THE GLANDS OF THE BILE AND PANCREATIC DUCTS: AUTORADIOGRAPHIC AND HISTOCHEMICAL STUDIES

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INTRODUCTION

'To obtain any idea of the histologic structure of the bile ducts one must consult books which have long since been swept by in the current of medical literature and are almost forgotten.' This sentence is largely as true to-day as it was when written by Burden in 1925. Since that time the majority of the investigations that have been carried out on the extra-hepatic bile ducts have been concerned with the musculature of the ducts, and its possible functional significance is being studied at the present time by Burnett & Shields (1958). In a recent series of papers, Boyden reported his observations on the choledocho-duodenal junction in a number of laboratory animals and in man (Boyden, 1955, 1957*a*, *b*; Eichhorn & Boyden, 1955), but no attention was paid to the mucous membrane, which was always destroyed during maceration of the specimens. Most text-books of histology make only a passing reference to the glands of the bile and pancreatic ducts. There is general agreement that there are mucous glands in the pancreatic duct, and most authors describe goblet cells as well. As far as the bile duct is concerned, Ham (1957) mentions the presence of tubulo-alveolar glands, which Garven (1957) states are not mucus-secreting, while Mann (1932) subscribes to the presence of glands with both a mucous and serous function. Maximow & Bloom (1957) assert that the columnar epithelium of the extra-hepatic biliary passages yields atypical mucus, and Deane (1954) describes the columnar cells as possessing a striated border and apical mucus droplets, apart from the presence of occasional goblet cells. Trautmann & Fiebiger (1952), dealing with domestic animals, state that mucous glands and goblet cells are found in pancreatic ducts, but that in the bile ducts goblets are found only in the pig, ox and horse; many glands are normally present, except in the pig.

The present work has been undertaken for the specific purpose of studying the mucosa of the lower ends of the bile and pancreatic ducts where they open into the lumen of the gut, with special reference to the glands that may be found in the walls of those ducts. Morphological studies on laboratory animals and on human specimens from the recently deceased have been supplemented by a number of histochemical observations, and by autoradiography using $^{35}SO_4$ *in vivo* and/or *in vitro*.

MATERIALS AND METHODS

A total of 48 adult animals of the following species was used: mouse (4), hamster (6), rat (11), guinea-pig (9), cat (7), dog (5) and Rhesus monkey (6). They were sacrificed in the morning having had access to water only overnight. The human material

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consisted of seven adult autopsy specimens, removed from 2 to 24 hr. post-mortem. The post-cibal status of the individuals was uncertain.

In the mouse, rat and hamster, the main pancreatic duct joins the bile duct before the latter opens into the small intestine. In the cat, monkey and man, the bile and main pancreatic ducts normally open on a common papilla, with an adjacent papilla for an accessory pancreatic duct. In the guinea-pig, the main pancreatic duct opens into the duodenum about 7 cm. distal to the bile duct, whose opening is very near the pylorus. In the dog, the main pancreatic duct opens distal to a papilla that receives the bile and accessory pancreatic ducts.

For this investigation, the regions of the gut bearing the duct openings were removed together with an adjacent segment of the appropriate duct or ducts. The fixatives used included neutral formalin, ice-cold 80% alcohol and Gendre's and Rossman's fluids; some specimens were freeze-dried or freeze-substituted, and fresh frozen sections were also used.

Apart from routine staining with haematoxylin and eosin, mucicarmine or iron haematoxylin and picrofuchsin (van Gieson), the following histological and histochemical staining procedures were used on selected serial sections:

(1) The cobalt sulphide or azo dye methods for alkaline phosphatase.

(2) The PAS technique, with and without diastase digestion, for protein-carbohydrate complexes and glycogen.

(3) Alcian blue, Hale's colloidal iron or toluidine blue for acid mucopolysaccharides.

(4) Toluidine blue or methyl green-pyronin, with and without ribonuclease digestion, for cytoplasmic basophilia, indicating ribonucleoprotein (RNA).

(5) The neotetrazolium method for succinic dehydrogenase carried out on fresh frozen tissue under anaerobic conditions, as an index of cellular metabolic activity.

Autoradiographic technique

For studies *in vivo*, animals were injected intraperitoneally with carrier-free $Na_2{}^{35}SO_4$, in doses of 4 μc./g. body weight for the mouse, 2 μc./g. for the hamster, and 1 μc./g. for the rat and guinea-pig. One cat of 2·5 kg. received a dose of 9 mc. The animals were killed 6 hr. after the administration of the isotope. For *in vitro* studies, pieces of duct tissue approximately 2 mm. long and 0·5 mm. thick from the hamster, rat, guinea-pig, cat, dog, monkey and man were incubated in roller tubes for 6 or 12 hr. at 37° C. in 2 ml. Tyrode's solution containing 2 μc. of radio-sulphate, 200 units of penicillin and 200 units of streptomycin (Curran & Kennedy, 1955).

