterations of the BC were seen and were similar to those observed in thin sections and described above.

C) Scanning Electron Microscopy. After fixation by immersion in solution *a*, the polygonal areas corresponding to the apical surface of the principal cells were frequently intermingled with less extended areas devoid of any surface differentiation or only occasionally displaying some microvilli. These were longer and thicker than those of the adjacent principal cells. The suggestion that the less extended areas corresponded to damaged BC was strengthened by observing the

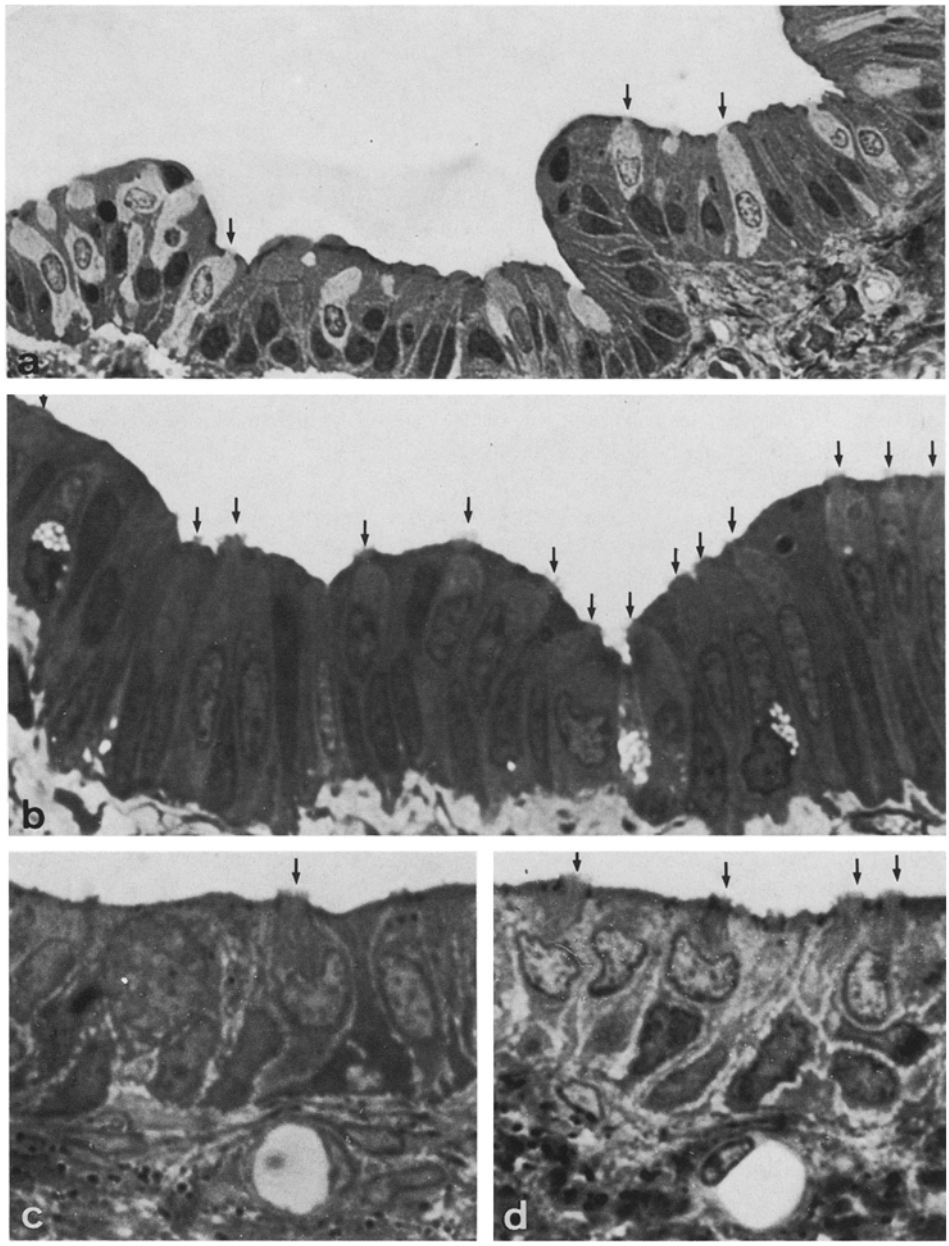

Fig. 1a–d. Light microscopy of 1 μm thick, toluidine blue stained sections of the distal region of the common bile duct. **a** Immersion fixation with an aldehyde solution (solution *a*) as primary fixative. The *arrows* point to some of the numerous brush cells which appear less strongly stained than the principal cells. In **b**, the same primary fixative solution as in **a** was used but the organ was fixed by perfusion. Principal cells and brush cells display a similar staining; the small tufts of microvilli of 14 brush cells (*arrows*) protrude into the lumen of the organ. In **c** and **d**, parallel sections prepared from a specimen fixed by perfusion with s-collidine-buffered aldehyde solution (solution *c*). The flask-like shape of some of the brush cells (*arrows*) and their apical filament bundles are recognizable. a × 600; b, c and d × 1,200

