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# IgA plasma cells in biliary mucosa: a likely source of locally synthesized IgA in human hepatic bile

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## SUMMARY

IgA synthesized in hepatobiliary tissues accounts for about one-half of the IgA present in human hepatic bile, but the location of the IgA synthesizing cells has been in doubt because few plasma cells are present in normal liver. Therefore, we immunocytochemically localized IgA, J chain and secretory component in bile duct tissues of six patients operated upon for biliary duct obstruction. Numerous plasma cells containing IgA and J chain were found surrounding the accessory glands of the major bile ducts and in the area just beneath the surface epithelium of the ducts. At the ultrastructural level, IgA and SC in the epithelial cells had the features characteristic of secretory component-mediated endocytic translocation of IgA. We conclude that plasma cells in biliary duct mucosa are the likely source of much of the locally synthesized IgA that is secreted into human hepatic bile. The IgA probably reaches the bile by direct transfer across biliary epithelium.

**Keywords** IgA secretory component J chain biliary mucosa immunohistochemistry

## INTRODUCTION

IgA is a major protein in the hepatic bile of several species, including man. Much of the biliary IgA is in the secretory form (Nagura *et al.*, 1981; Lemaitre-Coelho, Jackson & Vaerman, 1977; Delacroix *et al.*, 1982; Kutteth *et al.*, 1982) i.e. IgA polymers in complex with the glycoprotein secretory component (SC) (Tomasi *et al.*, 1965). In the rat, nearly all of the biliary IgA is derived from transport of serum IgA into bile, a process that is effectively carried out by the association of IgA polymers with their carrier molecule, SC, on hepatocytes (Fisher *et al.*, 1979; Mullock *et al.*, 1979; Socken *et al.*, 1979; Renston *et al.*, 1980; Takahashi, Nakane & Brown, 1982). Man differs from the rat in lacking an enriched source of polymeric IgA entering the blood circulation (Brown *et al.*, 1982). Hence, the proportion of IgA in human bile that is derived from local synthesis within hepatobiliary tissues is high (about 50%) compared to the corresponding proportion in the rat (Delacroix *et al.*, 1982). A related species difference is that SC is not present in human hepatocytes, only in biliary epithelial cells (Nagura *et al.*, 1981; Hopf *et al.*, 1978).

The origin of the locally synthesized IgA in human heptic bile has been in doubt because few plasma cells are present in biopsies of the normal human liver (Nagura *et al.*, 1981; Paronetto, Rubin & Popper, 1962; Hadziyannis *et al.*, 1969). In the present study, we found that IgA containing

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plasma cells are numerous in human biliary mucosa, especially in the vicinity of the extensive array of accessory glands that communicate with the major biliary ducts (Elias & Sherrick, 1969; McMinn & Kugler, 1961; Cohen, 1964). These cells are the likely source of much of the IgA in human hepatic bile.

## MATERIALS AND METHODS

*Patients.* Biopsies were taken from the extrahepatic biliary ducts of six patients at the time of surgery for diseases listed in Table 1.

*Antibodies.* The preparation of horseradish peroxidase (HRP) labelled Fab′ fragments of rabbit antibodies specific for the $\alpha$ chains, $\gamma$ chains, $\mu$ chains or J chains of human immunoglobulins and for human secretory component (SC) has been described (Nagura *et al.*, 1981; Nakamura *et al.*, 1982). For use in control experiments, Fab′ fragments of non-immune rabbit $\gamma$-globulin also were labelled with HRP.