Autoradiographs were prepared using the stripping-film technique (Heatley, Jerrome, Jennings & Florey, 1956), the exposure times being from 3 to 6 weeks.

RESULTS

The cytological findings in the duct epithelium and glands are detailed below under species headings. In general, it was noted that in any given animal, there were negligible morphological differences between the epithelial cells lining the bile and pancreatic ducts, or between the duct cells and those of the ampullae and papillae. However, the transition between papillary and intestinal epithelium was very

abrupt (Pl. 1, fig. 1). Also, there was considerable species variation in the size of the epithelial cells lining the ducts, those of the guinea-pig being by far the largest (Pl. 2, cf. figs. 14, 15).

Unless otherwise stated, material that was PAS-positive also gave positive reactions with Alcian blue and Hale's colloidal iron.

Mouse

During its course through the pancreas the bile duct proved to be a straight-walled tube, lined by columnar epithelium containing rounded basal nuclei. A number of small ducts from the pancreas opened into the bile duct at irregular intervals. On piercing the intestinal wall, numerous sac-like glands opened into the lumen of the duct (Pl. 2, fig. 9), and these were especially frequent near the papilla. Very occasional mitotic figures were noted in the duct epithelium.

Staining with mucicarmine and with the PAS technique revealed the presence of goblet cells scattered throughout the epithelium of the duct. The epithelial cells themselves and those of the glands stained strongly at their luminal borders and very often also in the supranuclear regions. The PAS-positive material was diastase-resistant. No metachromasia was noted, except in mast cells that were occasionally found in the periductal connective tissue. Compared with intestinal and pancreatic cells, the cells of the duct and glands contained only small amounts of RNA. Alkaline phosphatase activity was confined to a small number of stromal blood vessels, but a strongly positive reaction for succinic dehydrogenase was present in all epithelial cells.

Autoradiographs of ducts from animals that had received $^{35}SO_4$ showed that strong radioactivity was present in the goblet cells of the ducts and in most of the glands, but that there was no activity in the other epithelial cells.

Hamster

In the hamster, the distal end of the bile duct in its extra-intestinal course possessed a smooth lining of columnar cells without any glandular downgrowths. After piercing the muscular wall of the gut and while traversing the intestinal mucous membrane, the duct lining became thrown into folds but no diverticula comparable to those noted in the mouse were seen. Staining with mucicarmine and with the PAS method revealed many goblet cells in the duct lining; the remaining columnar cells possessed luminal borders and supranuclear regions that were PAS-positive. Diastase digestion did not alter the extent of PAS staining, hence none of this material could be identified as glycogen. The sites of PAS-positive material also gave a metachromatic reaction with toluidine blue. Some mast cells were noted in the periductal connective tissue. Treatment with ribonuclease removed the cytoplasmic basophilia of the duct cells almost completely, though some coloration persisted in the goblet cells and in the luminal borders. No alkaline phosphatase was found either in the duct epithelium or in its underlying connective tissue framework, but the epithelium gave a strongly positive reaction for succinic dehydrogenase.

Autoradiographs of duct tissue revealed strong activity in the mucin of the goblet

cells, and also in the borders and supranuclear regions of the remainder of the lining cells. Precisely similar activity was found in portions of duct that had been cultured for 6 or 12 hr., the longer period merely emphasizing the activity that could be seen after 6 hr.

Rat

The epithelial lining of the bile duct in the rat consisted of columnar cells, with a number of goblets that were less numerous than in the hamster and were chiefly found near the papilla. As the duct approached the intestine it was characterized by the presence of sac-like diverticula that became more numerous and test-tube-like as the duct pierced the intestinal wall. These downgrowths were lined by typical columnar duct cells interspersed with goblets. The goblets of the duct and in the downgrowths were PAS and mucicarmine-positive, and also showed metachromasia, but in the columnar cells themselves the luminal borders were only very faintly PAS-positive (Pl. 2, fig. 14). No significant staining reactions were noted in the supranuclear regions of these cells; there was no staining with mucicarmine, Alcian blue or Hale's method and no metachromasia. None of the PAS-positive material was removed by diastase digestion, but ribonuclease removed almost all the cytoplasmic basophilia of the columnar cells. Blood vessels in the subepithelial connective tissues contained alkaline phosphatase, but this enzyme was not observed in the epithelium. However, succinic dehydrogenase was present, both in the duct lining itself and in the diverticula.

Radioactivity in the duct and its glands both *in vivo* and *in vitro* was confined to the goblet cells, the ordinary epithelial cells (as in the mouse) showing no evidence of having incorporated the sulphur isotope (Pl. 1, fig. 2).

Guinea-pig

In this species the terminations of both the bile and pancreatic ducts were available for study, but the two ducts were essentially similar in structure, differing only in quantitative respects.

Both ducts were lined by columnar cells interspersed with goblets that were more numerous in the pancreatic than in the bile duct. The lining cells showed PAS-positive, diastase-resistant borders and supranuclear regions. The goblet cells were usually but not invariably metachromatic, and the cytoplasmic basophilia of the columnar cells was almost completely removed by digestion with ribonuclease.

