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Gall bladder: the predominant source of bile IgA in man?

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SUMMARY

The sedimentation profiles of IgA and Secretory Component (SC) and the concentrations of IgA, IgG, IgM, SC and albumin were evaluated after an overnight fast in gall bladder bile of six adult subjects without hepatobiliary disease. The sedimentation profiles differed from those previously obtained in hepatic bile in three ways: gall bladder bile contained a greater percentage of free-SC, a greater percentage of polymeric-IgA (p-IgA), and a major peak of 14 to 19 S p-IgA associated to SC. In contrast to hepatic bile in which IgG is the predominant Ig, IgA clearly was the predominant Ig in gall-bladder bile, its concentration averaging 92 μg/ml. Relative-to-albumin coefficients of excretion of proteins in gall bladder bile averaged 0·99 for IgG, 8·6 for monomeric IgA, 196 for p-IgA and 31 for IgM, indicating that there was a selective excretion of IgA and IgM into gall bladder bile. As compared to hepatic bile, the enrichment of gall bladder bile with IgA and IgM was respectively 6·5 and 11·5 times greater than with IgG. These results suggest that quite a significant amount of p-IgA could have been added to bile during its storage in the gall bladder which should therefore be regarded as the predominant source of bile IgA in humans.

Keywords Immunoglobulin A gall bladder bile secretory component

INTRODUCTION

Some animal experimental models (Hall & Andrew, 1980; Vaerman *et al.*, 1982) clearly exhibit bile as the predominant source of immunogloublin A for the upper intestine. In rats (Jackson *et al.*, 1978; Orlans *et al.*, 1979) and rabbits (Delacroix *et al.*, 1982), the daily amount of polymeric IgA (p-IgA) transported from plasma to bile via the hepatocytes and hence delivered to the duodenum, averages 35 mg/kg/day (Delacroix *et al.*, 1983b). When this pathway is interrupted, IgA concentration in duodenal washings drops to about 10% of its value in control animals (Lemaître-Coelho *et al.*, 1978) while IgA rapidly accumulates in the blood circulation (Lemaître-Coelho *et al.*, 1978a). The transport mechanism of plasma p-IgA depends on the presence of secretroy component (SC) located at the sinusoidal surface of hepatocytes (Orlans *et al.*, 1979; Renston *et al.*, 1980; Soeken *et al.*, 1979) where this glycoprotein corresponds to the extramembranous domain of the sacrificial receptor (Kühn & Kraehenbuhl, 1982; Mostov & Blobel, 1982; Mostov, Friedlander & Blobel, 1984) which is reponsible for the active endocytotic translocation of IgA into the bile canaliculi (Renston *et al.*, 1980).

The hepatobiliary transport of circulating p-IgA is, however, much less important in humans

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than in rats and rabbits (Delacroix *et al.*, 1982b, 1983a,b; Dooley *et al.*, 1982). In humans, the amount of hepatic bile p-IgA delivered to the intestine averages less than 1 mg/kg/day (Delacroix *et al.*, 1983b) and interruption of the bile flow does not lead to significant elevations of the levels of p-IgA in plasma (Delacroix *et al.*, 1982b, 1983a). Differences between various species were also observed when rats were compared to dogs (Delacroix *et al.*, 1983b), guinea pigs (Hall, Gyure & Payne, 1980; Delacroix *et al.*, 1984b) or humans (Nagura *et al.*, 1981; Shandy *et al.*, 1983; Delacroix *et al.*, 1984b). In these three species as opposed to rats, neither the surface and the cytoplasm of hepatocytes nor the bile canaliculi carry any detectable amounts of SC although the presence of the receptor has been clearly demonstrated in/on the cells of the biliary epithelium (Nagura *et al.*, 1981; Dalacroix *et al.*, 1984a,b).

In the past, all the studies devoted to IgA in human bile were performed using bile collected in cholecystectomised individuals or in the upper common bile duct of normal individuals (Dive & Heremans, 1974; Kutteh *et al.*, 1982; Delacroix *et al.*, 1982b). Gall bladder bile is highly viscous, and difficult to handle and, except in one case (Chordirker & Tomasi, 1963), it has not been studied although it was likely that hepatic bile became enriched with IgA while stored in the gall bladder. Indeed, as an integral part of the biliary tree, the gall bladder is covered by biliary epithelial cells expressing SC (Tourville *et al.*, 1969), themselves being surrounded by plasma cells containing predominantly IgA (55%) and IgM (32%) (Green & Fox, 1972) in the same manner as observed in most mucosas (Crabbé *et al.*, 1970; Brandtzaeg, 1981). So far, the quantitative enrichment of human gall bladder bile with IgA has not been quantitated: therefore we designed the present study to address this question. The concentrations of monomeric (m) and p-IgA as well as other plasma proteins were measured in gall bladder bile obtained from six individuals after a fasting night. The immunoassays, standards and techniques used were the same as previously described in the study of hepatic bile (Delacroix *et al.*, 1982b) allowing us to compare as accurately as possible the data for gall bladder bile to those previously obtained for hepatic bile.

MATERIALS AND METHODS

Subjects and samples. The total bile content of the gall bladder was collected by direct puncture of the gall bladder after a fasting night at the beginning of a surgical operation for a disease of the upper digestive tract in six men without hepatic or biliary disease. Conditions of the study and number of patients selected for the study were determined by the Ethical Committee of the University Hospital. The type of operation, ages of the patients and histological features of the liver obtained by surgical biopsy are shown in Table 1. Prothrombin time, serum Alkaline Phosphatases, Alanine Amino Transferase, total and conjugated bilirubin were normal in all the patients. Serum samples and bile samples were obtained simultaneously.