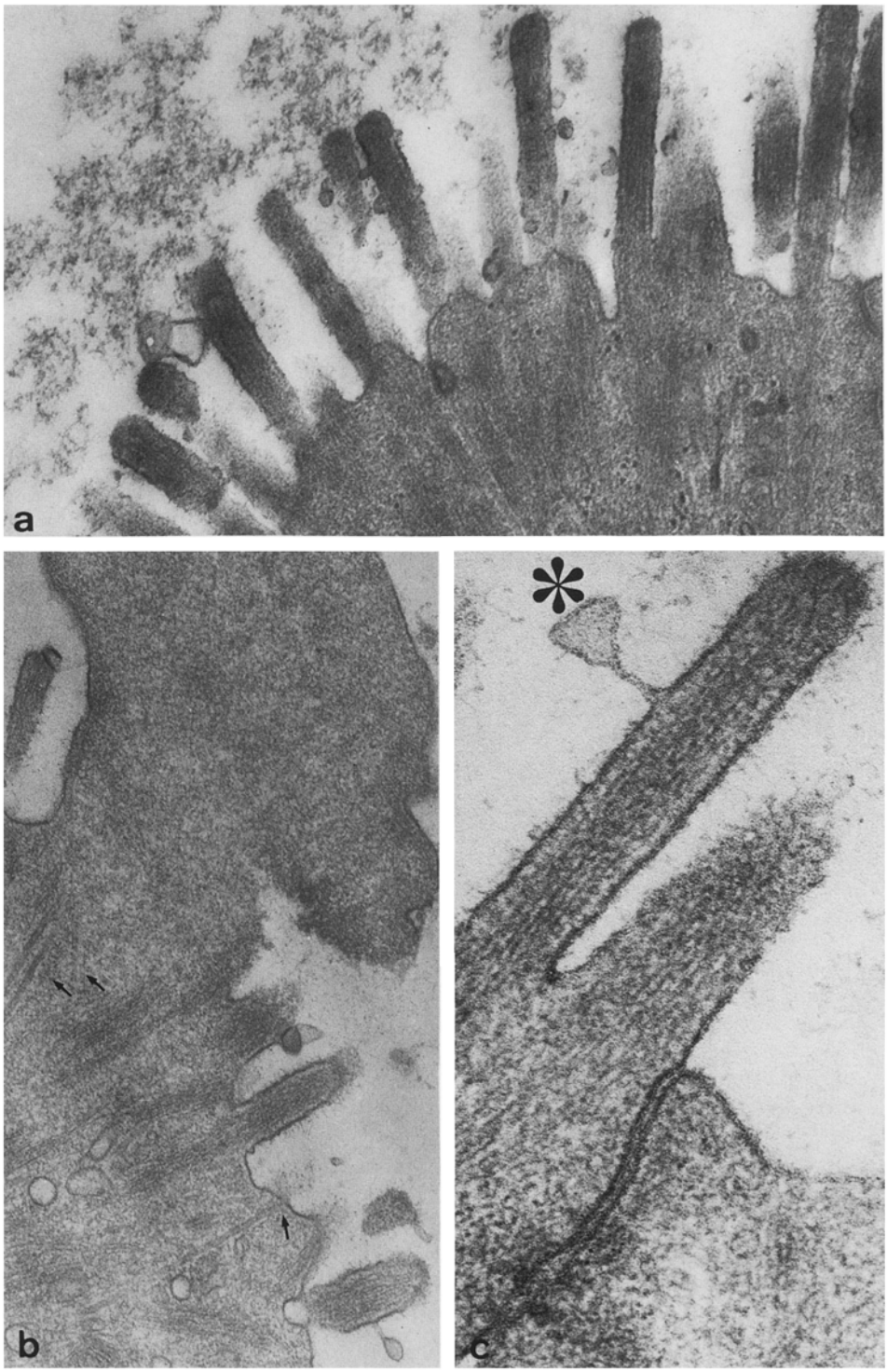

Fig. 2a–c. Most frequent artefacts presented by brush cells fixed by immersion. In **a,** small vesicles and flocculent material are seen between and over the microvilli. The small vesicles probably originate from swellings of the plasma membrane as visible in **c** (*asterisk*). In **b**, a large bleb, instead of some microvilli, is seen; nevertheless, microtubules within the apical cytoplasm of the BC are still recognizable (*arrows*). a and b ×40,000; c ×120,000

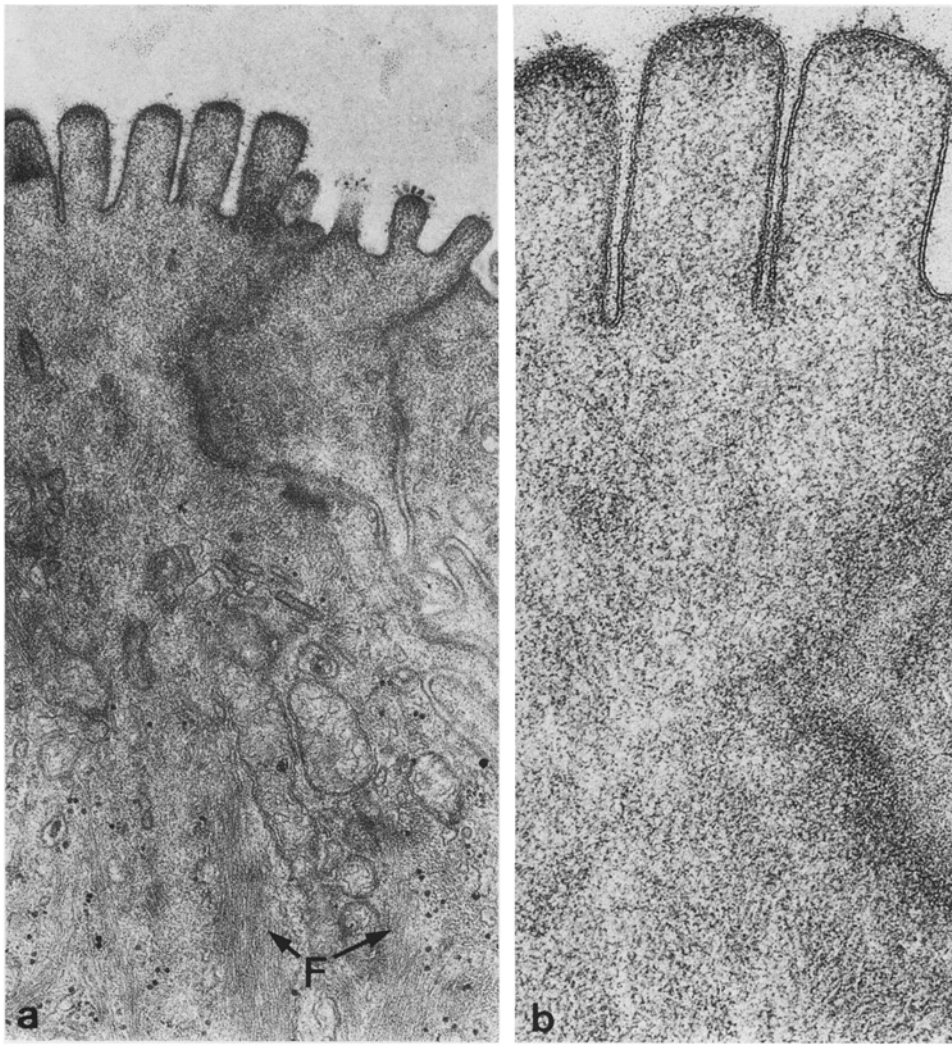

Fig. 3a, b. Common bile duct after fixation by immersion in an OsO_4 solution (solution *d*). Within the microvilli and the apical region of the brush cell the bundles of tiny filaments are dissolved and, as seen in parallel section **b**, only material with fine granular appearance can be observed. The thicker 10nm filaments (*F*), however, remain. a ×28,000; b ×74,000

samples fixed by immersion in the OsO_4 solution *c*, where the preservation of the BC surface morphology was superior although not yet optimal.