Opening into the lumen of the intra-mural and the adjacent extra-mural portions of both ducts was a large number of tubulo-alveolar glands (Pl. 1, fig. 3). The bile duct was somewhat thicker in section than the pancreatic duct due to the greater depth of these glands in the former. The upper, tubular parts of the glands were lined by columnar cells and goblets that gave the same histochemical reactions as the epithelium of the duct lining (Pl. 1, fig. 6). The lower parts appeared as typical glandular alveoli whose cells were packed with PAS and mucicarmine-positive material that was also metachromatic (Pl. 1, figs. 4 and 6–8). In some specimens the more superficial portions of the alveoli, while still PAS-positive, did not stain with mucicarmine and showed no metachromasia. Alkaline phosphatase was absent from the duct and its glands, but there was strong succinic dehydrogenase activity.

Radioactivity was present in both ducts in the mucin of the goblet cells, but was strongest in the basal parts of the alveoli (Pl. 1, fig. 3), i.e. the parts that were constantly metachromatic and mucicarmine-positive as well as PAS-positive. The remaining more superficial parts of the alveoli did not exhibit radioactivity.

Cat

The most striking cytological feature in this species when compared with the rodents examined was the complete absence of goblet cells from all the ducts (Pl. 2, fig. 10). The bile and pancreatic ducts, opening on their common papilla through the ampulla, were lined as in the rodents by columnar cells with borders and supranuclear regions that were PAS and mucicarmine-positive, but there was no metachromasia. Many mucosal folds were present in the ampullary and papillary regions. A number of sac-like glands opened into the lumina of both ducts (Pl. 2, fig. 10). They contained no goblet cells, but those in the pancreatic duct nearest the papilla contained alkaline phosphatase in their cytoplasm. The enzyme was not seen in the glands of the bile duct itself, but those glands that opened into the ampulla common to both ducts gave a strongly positive phosphatase reaction (Pl. 2, fig. 11). The cells of the glands of both the ducts and the ampulla usually stained with PAS and mucicarmine, and a considerable amount of RNA was present. In one specimen a number of epithelial cells, on the very summit of the papillary opening into the lumen of the gut, contained perinuclear granules of glycogen. This was the only instance throughout the course of the present investigation in which glycogen was detected.

Autoradiographs of portions of cultured ducts revealed an uptake of sulphur by all the epithelial cells lining the ducts, and radioactivity was usually but not invariably present in the glands (Pl. 2, fig. 17). A precisely similar distribution, but of greater intensity, was noted in the duct tissues of the cat that had received a large dose of isotope *in vivo*.

Dog

As in the cat, numerous folds of mucous membrane were found in the lower ends of the three principal ducts and their associated ampullae and papillae (Pl. 1, fig. 5). The columnar epithelial cells in this species were particularly tall and closely packed (Pl. 2, fig. 16), though in the many saccular glands that opened into the lumina of the ducts they were more cubical in form. Mitotic figures were frequent, especially in the epithelium of the papillary regions, and numbers of infiltrating lymphocytes were also noted.

The cells of both the ducts and glands were PAS and mucicarmine-positive, and occasionally but not always exhibited metachromasia. In one dog the glands associated with the bile duct did not stain with Alcian blue or mucicarmine, although they were PAS and Hale-positive. No goblet cells were found in any of the ducts (Pl. 1, fig. 5), but, unlike any other species examined, the cells lining all the ducts, as well as those in the glands, gave a strongly positive reaction for alkaline phosphatase (Pl. 2, fig. 16). Succinic dehydrogenase was also present in duct lining cells and glands.

Culture of the ducts with radio-sulphur showed that the cells lining all the ducts

were radioactive at their luminal borders. Only a few of the glands were overlaid by granules in the autoradiographs, and such radioactivity as was present was in the superficial and not in the deep portions.

Monkey

The general morphological pattern of the ducts closely resembled that of the cat and dog. Sac-like glands were present in the bile, pancreatic and accessory pancreatic ducts. The columnar epithelial lining of the ducts and glands contained goblet cells that were much more abundant in the pancreatic than in the bile ducts. The mucus of the goblet cells, together with the borders and supranuclear regions of many of the columnar cells, stained deeply with PAS, and rather faintly with mucicarmine, metachromasia being often but not constantly present in these regions. Alkaline phosphatase was not present in duct or glandular epithelium, but was found in the stroma surrounding the papilla and in some subepithelial and periglandular blood vessels.

Following culture with radioactive sulphur, autoradiographs revealed activity in the luminal borders of the duct epithelium (Pl. 2, fig. 18), and in the region of the necks of the glands but not at deeper levels.

Man

In all the human specimens, even that removed 2 hr. post-mortem, most of the epithelium lining the lower ends of the main ducts had undergone autolysis and only small fragments were available for study. However, the glands appeared to be well preserved in most instances in both bile and pancreatic ducts.