*Immunohistochemical methods.* Immunoglobulins, J chain and SC were localized in the bile duct tissues by the direct peroxidase labelled antibody technique as described previously (Nagura *et al.*, 1981; Nakamura *et al.*, 1982). The tissue specimens were promptly fixed in periodate–lysine–4% paraformaldehyde (PLP) (McLean & Nakane, 1974), washed in increasing concentrations of sucrose in phosphate-buffered saline (PBS), frozen in Tissue-Tek II OCT compound (Lab Tek Products, Naperville, Illinois, USA), and sectioned (8 $\mu$m thick) on a cryostat microtome. Sections to be observed by light microscopy were treated with 5 mM periodic acid and 3 mM sodium borohydride to inactive endogenous peroxidase. The conjugated antibodies were applied to the sections for 2 h at room temperature. Control sections were reacted either with the conjugated Fab′ fragments of non-immune rabbit $\gamma$-globulin or with the conjugated antibodies absorbed with purified relevant antigens. After excess antibody reagents were washed off with PBS, the sections were dipped in 0·25% diaminobenzidine (DAB) solution in 50 mM Tris-HCl buffer, pH 7·6, containing 10 mM hydrogen peroxide and 10 mM sodium azide for 10 min and counterstained with methyl green. For the detection of J chain, whose antigenic determinants are partially masked in IgA or IgM (Brandtzaeg, 1976a, 1976b), cryostat sections were treated with 6 M acid–urea in glycine-HCl buffer, pH 3·2, for 20 h at 4°C before the immunostaining (Brandtzaeg, 1976a, 1976b; Nagura *et al.*, 1979a; Nakamura *et al.*, 1982).

Cryostat sections adjacent to those used for light microscopy were selected for electron

**Table 1.** IgA cells in bile duct wall

| Patient | Diagnosis | Age/sex | Areas of bile duct wall A* (Cells per 0·0025 mm² bile duct wall) | B* | C* |
|---|---|---|---|---|---|
| 1 | Gall bladder carcinoma | 81/F | 1·45 | 0·05 | 0·72 |
| 2 | Common bile duct carcinoma | 70/M | 1·20 | 0·15 | 0·25 |
| 3 | Common bile duct carcinoma | 68/F | 1·27 | 0·15 | 1·09 |
| 4 | Pancreas head carcinoma | 69/M | 0·58 | 0·05 | 0·19 |
| 5 | Common bile duct carcinoma | 71/F | 1·21 | 0·05 | 0·83 |
| 6 | Pancreas head carcinoma | 61/M | 2·34 | 0·11 | 0·17 |
| Mean ± s.e. | | | 1·34 ±0·52 | 0·09 ±0·05 | 0·54 ±0·36 |

*A = periglandular zone; C = zone beneath the surface epithelial layer; B = zone between A and C. See also Fig. 2b.

microscopic study. The sections were reacted with the HRP-labelled antibodies or control HRP-Fab′ fragments at 4°C for about 12 h. After washing in PBS, the sections were post-fixed with 1% glutaraldehyde in PBS and incubated sequentially in 0·25% DAB solution containing 1% dimethylsulphoxide for 30 min and 0·25% DAB solution containing 10 mM hydrogen peroxide for 10 min. The sections were washed in PBS, dehydrated in graded alcohol, and embedded in Epon. Ultrathin sections, either unstained or stained with lead citrate, were viewed with a JEM 100C electron microscope.

*Enumeration of IgA containing cells.* IgA containing cells in the stained sections of bile duct were counted during light microscopic observation by use of a reticular grid. The number of cells was expressed as mean cells per 0·0025 $mm^2$ tissue in each of three areas: (a) a 0·05 mm wide zone immediately surrounding the accessory glands, referred to as the periglandular zone; (b) an area adjacent to the 'periglandular zone'; (c) a zone just beneath the surface epithelium. A minimum of 10 each such areas were observed per patient.

## RESULTS

*Light microscopy*

A large number of tubulo-alveolar (accessory) glands opening into the lumen of the common bile duct were seen. The tubular parts of the glands were lined by columnar cells, and glandular alveoli formed the lower parts (Fig. 1).

Numerous IgA containing cells, most with typical plasma cell morphology, were present in the biliary mucosa. The cells were most numerous in the periglandular zone of the accessory glands, where there were about 1·3 cells per 0·0025 $mm^2$ tissue (Fig. 2b; Table 1). J chain containing cells were about as numerous as IgA containing cells (Fig. 2c). By contrast, too few IgM or IgG containing cells were present to justify counting them; together they appeared to be less than 5% as numerous as IgA or J chain containing cells.

SC was prominently present in the epithelial cells of the accessory glands and the surface epithelial cells (Fig. 2a). The epithelial cells were positive also for IgA and, after acid–urea treatment, for J chain (Fig. 2b & 2c). The IgA and J chain appeared to be present most prominently along lateral margins of the epithelial cells.

Control sections were negative for specific staining (Fig. 2d).