Independently from the solution used, after immersion fixation the mucosal surface of all specimens examined in the scanning electron microscope showed debris of various shape and size, probably mucus.

II. Perfusion Fixation

A) Light Microscopy after perfusion fixation of the common bile duct with the aldehyde solutions *a*, *b*, and *c*, and postfixation in the OsO_4 solutions *e* and *f*

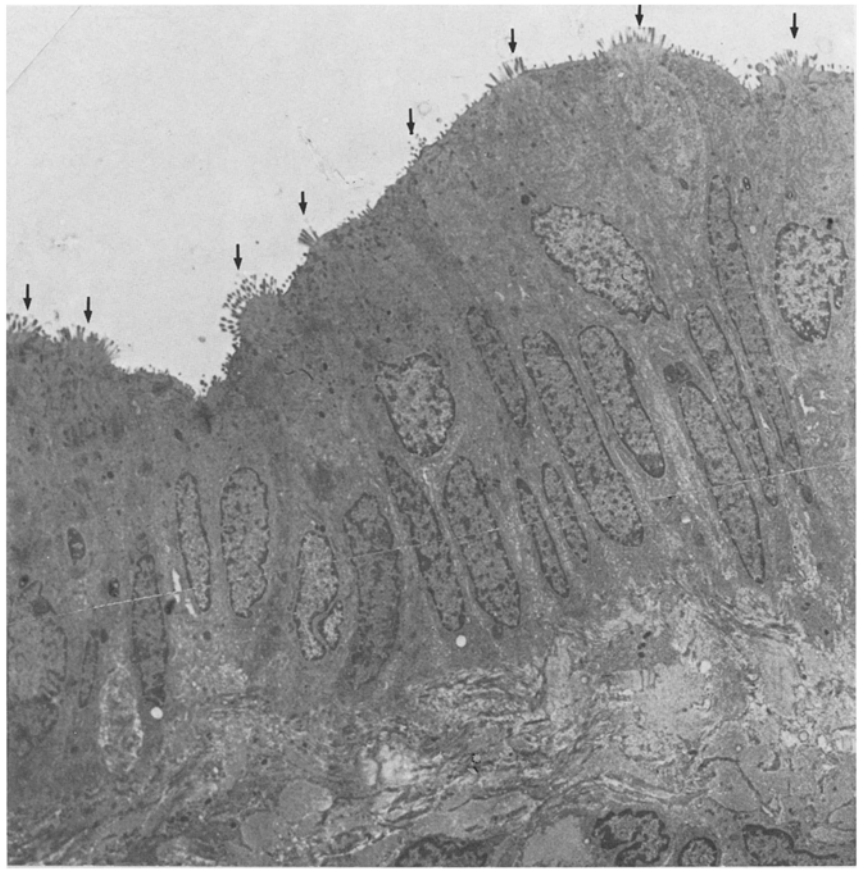

Fig. 4. Low magnification of the common bile duct mucosa. Same specimen as in Fig. **1b** showing 8 brush cells (*arrows*) protruding into the organ lumen. ×2,400

revealed a uniformly well preserved epithelium. The BC could be identified because of their shape and especially their microvilli were clearly larger than those of the neighbouring cells (Fig. 1b – d). Fig. 1b shows 1 BC to every 2 principal cells as scanning electron microscopy (Fig. 9a).

B) Transmission Electron Microscopy. 1. *Thin sections* of the same samples also indicated a successful fixation (Figs. 4, 5, 6, 7). After perfusion with solution *c* (glutaraldehyde dissolved in s-collidine buffer) the BC showed a high number of microfilaments in their cytoplasm. In each microvillus an axially located bundle of 11 – 13 filaments each measuring about 5 nm diameter and 7.5 nm apart was seen (Fig. 5a, b). These filament bundles extended from the summit of each microvillus to the supranuclear region of the cell. Here, they intermingled with other filaments measuring from 8 to 10 nm diameter (Figs. 5a, 6a). These larger filaments were numerous, arranged in bundles and ran without a predominant direction throughout the cytoplasm (Fig. 6b).

Microtubules of 25 nm outer diameter were oriented parallel to the bundles of 5 nm filaments within the apical cytoplasm (Fig. 5a). Some of these microtubules