The available surface epithelium was of the tall columnar variety, and the luminal borders and supranuclear regions stained strongly with PAS and mucicarmine. No metachromasia was encountered, nor were any goblet cells found. Ribonuclease removed most of the cytoplasmic basophilia. The glands, as previously noted by Burden (1925), were arranged in irregular groups and not evenly distributed throughout the mucosa as was the case in all other species. Tubulo-alveolar in nature, their epithelium was more cuboidal than that of the duct lining itself, but gave the same staining reaction, without metachromasia or goblet cells (Pl. 2, fig. 12). No epithelial or glandular pathology was found in the present series, although studies by Bagenstoss (1938) and Birnstingl (1959) on much larger numbers of specimens indicate that minor pathological processes such as epithelial hyperplasia or metaplasia are not uncommon. Alkaline phosphatase was present only in mucosal blood vessels, but both surface epithelium and glands contained succinic dehydrogenase (Pl. 2, fig. 19).

Most but not all of the glands in the bile and in the pancreatic ducts showed radioactivity after culture with $^{35}SO_4$ (Pl. 2, fig. 13). No conclusions about sulphate in the lining cells could be reached owing to the unsatisfactory nature of the particular specimens incubated.

DISCUSSION

These studies reveal morphological differences between species with regard to the lower ends of the bile and pancreatic ducts and their associated glands. The simplest bile duct of this series is that of the hamster—a straight tube, the mucosa being thrown into folds only a little distance from the papillary opening. The most complex is that of the guinea-pig, which like the pancreatic duct in this species possesses a thick mucosa due to the presence of many tubulo-alveolar glands.

The histochemical findings indicate that all the ducts are capable in some way or other of secreting protein-carbohydrate complexes, some of which is mucin that can be shown by autoradiography to be of the sulphated variety, though the sites of origin of these secretions show species differences. In the rat, goblet cells appear to be the sole source of duct secretion, whereas in all other species examined the epithelial cells lining the ducts are secretory. The cat and dog are the only species that do not possess goblets, but our human material does not show goblet cells either. A larger series of fresh specimens is required before a definite answer can be given to this question in man. The hamster alone possesses no glands comparable with any other species, although in the rat there are no alveoli and the 'glands' are simply downgrowths of lining epithelium containing scattered goblet cells.

The autoradiographic evidence indicates that some at least of the mucin secreted by the ducts is of the sulphated variety. The autoradiographs of cultured material from the smaller animals give results that very closely resemble those obtained from the same animals *in vivo*, and it may be that the ducts in question could well be added to the structures listed by Trowell (1959) as suitable for organ culture. With the reservation that the behaviour of cells in culture has not always proved to be a reflexion of their behaviour *in vivo*, it is considered reasonable to assume that the *in vitro* findings in the dog, monkey and man give sufficient indication of whether or not duct cells and glands are capable of secreting sulphated material, although whether the sulphur in culture is being actively metabolized or merely exchanged is a question that need not be debated here.

All the goblet cells of this series show radioactivity following the administration of $^{35}SO_4$, and in this respect they are identical with the goblets of the intestine (Jennings & Florey, 1956) and of the gall-bladder and trachea of some species (Jennings, 1958). Wherever alveolar glands are present, i.e. in all species except the rat and hamster, some or all of their cells show radioactivity. Although, like all other species except the rat, the lining cells of the mouse and guinea-pig ducts are secretory, as evidenced for example by positive staining with PAS, Alcian blue and Hale's colloidal iron, there is no suggestion of radioactivity in these cells, whereas those of the hamster, cat, dog, monkey and man do give positive autoradiographs. In some situations, e.g. in most goblet cells, the site of formation of sulphated mucin can be correlated with metachromasia and positive staining with mucicarmine, but in other situations this is not always so. For example, in some guinea-pig specimens, where only the deeper portions of the alveoli are radioactive, there is in these deeper parts metachromasia and positive mucicarmine staining, while in more superficial parts there is no metachromasia, no mucicarmine staining and no radioactivity. But in the glands of the mouse, cat and dog, sites that are mucicarmine

positive and radioactive are not metachromatic. Many factors, as yet little understood, influence the demonstration of metachromasia (cf. the discrepancies noted by Jennings & Florey, 1956), and the interest that is being taken in this matter is reflected in recent papers dealing with its fundamental aspects (e.g. Walton & Ricketts, 1954; Bergeron & Singer, 1958). Furthermore, Pearse (1960) points out that the former belief that the presence of metachromasia indicated specific chemical groups is now untenable. The precise chemical structure of the various forms of mucopolysaccharide still remains to be elucidated despite recent progress (Wolstenholme & O'Connor, 1958; Young & Maw, 1958), and their function too is largely speculative. The evidence suggests that mucins serve as a protective coating for body surfaces (Florey, 1955). If they exercise this function in bile and pancreatic ducts, they are warding off possible injury to the mucosa caused by ascending duodenal contents, or even by the normal hepatic and pancreatic secretions. While some mucins may aid bacterial growth, others may inhibit the proliferation of microorganisms, and this is a possible additional form of protection.