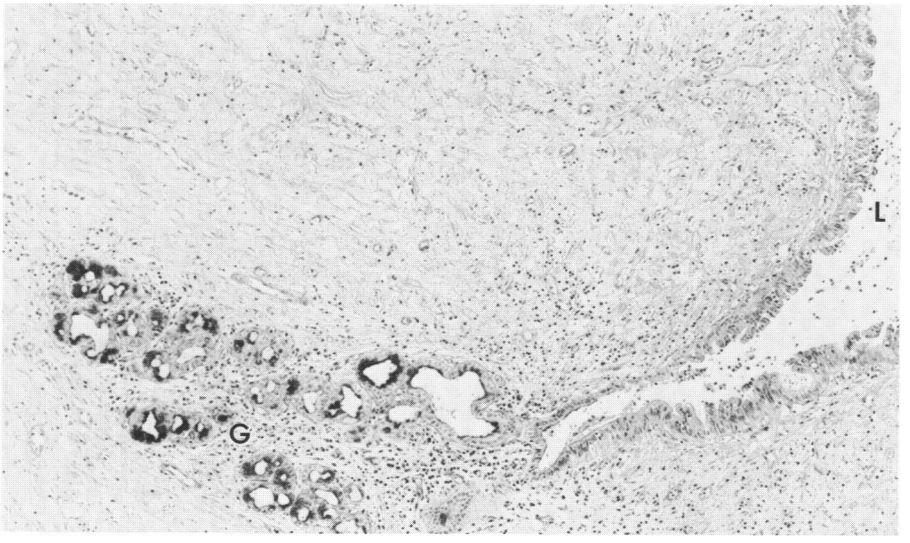


**Fig. 1.** Light micrograph of human bile duct (case 3) stained with PAS hematoxylin. A tubulo-aveolar gland (G) is opening into the lumen (L) of the duct. Numerous mononuclear cells are present in the periglandular zone (× 75).