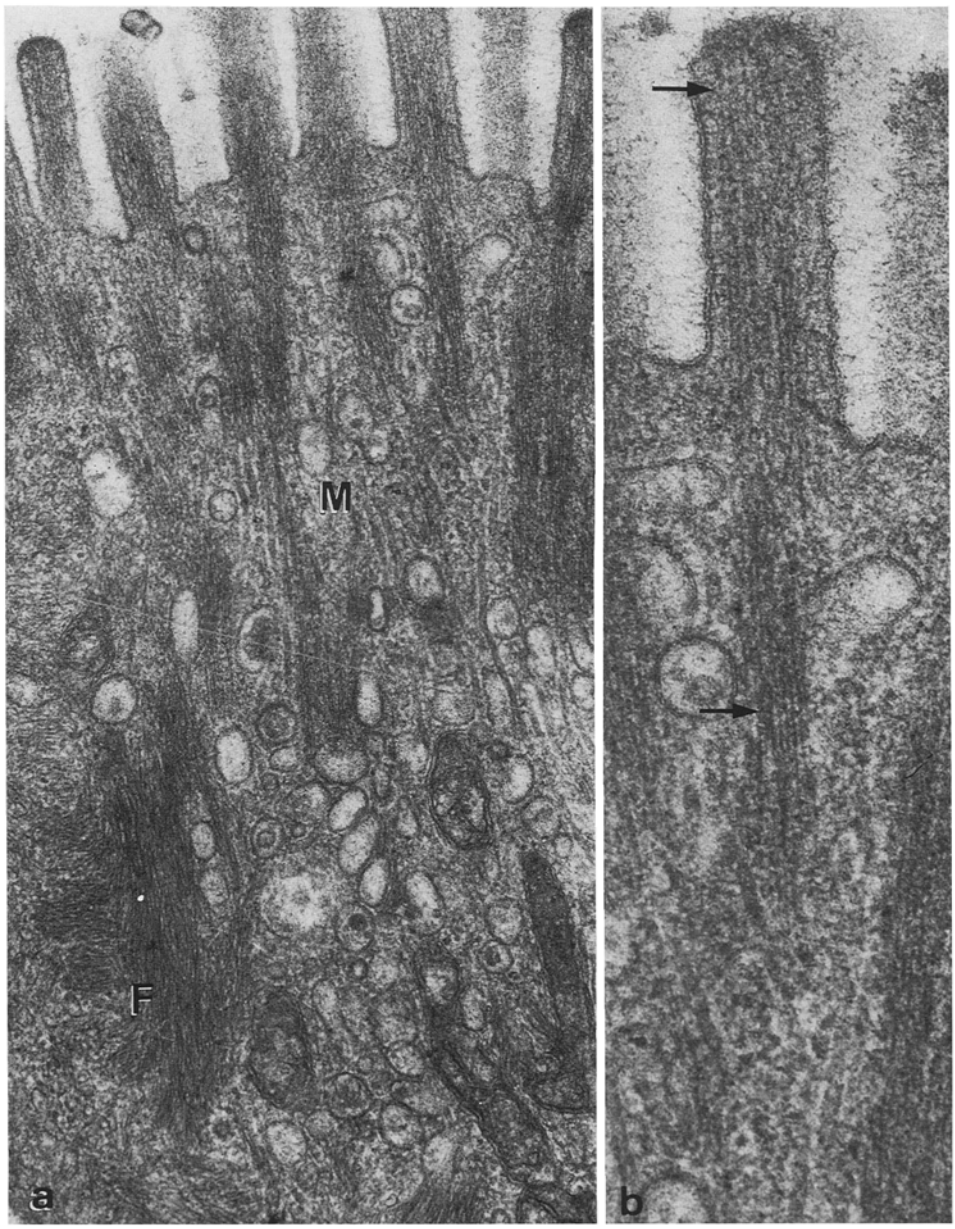

Fig. 5a, b. Microvilli and apical region of a brush cell after fixation by perfusion in solution *c*. In **a**, bundles of tiny filaments within the microvilli and the apical cytoplasm, microtubules (*M*), numerous vesicles and large bundles of thick filaments (*F*) are seen. The regular spacing of the tiny filaments (*arrows*) is visible in **b** (higher magnification of a microvillus of Fig. 5a). a ×40,000; b ×80,000

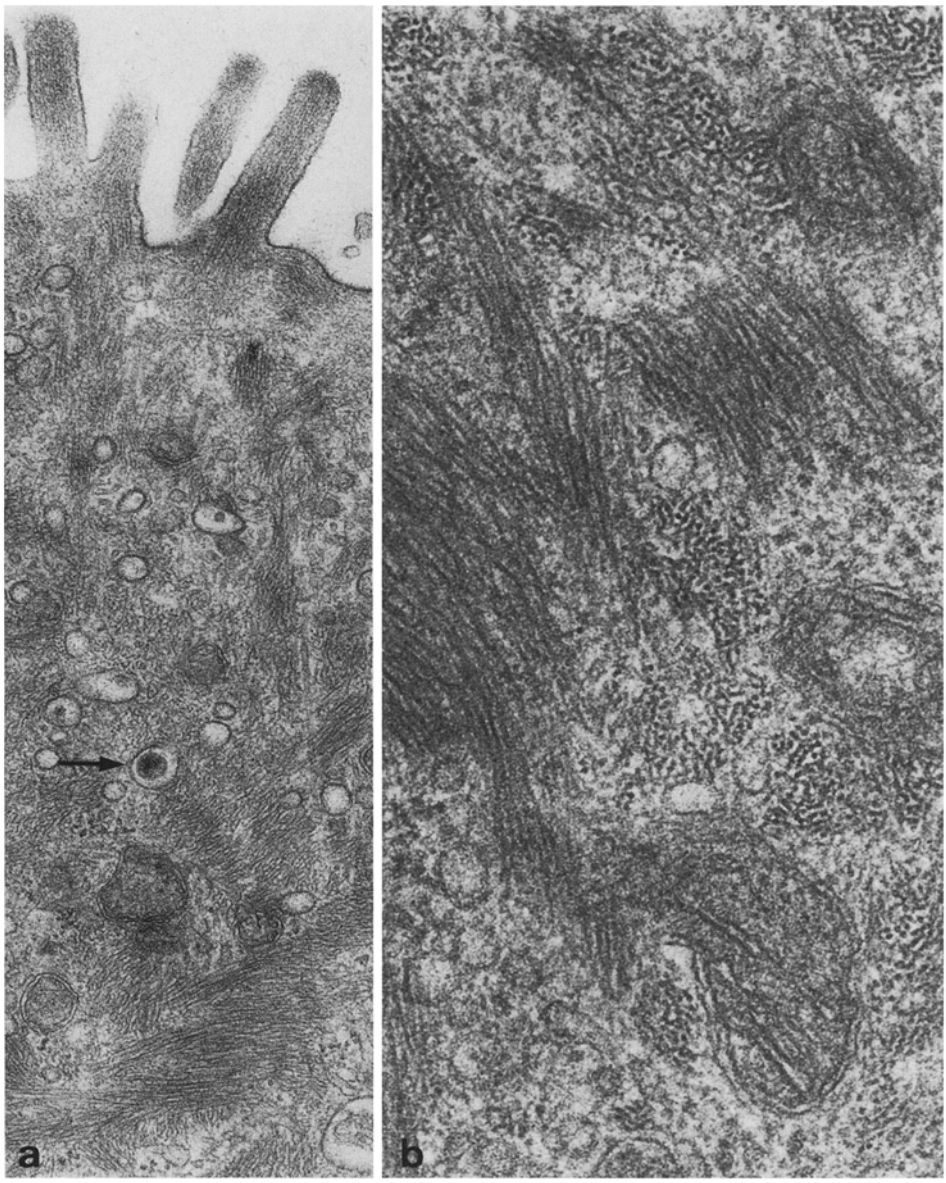

Fig. 6a and b. Oblique section through brush cells after fixation by perfusion in solution 1. In **a** large amount of 10 nm filaments running in all directions within the cytoplasm. The *arrow* points to a vesicle containing an electron dense granule. In **b** higher magnification of bundles of 10 nm filaments in cross and oblique section. a ×41,500; b ×80,000

lay with one extremity very close or in apparent continuity with the inner surface of the plasma membrane interposed between adjacent microvilli (Fig. 7).