Glycogen is found in the duct epithelium of only one animal in the present series—a cat—in the form of infranuclear granules in cells on the very summit of the duodenal papilla. From the absence of this substance elsewhere it may be inferred that it is not a normal constituent of duct cell cytoplasm, at least in histochemically detectable quantities. Its presence in one animal is difficult to explain, but it may indicate that the cells in question have regenerated as a result of trauma, for Johnson & McMinn (1960) have shown in the cat that regenerating epithelium in both the small and large intestines is characterized by a small accumulation of glycogen in the new cells within the first week after injury. However, Kugler & Wilkinson (1959, 1960) have suggested that of the two fractions in which cellular glycogen exists—protein-bound and trichloracetic acid-soluble—only the latter fraction is in fact detected histochemically. Thus considerable quantities of glycogen may still be present in cells and yet remain undetected cytologically. The factors influencing a change from one form to another are not known.

While it is well established that alkaline phosphatase can be found in the smaller blood vessels of many species, as for example those in the subepithelial connective tissue of the rat bile duct, its presence in such striking degree in the ducts and glands of the dog, and in the ampullary and pancreatic glands of the cat, is an unexpected finding, especially in view of its complete absence in all other species examined. The presence of phosphatase in bile and pancreatic ducts in the dog has been noted previously by Gomori (1941), Jacoby (1946) and Jacoby & Martin (1951), and the latter authors also found the enzyme to be absent from guinea-pig ducts. Phosphatase in other epithelial sites, e.g. in the intestine and proximal convoluted tubules of the kidney, has been correlated with absorptive processes, but the absorption at least of water can take place without phosphatase, as in Henle's tubules of the kidney. The reason for the presence of phosphatase in duct linings and glands is so far unknown. It is a notable fact that succinic dehydrogenase has been found in all species examined. The presence of this enzyme indicates that considerable metabolic activity is taking place, and that the ducts are not merely serving as passive conduits. In this respect there is a close resemblance between the ducts under discussion and those of the salivary glands of a number of species (Padykula, 1952;

Hill & Bourne, 1954). Babkin (1950) has reviewed the earlier evidence for the existence of a secretory function on the part of salivary gland ducts, and Grossman & Ivy (1946) suggested that the ducts of the pancreas contributed to the exocrine secretions of that organ. Whether the substance secreted by the salivary duct cells is water alone or some other substance has not been established, but it would appear that a similar activity is occurring in bile and pancreatic ducts. The secretions need not of course be identical in all species. It might be speculated that water is being passed from the blood stream into the ducts in order to dilute bile that has been concentrated in the gall-bladder, but for the fact that in the rat that possesses no gall-bladder there appears to be just as much succinic dehydrogenase activity as in other species. There is a considerable field here for biochemical and physiological investigation. 'Bile' collected from the lower end of the bile duct should not always be assumed to contain merely a mixture of mucin and hepatic secretion, since in the guinea-pig, for example, the extensive glandular arrangements suggest that much more than simply mucin is being secreted by the duct. Studies with the electron microscope would also be of interest, to determine whether microvilli are present at the luminal surface of duct cells, and also to establish whether the basal plasma membranes show the characteristics that have been considered by Pease (1956) to be associated with water transport.

SUMMARY

1. The epithelium and glands at the lower ends of the bile and pancreatic ducts of the mouse, hamster, rat, guinea-pig, cat, dog, Rhesus monkey and man have been studied by various histological and histochemical methods, and also by autoradiography using $^{35}SO_4$ *in vivo* and /or *in vitro*.

2. The ducts of all species were found to be capable of secreting protein-carbohydrate complexes, some of which was mucin of the sulphated variety.

3. The secretions were derived from one or more of three sources—goblet cells, duct epithelium (excluding goblets) and alveolar glands—according to species.

4. Goblet cells were not found in the ducts of the cat or dog, nor in the available human specimens. In all other species goblets were present and secreted sulphated mucin.

5. With the exception of the rat, the cells lining the ducts were secretory in all species, the secretion being sulphated except in the mouse and guinea-pig where it was non-sulphated.

6. The hamster and rat possessed no true alveoli. In all other species examined, alveolar glands were present, secreting both sulphated and non-sulphated material.

7. The ducts of all species showed strong succinic dehydrogenase activity, suggesting that, like those of the salivary glands, the ducts are not merely passive conduits but are actively engaged in transport mechanisms.