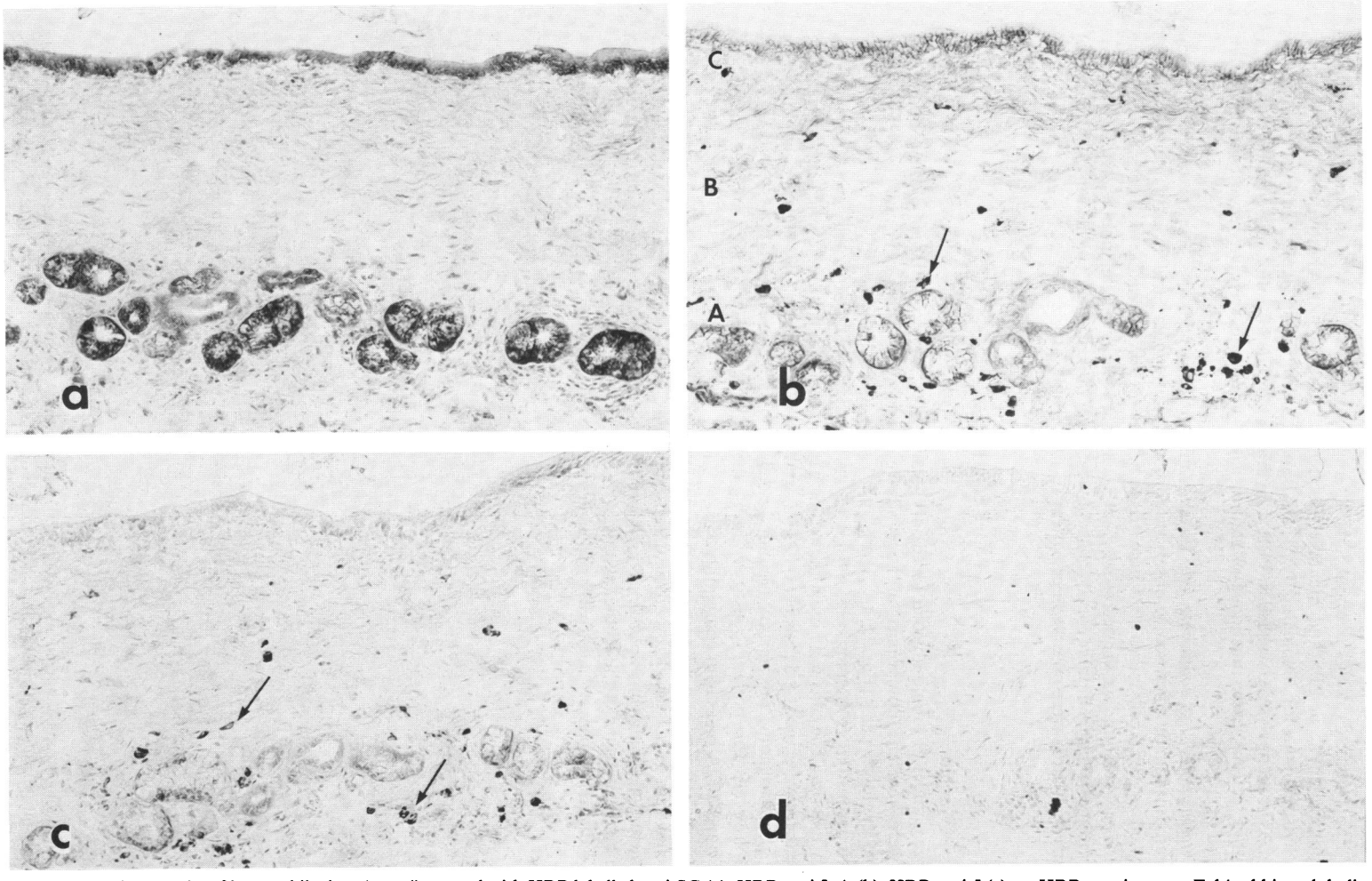


**Fig. 2.** Light micrographs of human bile duct (case 4) reacted with HRP labelled anti-SC (a), HRP-anti-IgA (b), HRP-anti-J (c), or HRP-non-immune Fab′ rabbit γ-globulin fragments (d). In (a), reaction products of HRP, indicating the sites of SC, are present in epithelial cells of both the duct surface and the accessory glands. IgA and J chain are present in cells with typical plasma cell morphology (→). IgA and J chain are present also in epithelial cells, but less prominently than is SC. In b, A designates the periglandular zone, C the zone beneath the surface epithelium, and B the zone between A and C (× 150).