Vesicles of about 125 nm in diameter were aligned between the bundles of 5 nm filaments and the microtubules (Figs. 5a, 6a, 7). Occasionally some of these vesicles showed an electron dense granule of about 90 nm in diameter (Fig. 6a).

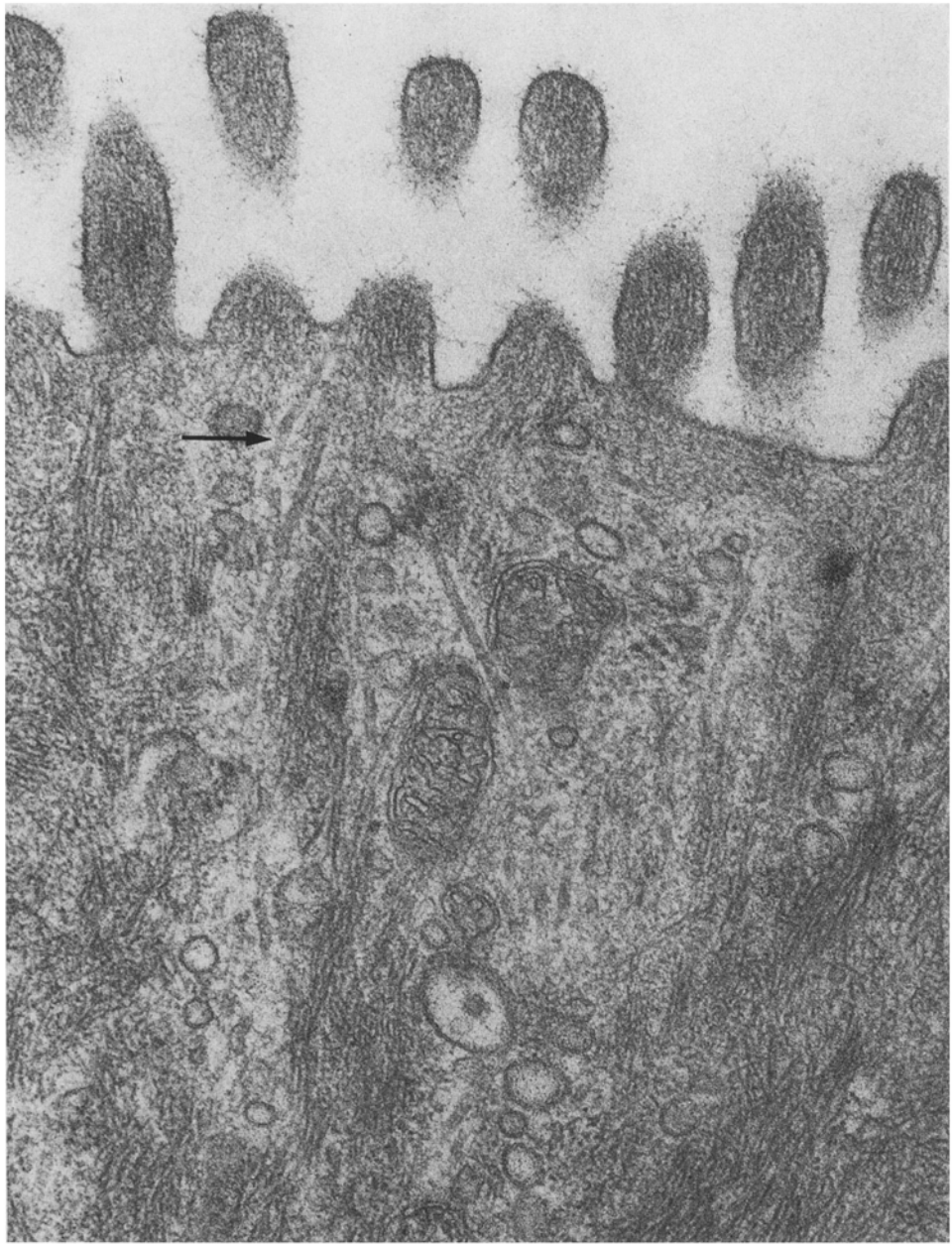

Fig. 7. Apical region of a brush cell after fixation by perfusion in solution *c* showing numerous microtubules. One of these (*arrow*) can be seen close to the plasma membrane. ×66,000