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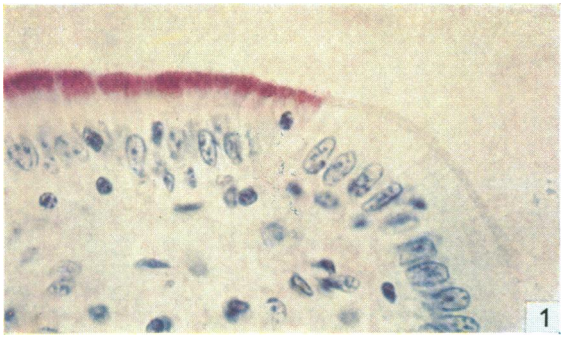
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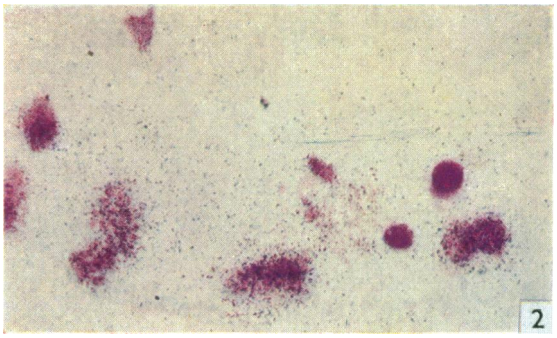
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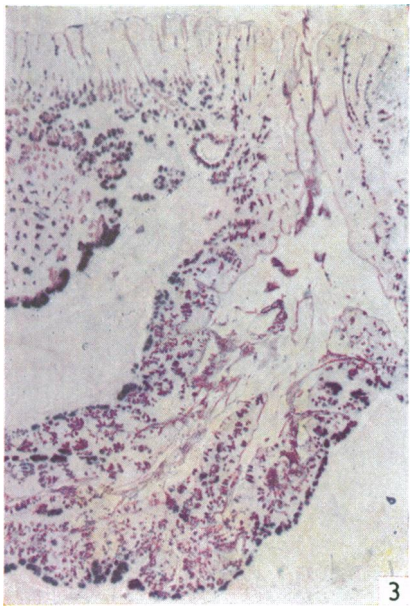
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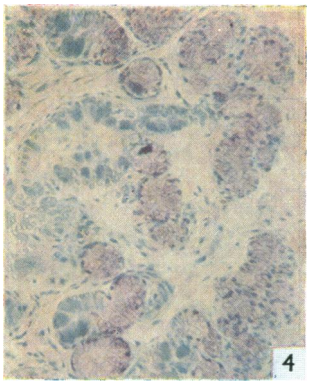
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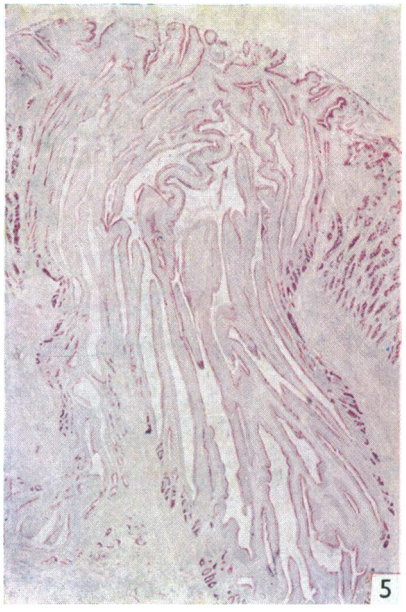
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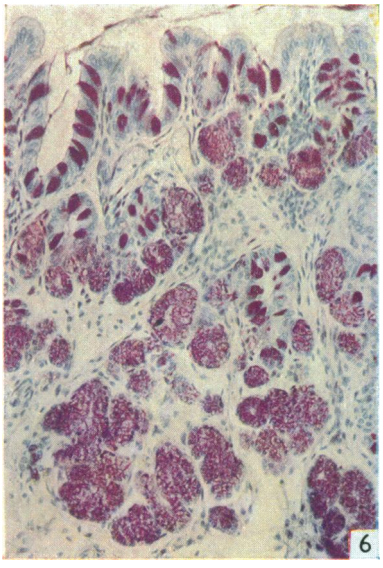
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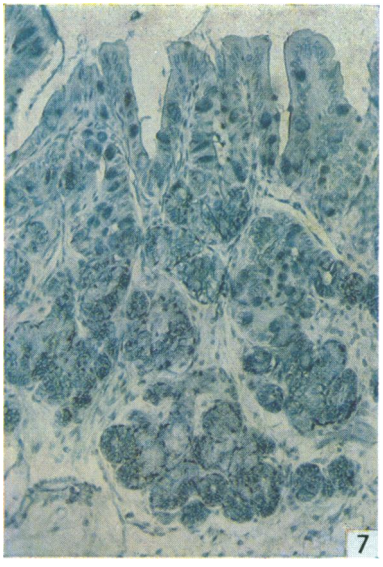
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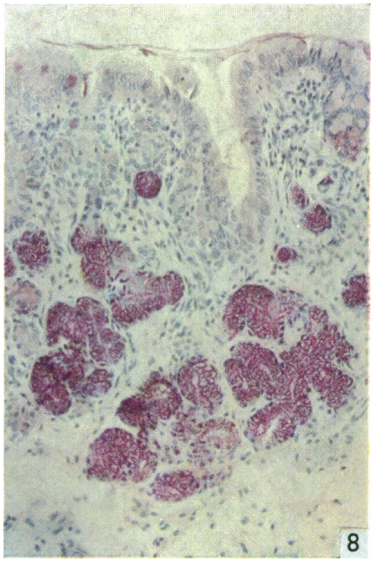
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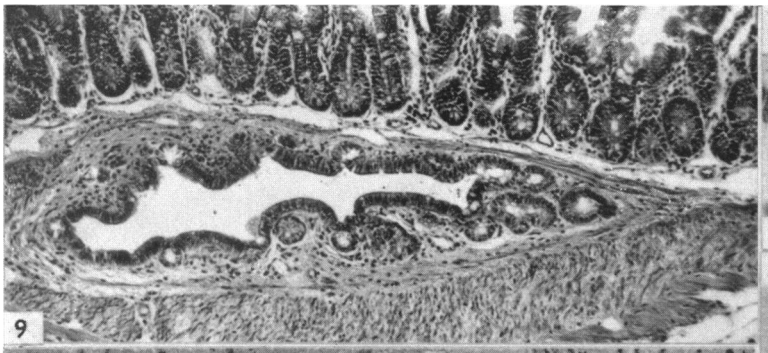