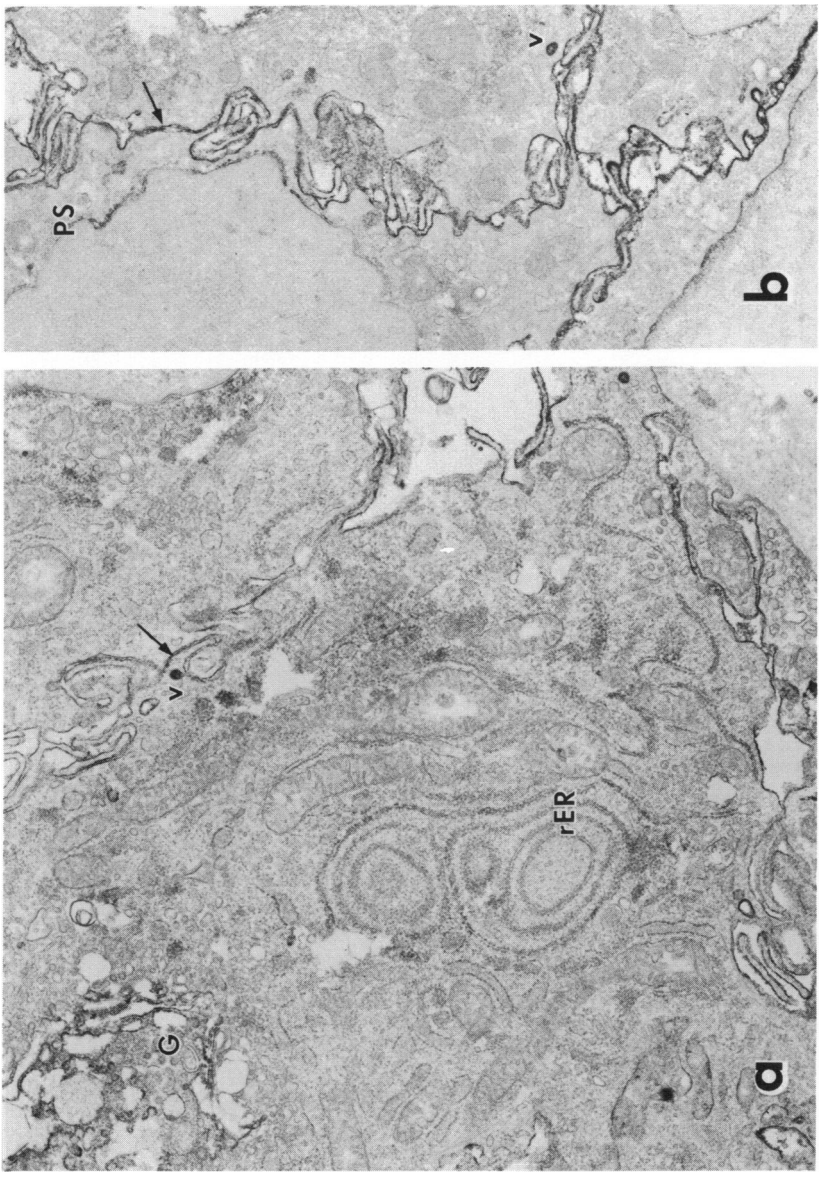


**Fig. 3.** Electron micrographs of epithelial cells of an accessory gland reacted with HRP-anti-SC. SC is present in the perinuclear spaces (PS), rough endoplasmic reticulum (rER). Golgi complexes (G), and endocytic vesicles (V). SC also is prominently present along the lateral plasma membrane (→) and faintly present along the basal plasma membrane (a, × 13.000; b, × 12.000).

*Electron microscopy*

Electron microscopy confirmed that most of the cells containing IgA and J chain were typical plasma cells. IgA and J chain were present in sites of protein synthesis, i.e. perinuclear spaces and rough endoplasmic reticulum, in the cells.

In epithelial cells of the accessory glands, SC was associated with the perinuclear spaces, rough endoplasmic reticulum, and Golgi complexes (Fig. 3). In addition, SC was present along the external surface of the basolateral plasma membrane to the level of the intercellular junctional complexes, in endocytic like invaginations of the membrane, and in vesicles in the apical cytoplasm.

Like SC, IgA was present along the basolateral surfaces of the epithelial cells of the accessory glands, in endocytic like invaginations of the membrane, and in vesicles of the apical cytoplasm