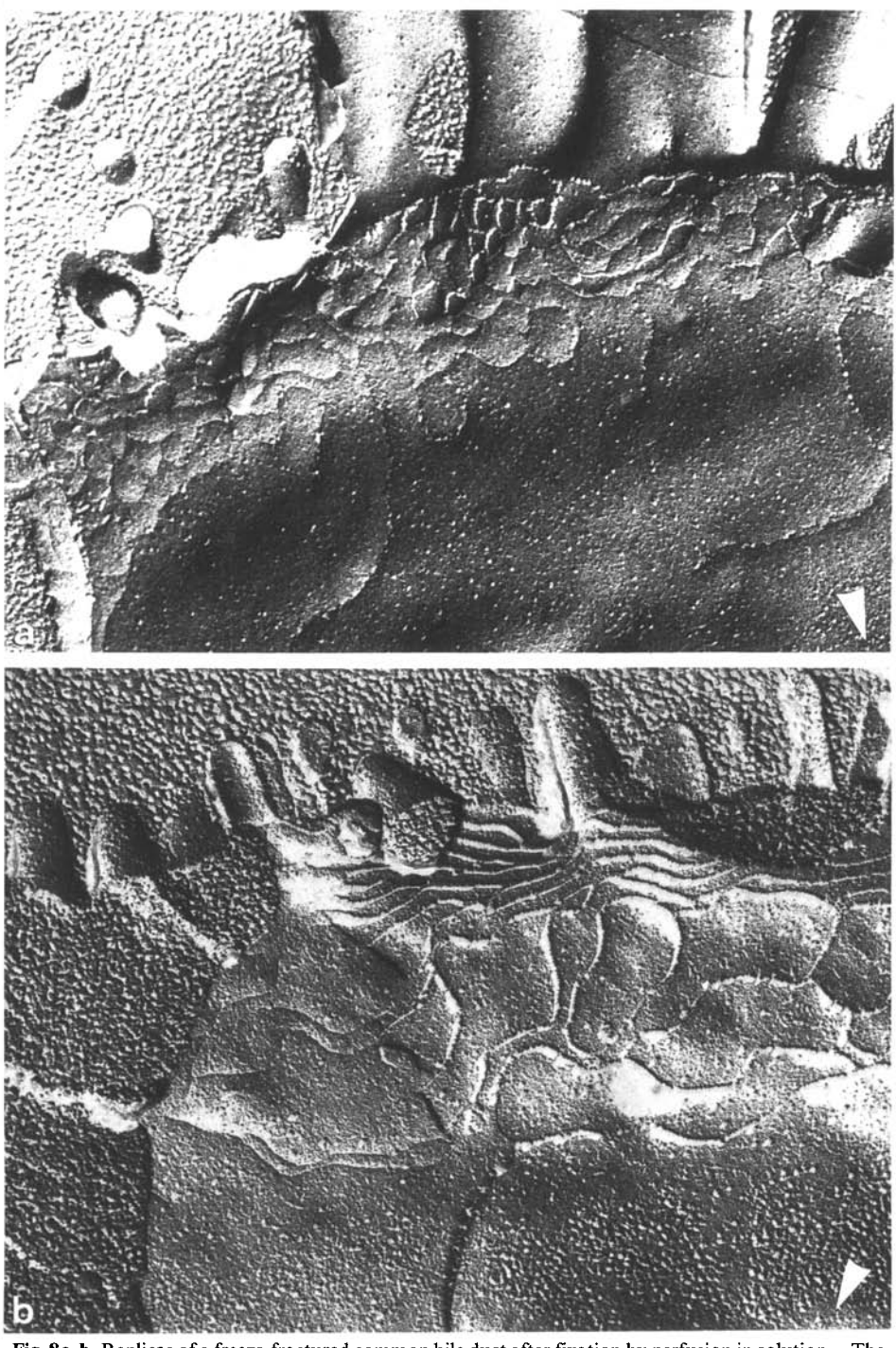

Fig. 8a, b. Replicas of a freeze-fractured common bile duct after fixation by perfusion in solution *a*. The network of ridges and grooves forming the zonula occludens of a brush cell (**a**) shows numerous, small polygonal meshes. The network of the zonula occludens between adjacent principal cells (**b**) displays large, flattened meshes. In **b**, below the occluding junction, desmosomes are seen. The *arrowheads* indicate the shadowing direction. a × 59,000; b × 63,500

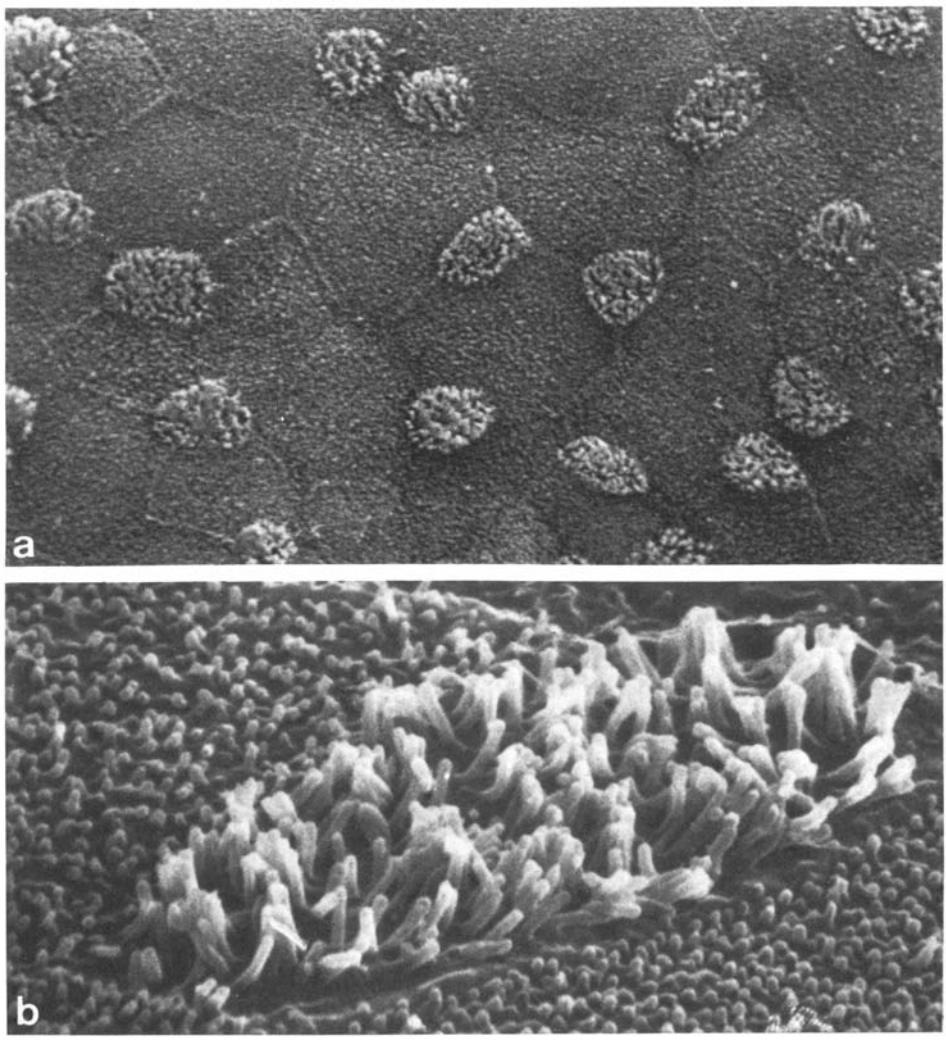

Fig. 9a, b. Scanning electron microscopic aspects of the distal segment of the common bile duct after fixation by perfusion in solution *a*. The brush cells protrude with a tuft of microvilli into the organ lumen. In **a** 19 brush cells and 30 principal cells are recognizable. The apical surface of the brush cells is considerably smaller than that of the principal cells. The different size of the microvilli of the brush cells and of the principal cells can be seen in **b**. a ×3,000; b ×15,000

The lateral surface of the BC displayed numerous folds. However, lateral microvilli, similar to those recently described in the BC of the gallbladder epithelium of the mouse (Luciano and Reale 1979) were rarely detected and were in general poorly developed.