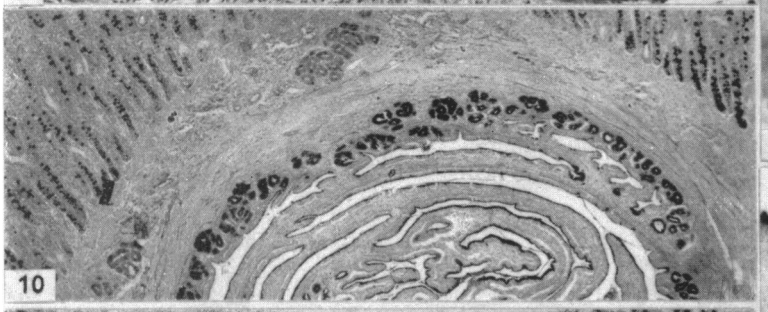

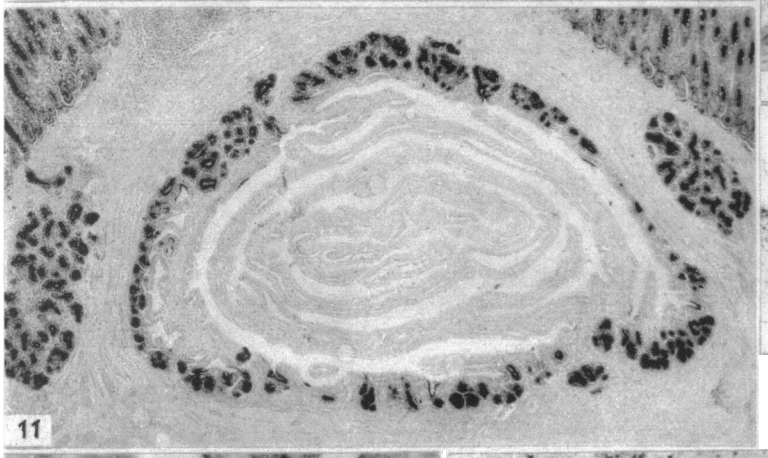

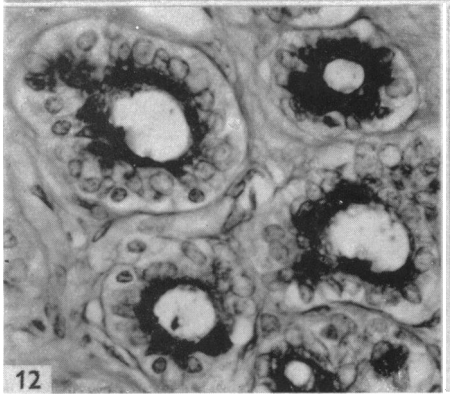

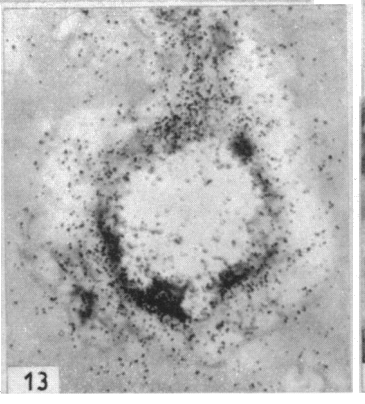

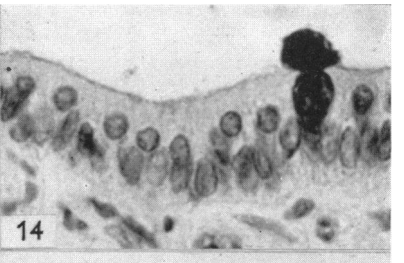

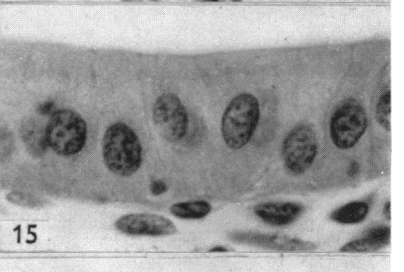

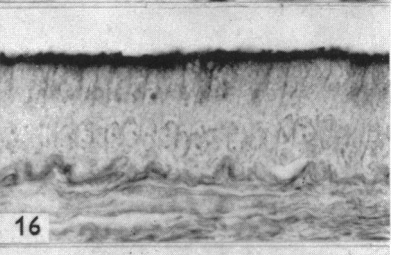

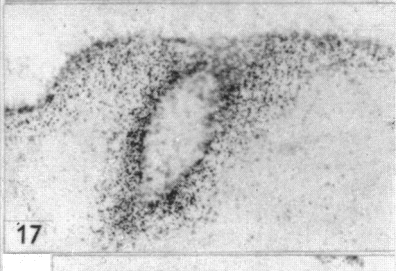

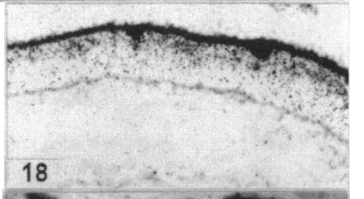

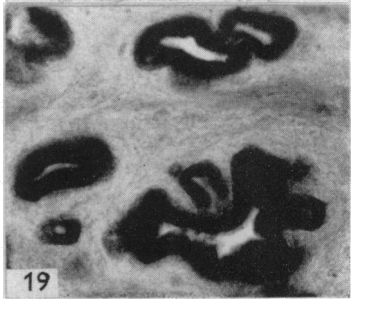

McMINN AND KUGLER—GLANDS OF THE BILE AND PANCREATIC DUCTS

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Cat. Junction between papillary epithelium (on the left) and duodenal epithelium (right). PAS, haematoxylin. ×460.

Fig. 2. Rat. The bile duct surface epithelium (barely visible near the top margin of the figure) shows no radioactivity, whereas the mucin of the goblet cells in the glands gives a strong reaction. Autoradiograph, counterstained PAS. ×480.

Fig. 3. Guinea-pig. Bile duct opening into duodenum. The numerous duct glands show strong radioactivity at their bases, like the pyloric glands of the stomach (upper, left). Autoradiograph, counterstained PAS. ×19.

Fig. 4. Guinea-pig. Glands of the bile duct, showing metachromasia. Toluidine blue. ×58.

Fig. 5. Dog. Accessory pancreatic duct (left) and bile duct opening into the duodenum. Note the deeply staining glands at the periphery of the ducts; compare the duct epithelium with intestinal epithelium (right) and note the absence of goblet cells in the former. PAS, haematoxylin. ×12.

Fig. 6. Guinea-pig. Wall of bile duct, showing lining epithelium with goblet cells, and tubulo-alveolar glands. PAS, haematoxylin. ×58.