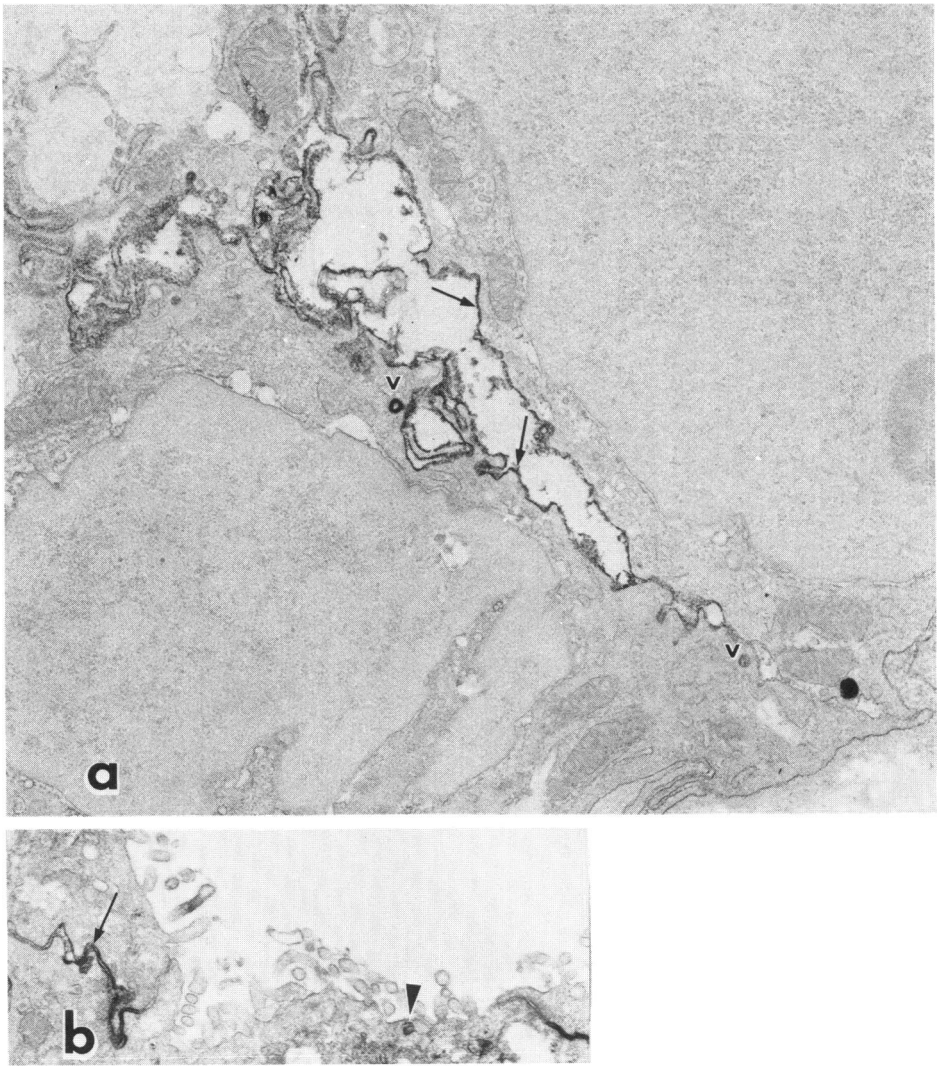


**Fig. 4.** Electron micrographs of epithelial cells of an accessory gland reacted with HRP-anti-IgA. IgA is deposited prominently along the lateral plasma membrane (→) to the level of the intercellular junction and less prominently along the basal plasma membrane. IgA is present also in endocytotic like vesicles (V) just inside the lateral plasma membrane and in vesicles (►) beneath the luminal surface of the cells (a, × 15,000; b, × 15,000).