2. In *replicas* of freeze-fractured specimens which were fixed by perfusion with the solution *a*, the BC were well preserved. In the apical region, their split lateral plasma membranes revealed a zonula occludens composed of 5 to 8 anastomosing strands. The network they formed had close and small meshes towards the luminal

cell surface, and free-ending strands abluminally. Short ridges or segments of fibrils intercalated with particles were seen on the P face, grooves with scattered particles on the E face (Fig. 8a). Zonulae occludentes with this morphology occurred between BC and principal cells. Where the lateral plasma membranes of two adjacent principal cells were split, the zonula occludens was formed by 4 to 6 strands which ran parallel to the luminal cell surface; abluminally other strands formed large polygonal meshes. The strands displayed on face P almost continuous ridges and on face E grooves devoid of particles (Fig. 8b).

Scanning Electron Microscopy. After perfusion fixation with solution *a*, the BC were easily identified since their long and thick microvilli emerged as a bush or tuft over the microvilli of the adjacent principal cells (Fig. 9a, b). In surface views these appeared as polygonal areas 10 to 15 µm wide and were covered by short and tight microvilli (Fig. 9a, b). The borders of the BC were difficult to detect because, as is known from thin section observations, their apical region is usually narrow (from 5 to 8µm) and their microvilli protrude fan-like into the organ lumen thus masking the cell limits in surface views (Fig. 9a). Through this peculiar arrangement of the microvilli, the luminal surface of the BC always was smaller than those of the principal cells (Fig. 9a).

SEM was particularly useful for evaluation of the frequency of the BC and their preferential location along the common bile duct. The BC in the mucosal folds were not considered since in general they are not favourably exposed for observations in SEM. In the middle segment the BC represented 8 to 10% of the epithelial cells, in the proximal and distal segments they were about 35% (Fig. 9a).

Discussion

I. Effect of the Composition of the Fixative Solution and of the Fixation Procedure

Different types of cells occur in the epithelium of the common bile duct of the rat (Luciano 1972). In this organ the fixation is frequently unsuccessful. As a consequence of this, the BC probably have been overlooked by Leeson and Melax (1969) and have been described as dark cells by Yamada (1969), or as light agranular cells by Riches (1972). It is conceivable that these various and divergent results depend upon both the fixative solution and the fixation procedure used. This was suggested by previous investigations on other epithelia demonstrating BC with different morphological characteristics after fixation of the organ in OsO_4 or in glutaraldehyde solutions (Luciano et al. 1968; Luciano and Reale 1969). To support this hypothesis, in the present investigation we compared 4 fixative solutions with different composition and osmolarity and 2 fixation procedures. The results show that fixation procedure – immersion versus perfusion – is more important than osmolarity – isotonic versus moderately or highly hypertonic – for a satisfactory preservation of the BC. In fact, using the same fixative solution (sol. *a*, 1080 mOsm) we observed badly damaged BC after immersion fixation, and well fixed BC after perfusion. In the last case the fixative solution washes out the bile from the duct and the epithelium will be immediately and uniformly fixed. In the first case (immersion fixation), the size of the duct which measures 3 cm long and

1 mm diameter (Mann et al. 1920), and its content will delay the fixation of the epithelium. This delayed fixation does not apparently influence the principal cell morphology. However, the BC show various degrees of swelling of their apical region with occasional fragmentation of the plasma membrane and consequent damage of the cytoplasmic structures. The principal cells, nevertheless, can be altered by a hypertonic fixative solution since they became shrunken (dark) after such treatment. In conclusion, although the osmolarity of the bile present within the common bile duct measures 300 mOsm, well preserved principal cells *and* BC can be obtained after perfusion fixation of the duct with a solution measuring 500–600 mOsm (sol. *b* and *c*).

II. Morphology of the BC

In the common bile duct of the rat the BC display the same general aspect as in other organs (review by Luciano and Reale 1979). Therefore, only some structures will be considered here which have been revealed by improving the fixation.

a) Microfilaments. In this investigation we used, as fixative solution, glutaraldehyde dissolved in s-collidine buffer (solution *c*). This buffer, introduced by Bennett and Luft (1959) as a substitute for veronal-acetate to dissolve OsO_4, has only occasionally been used in association with other fixatives, especially with aldehydes. However, since purified trimethylpyridine is brought to pH 7.2 with HCl before addition of glutaraldehyde, there exist no chemical reasons against its use in the preparation of fixative solutions. This solution has been employed, for example, for both morphological (Fawcett et al. 1969) and histochemical (Simson and Spicer 1975) investigations. After perfusion of the common bile duct with s-collidine buffered fixative solution, the BC exhibit more microfilaments than with other fixatives (solution *a*, *b*) and are recognizable even by light microscopy as a wide structure which is luminally oriented and deeply stained in the apical region of the cells (Fig. 1c and d).

The microfilaments of the BC belong to two morphologically diverse categories. Those of the microvillus core measuring 5 nm diameter could correspond to actin filaments. The filaments of the second type, with a diameter from 8 to 10 nm, could be identified with the intermediate size filaments. These probably contain prekeratin-like protein which should be produced by all the epithelial cells (Franke et al. 1979). In addition, the different nature of the two filaments of the BC is supported by our observation that after the primary OsO_4 fixative the thin filaments (5 nm) forming and coming from the axis of microvilli disappear whereas the numerous thicker filaments (8–10 nm) remain. Szamier et al. (1975) described a partial solubilization of actin filaments by OsO_4 even after glutaraldehyde fixation. Our findings demonstrate that OsO_4 does not preserve the 5 nm filaments well but these are still visible if the OsO_4 follows the primary fixation in aldehyde, as noted also by Temmink and Spiele (1978). Immunochemical investigations could definitively characterize both these categories of filaments.

b) Microtubules. These are labile structures since one of their most extraordinary properties is their rapid disassembly (in a few seconds) and reassembly (review by Dustin 1978). In addition, it has been shown that traditional buffers such as

cacodylate or phosphate used to dissolve aldehydes destabilize the microtubules hindering their identification in thin sections (Luftig et al. 1977). The microtubules of the BC apparently are more resistent than those of other cells. Indeed, the microtubules of the BC were always seen, independently from the alteration induced in these cells by fixation procedures and by aldehyde solutions. Also if the mitochondria were swollen, the microfilaments disorganized and the apical plasma membrane damaged, the microtubules were still recognizable and occasionally they even maintained their relationship with the inner surface of the plasma membrane between the microvilli (Fig. 2b). As noted by Dustin (1978), the microtubules of nerve cells belong to the resistent category of microtubules. Therefore it is conceivable that those of the BC are more similar in their nature to the microtubules of the nerve cells than of other tissues.

c) Plasma Membrane. The plasma membrane appears the most sensitive structure of the BC, i.e., the first which undergoes the deleterious effects of inadequate fixation procedures and/or fixative solutions, the extent of cytoplasmic alterations depending on the degree of plasma membrane damage. Thus, for example, the detachment of small vesicles from the plasma membrane at the summit and at the lateral surfaces of the microvilli (Fig. 2a, b, c) represents, according to our findings, a fixation artefact. This artefact which has been observed also in BC of other epithelia and has been interpreted as a special form of secretion (Nabeyama and Leblond 1974), should be considered as a reaction of the BC to inadequate milieu. This can generate more obvious and extensive alterations if the fixation is delayed.