Fig. 7. Guinea-pig. As fig. 6. Hale's collodial iron. ×58.

Fig. 8. Guinea-pig. As fig. 6. Mucicarmine. ×58.

PLATE 2

Fig. 9. Mouse. Bile duct showing sac-like glands. Haematoxylin and eosin. ×88.

Fig. 10. Cat. Transverse section through half an ampulla showing circumferential glands. Compare ampullary epithelium with that of the intestine (left and upper right) and note the absence of goblet cells in the former. PAS, haematoxylin. ×37.

Fig. 11. Cat. Transverse section through an ampulla. The ampullary glands (and Brunner's glands, left and right) give a strong reaction for alkaline phosphatase, but the ampullary epithelium is negative.

Fig. 12. Man. Glands of the bile duct. PAS, haematoxylin. ×460.

Fig. 13. Man. Gland of bile duct showing maximal radioactivity at the luminal border of the epithelium. Autoradiograph, counterstained PAS. ×510.

Fig. 14. Rat. Epithelium of bile duct. Note the absence of supranuclear and apical staining. PAS, haematoxylin. ×650.

Fig. 15. Guinea-pig. Epithelium of bile duct, at same magnification as fig. 14. Haematoxylin and eosin. ×650.

Fig. 16. Dog. Epithelium of bile duct, showing a strong phosphatase reaction at the luminal border. Gomori technique, incubation time 30 min. ×380.

Fig. 17. Cat. Unstained autoradiograph of bile duct epithelium and gland. ×380.

Fig. 18. Monkey. Bile duct epithelium showing maximal radioactivity in the region of the luminal border. Autoradiograph, counterstained PAS. ×350.

Fig. 19. Man. Glands of the bile duct showing a strong reaction for succinic dehydrogenase. ×30.