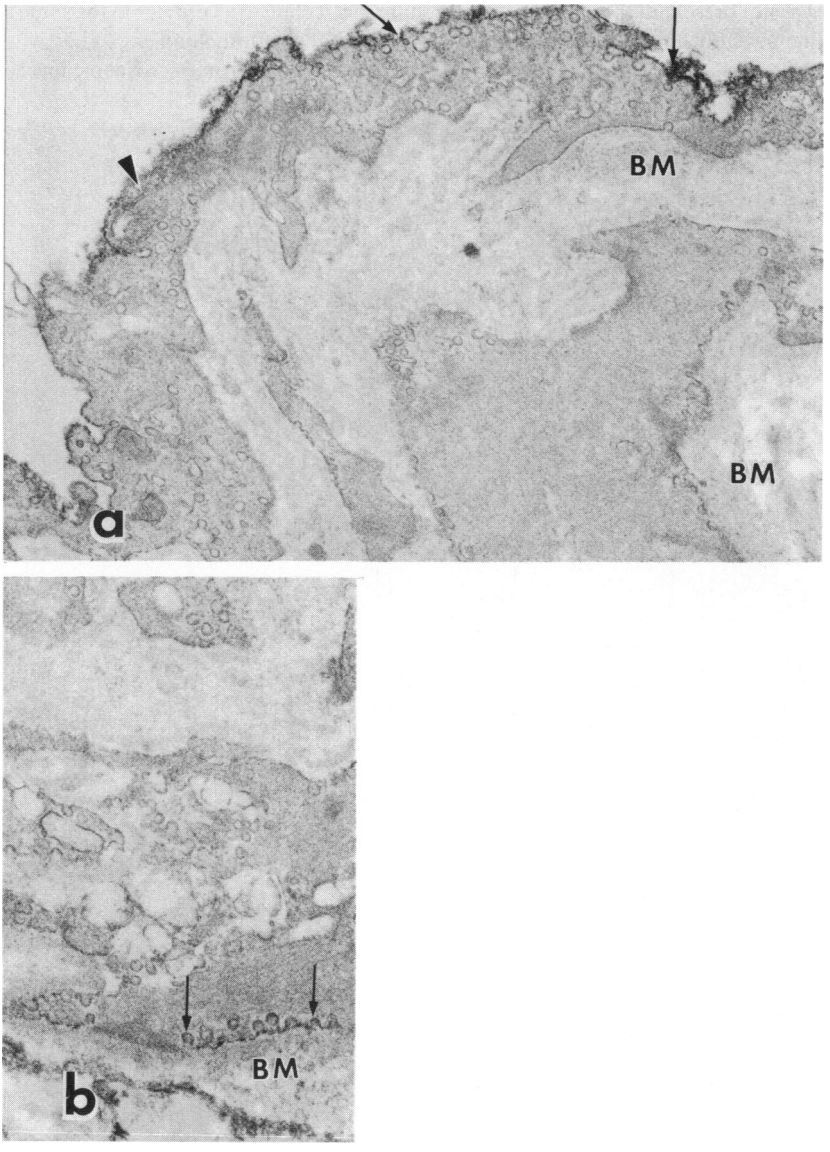


**Fig. 5.** Electron micrographs of a periglandular capillary reacted with HRP-anti-IgA. IgA is present in endocytic vesicles (→) of the endothelial cells (a) and smooth muscle cells (b), in spaces between adjoining endothelial cells (►), and on the luminal surface of endothelial cells. IgA is present also in the basement membranes (BM) (a, × 20,000; b, × 20,000).

(Fig. 4); IgA was absent from protein synthesizing organelles of these cells. Some reactivity for J chain along the basolateral plasma membrane was observed in acid–urea treated sections.

IgA was associated also with the endothelium of capillaries in the vicinity of the accessory glands (Fig. 5). The IgA was present along the lumenal and basolateral surfaces and in cytoplasmic vesicles of the endothelial cells; occasionally, it was present also in spaces between adjoining endothelial cells. SC was not present in the capillary endothelium, but J chain was present in the same locations as IgA (Fig. 6). Only a little IgM staining was present in the endothelium.

Control sections were negative for specific staining at the electron microscopic level.

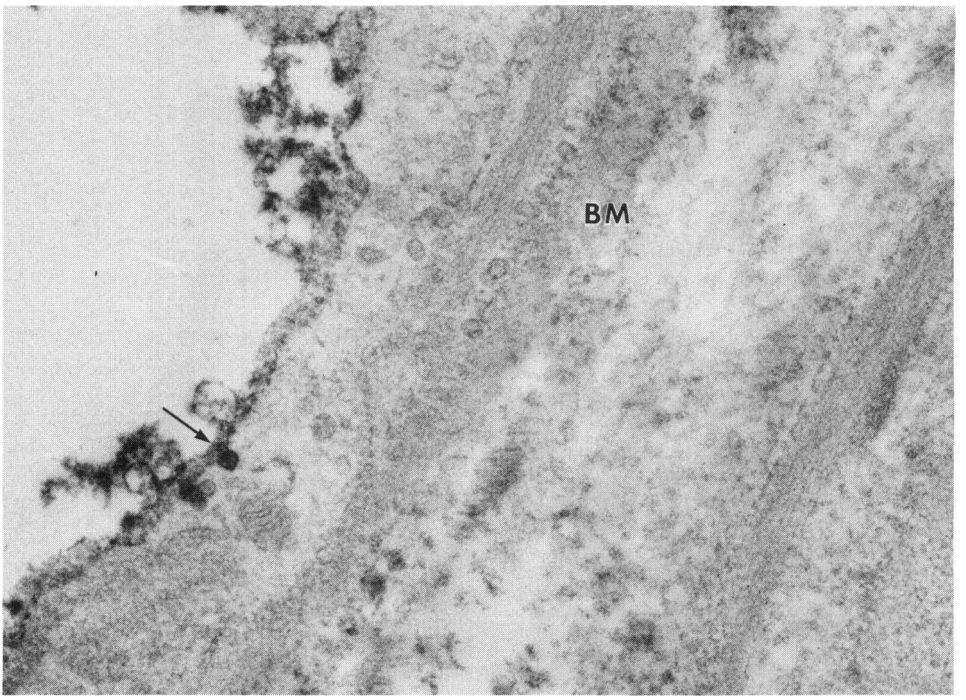


**Fig. 6.** Electron micrograph of a periglandular capillary reacted with HRP-anti-J after acid–urea treatment. J chain is present in endocytic vesicles of the endothelial cells (→), on the luminal surface of endothelial cells, and on the basement membrane (BM) ( × 25,000).