Plasma membranes, microtubules, microfilaments and probably the apical vesicles represent the special functional entities of the BC. We believe (review by Luciano and Reale 1979) that functionally these cells are receptors. In the BC, the strong parallel course of their apical cytoplasmic structure reaching from the apex of the microvilli to the supranuclear region without apparent interruption (the BC do not possess or have only a poorly developed terminal web) suggests a direct pathway from the external (lumen-interstitium) to the internal (cellular body) environment. According to previous research (Atema 1973, review by Atema 1975), the microtubules play a role in the transmission of sensory (mechanical and chemical) impulses in invertebrates and higher animals as well. Therefore, the microtubules of the BC could represent an essential link in the transmission of stimuli rather than a mechanical scaffolding for cytoplasmic structures. This hypothesis is supported both by our observation of a close relationship or even a contact between microtubules and plasma membrane and by the findings of Matsumoto and Sakai (1979). These authors demonstrated that, in the squid giant axon, a correlation exists between preservation of microtubules and excitability of the neural plasma membrane. They concluded that "it is probable that many microtubules are directly or indirectly associated with the plasma membrane. The most probable function of cytoskeletal microtubules close to the membrane is that they control the spatial interaction among functional or integral proteins regulating the ion permeability".

d) Lateral Microvilli. These were rare and poorly developed in the specimens examined in the present investigation, notwithstanding the different techniques we applied and the high number of BC we observed. The reason for this finding is not

clear. However, we recently remarked upon some morphological analogies between the lateral microvilli of the BC and the "spine-like processes" of the Merkel cells (Luciano and Reale 1979). The question of whether the spine-like processes are a constant morphological feature is also posed for the Merkel cells. In connection with this, Fox and Whitear (1978) observed "... nor do the microvilli (of the Merkel cells) always look stiff. They may for instance bend around desmosomal bridges ..."

e) Freeze-Fracture Replicas of the common bile duct epithelium reveal a new and important aspect of the BC, i.e., the presence of a zonula occludens differing in some morphological aspects from that of the principal cells. The junctional strands show many particles in the zonula occludens of the BC, but they are composed of almost continuous ridges in the principal cells. This suggests a different permeability of these junctions (see Luciano et al. 1979). Since previously as well as in the present investigation, we never observed BC reaching the luminal surface close to each other, it is conceivable that the zonulae occludentes of the BC influence or even determine the pattern of the strands in the interposed principal cells. In addition, the regions of the common bile duct with numerous BC (proximal and distal regions) could have different permeability characteristics from the middle region where the BC are less frequent. A similar situation – i.e. prevailing of the zonula occludens configuration of one cell type over that of adjacent cells – has also been observed in sensory epithelia (e.g., organ of Corti; vestibular labyrinth) where the zonula occludens between sensory and supporting cells is different from that between adjacent supporting cells (Iurato et al. 1976; Luciano et al. 1977b).

III. Occurrence of the BC

We observed a consistently high number of the BC in the proximal and distal part of the common bile duct. This suggests a peculiar common function of the BC in these two locations, which probably also have some common functional aspects.

It is known that physiological changes in pressure and movement of bile take place within the biliary system (Hallenbeck 1967). These changes depend upon the following major factors; the amount of bile entering the biliary system; the presence or absence of a gallbladder; and the resistance hindering the flow of bile towards the intestine. In turn, the last factor is influenced by the viscosity of the bile, the length and diameter of the common biliary duct, and the sphincteric structures surrounding the terminal segment of this duct (Hallenbeck 1967).

Since a large anatomical variability of the biliary system exists between species (Mann et al. 1920; Hallenbeck 1967), the functions of the various regions of the biliary system may also be different.

From the anatomical point of view, it should be noted that, in the rat, a gallbladder is absent. An extensive hepatic plexus around the branches of the portal vein exists from which the common bile duct originates (Higgins 1926). This measures about 3 cm length and 1 mm diameter and receives the pancreatic duct in its distal region. A common channel is provided that carries both the pancreatic secretion and bile to the duodenum (Hallenbeck 1967). Before its entrance into the duodenum, this common channel is surrounded by layers of smooth muscle cells which, according to Mann (1920), are present in the rat as in other species.

From a functional point of view, it is known that the liver of the rat produces a large amount of bile. We found 14 ml/24 h, a value which is in agreement with that reported by McMaster (1922) and Schmidt and Ivy (1937). The bile is not concentrated along the common bile duct of the rat either normally or during prolonged stasis (McMaster 1922). The efflux of the bile into the duodenum is continuous because the sphincteric resistance (i.e., the resistance offered to the passage of fluid from the common duct into the intestine) in the rat is low, usually not more than 30 mm water (Mann 1919; Schmidt and Ivy 1937; review by Hallenbeck 1967).

Considering the anatomical and functional aspects reported above, it is conceivable that proximal and distal regions of the common biliary duct of the rat are the sites where intraluminal pressure alterations can occur. Thus changes in intraluminal pressure in the proximal region (which receives the branches of the hepatic plexus) could occur through changes in the amount of bile production; and in the distal region (which receives the pancreatic duct) by changes in the flow of pancreatic juice and by the sphincteric resistance.

In conclusion, proximal and distal regions of the common bile duct of the rat could undergo more marked transient pressure changes (and therefore distortion of the wall) than the long intermediate part. The occurrence of numerous BC in the proximal and distal regions suggests that these cells function as barorecptors, i.e., they are implicated in a process of registration and/or regulation of internal pressure alterations within the common bile duct.

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