## DISCUSSION

The major observation made in this study was that IgA containing plasma cells are numerous in the extrahepatic biliary ducts of man. The cells are most plentiful in the vicinity of the accessory glands of the ducts.

Previously, we reported immunocytochemical evidence that translocation of IgA in the human liver occurs only across biliary epithelial cells (Nagura *et al.*, 1981); only those cells, not hepatocytes, contain both SC and IgA. However, we felt that the few IgA containing plasma cells present in the periportal connective tissue were inadequate to account for all of the IgA present in human hepatic bile (Nagura *et al.*, 1981; Delacroix *et al.*, 1982; Kutteh *et al.*, 1982). In one of the specimens we examined, extrahepatic bile ducts in the hilus of the liver were available, and many IgA containing cells were found in the bile duct wall. This finding prompted the present more extensive search of the biliary mucosa for IgA forming cells.

Indeed, a rich supply of IgA containing cells was found all along the major extrahepatic biliary ducts. The number of cells adjacent to the accessory glands appeared to be in the range of that in the intestinal lamina propria surrounding gland crypts (Brown, Isobe & Nakane, 1976), which is

perhaps the most densely populated IgA plasma cell containing mucosa in the body. Of course the amount of IgA secreted by plasma cells in the biliary mucosa was not quantitated. Nevertheless, it seems reasonable that such cells contribute a sizeable proportion of the IgA that is produced locally in human hepatobiliary tissues. Accessory glands in the biliary tracts are present all around the large intrahepatic and extrahepatic ducts (Elias & Sherrik, 1969; McMinn & Kagler, 1961). The glands have been reported to be the centres of cell division within the biliary epithelium (Cohen, 1964) and to be capable of secreting protein–carbohydrate complexes (McMinn & Kagler, 1961).

The findings of the present study are compatible with direct transfer of polymeric IgA from plasma cells in the biliary mucosa across the ductular epithelial cells by SC-mediated endocytosis. Such a mechanism has been defined in detail in intestinal epithelial cells (Brown *et al.*, 1976; Nagura, *et al.*, 1979a, Nagura, Nakane & Brown, 1979b; Brandtzaeg, 1974; Crago *et al.*, 1978) and appears to be operative in several other mucosae as well (Nakamura *et al.*, 1982; Goodman *et al.*, 1981). Evidence favouring this pathway for IgA secretion in the biliary mucosa includes the presence of J chain as well as IgA in plasma cells, SC on the basolateral plasma membrane of biliary epithelial cells, and SC as well as IgA associated with endocytic appearing vesicles in the biliary epithelial cells. These findings suggest that: first, IgA synthesized in the biliary mucosa is in polymeric form linked by the J chain, a molecular configuration that is necessary for efficient, non-covalent binding of IgA polymers to SC (Brandtzaeg, 1976a). Second, SC on the surface of epithelial cells in close proximity to the IgA synthesizing cells is well positioned to serve its role as a receptor for J chain containing IgA polymers (Nagura *et al.*, 1979a, 1979b).

We observed also that IgA is associated with capillaries that surround the accessory glands in the biliary ducts. The IgA was present in a pattern suggestive of transfer of IgA across or between the endothelial cells. Since J chain was found in the same locations as IgA, at least some of the IgA might be polymeric. Although association of the J chain with IgM cannot be excluded, little IgM was present in the endothelium. As we have suggested previously (Nagura *et al.*, 1981), the portion of biliary IgA that is derived by transfer from plasma might travel across the endothelium of capillaries surrounding biliary epithelium, thence across the biliary epithelial cells into bile. Transudation of IgA polymers from capillaries through intercellular spaces into bile also must be considered. Such an SC-independent pathway might account for the presence of the fraction of biliary polymeric IgA that is unassociated with SC (Delacroix *et al.*, 1982; Kutteh *et al.*, 1982; Nagura *et al.*, 1981).

Whether IgA in hepatic bile has important biological functions is yet uncertain. Nevertheless, the presence of IgA synthesizing cells in the biliary mucosa suggests that IgA antibodies produced at that site participate in immunological protection of the biliary tract.

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