Measurements of protein concentration. Serum and bile samples were frozen at −20°C immediately after collection. Bile samples were checked for absence of blood contamination by the

Table 1. Clinical data and liver histology in the 6 men under study

Patient No.	Diagnosis	Surgical operation	Liver histology
1	Gastric Peptic Ulcer Gastro- esophageal reflux	Vagotomy + antrectomy + Nissen fundoplication	Minimal steatosis
2	Adenocarcinoma of the cardia	Esophagogastrectomy	Minimal steatosis
3	Achalasia of the cardia	Esophagomyotomy	Normal liver
4	Adenocarcinoma of the cardia	Subtotal gastrectomy + Polya jejunogastrostomy	Discrete mononuclear infiltration in some portal spaces
5	Duodenal peptic ulcer	Supra-selective vagotomy Nissen fundoplication	Normal liver
6	Esophageal reflux	Nissen fundoplication	Minimal steatosis

benzidine test. Serum Albumin, IgG, IgA and IgM concentrations were assayed by immuno-nephelometry (Ritchie *et al.*, 1973). The same proteins and SC in the bile samples were measured by immuno-radiometric assay (IRMA) as described previously in details (Delacroix, Dehennin & Vaerman, 1982a; Delacroix *et al.*, 1982b). Both serum and bile (m-IgA) and p-IgA concentrations as well as bile free- and IgA bound-SC concentrations were measured separately by ultracentrifuging all the samples for 16 h in 5–21% isokinetic sucrose density gradient run in a Beckman SW41 T1 rotor (190,000 *g* in the gradient bottom). Both IgA and SC concentrations in the 30 fractions eluted from the gradients were measured by IRMA and the proportion of m- and p-IgA or free- and IgA bound-SC were determined by planimetry, carefully taking into account the influence of the size of IgA in its immunoassays (Delacroix, Dehennin & Vaerman, 1982a; Delacroix *et al.*, 1982b).

Mathematical expression of the results. All the results are presented as compared to those previously obtained under the same conditions for hepatic bile. Data are expressed in absolute protein concentration or in relative (to albumin) coefficient of excretion (RCE).

$$\text{RCE} = \frac{[\text{Protein}]^{\text{bile}}}{[\text{Protein}]^{\text{serum}}} \times \frac{[\text{Albumin}]^{\text{serum}}}{[\text{Albumin}]^{\text{bile}}}$$

This coefficient (Dive & Heremans, 1974; Delacroix *et al.*, 1982b) expresses the secretion rate of a protein by reference to albumin, thus eliminating the influence on the results of (a) the plasma protein concentration, (b) the degree of dilution or concentration of the secretion, (c) the presence of a spontaneous protein precipitation phenomenon during storage. Therefore, it allows us to compare the parameters of secretion of proteins into gall bladder bile to those in hepatic bile of either humans or other species.

Statistical comparisons were made using the non-parametric Mann and Whitney test.

RESULTS

Molecular distribution of IgA and SC in gall bladder bile

As illustrated by Fig. 1, for one individual the sedimentation profile of IgA and SC in gall bladder bile resolved into (a) a 4·5 S peak of free SC (30–60% of total SC), (b) a 7 S Peak of monomeric IgA, (c) a poorly defined 10·2 S peak of serum-type dimeric IgA uncomplexed to SC, and (d) a >11 S peak of polymeric IgA (62–86%, mean 77% of total IgA) sedimenting with SC.

These profiles were roughly similar to those previously obtained in hepatic bile (Delacroix *et al.*, 1982b; Kutteh *et al.*, 1982) but nevertheless differed by three points. Gall bladder bile contained a greater percentage of free SC ($P<0·05$), a greater percentage of p-IgA ($P<0·05$), and its peak of p-IgA associated to SC also comprised a greater proportion of p-IgA sedimenting in the 14 to 19 S position corresponding either to tetramers and pentamers or to p-IgA complexed to lipoproteins (Nalbone *et al.*, 1979; Vigne *et al.*, 1981).

Protein concentrations in gall bladder bile

Spontaneous precipitation of mucin was always observed when bile samples were thawed prior to the assays (performed on the supernatant). The concentration of albumin, IgG, m-IgA, p-IgA, IgM and SC in serum and gall bladder bile are listed in Table 2 together with the results previously obtained with the same assays and standards in hepatic bile. As can be seen, the concentrations of albumin and IgG in gall bladder bile were markedly lower than in hepatic bile (13% of the hepatic bile value) whereas those of the other Ig and SC were similar (m-IgA) or even greater in gall bladder bile (p-IgA, 135%; IgM, 230%; SC, 283% of the mean value in hepatic bile). In contrast to human hepatic bile in which IgG was the predominant Ig, IgA clearly was the predominant Ig in gall bladder bile. These results did not reflect a more important coprecipitation of albumin than IgA or SC with mucins in the gall bladder bile. In fact, measurement of individual proteins released from mucin precipitates (three samples) after a 48 h incubation, with continuous shaking, in IRMA dilution buffer revealed that, relative to albumin, IgA and SC concentrations were respectively 2·4 and 1·6 times greater in the precipitate than the supernatant.

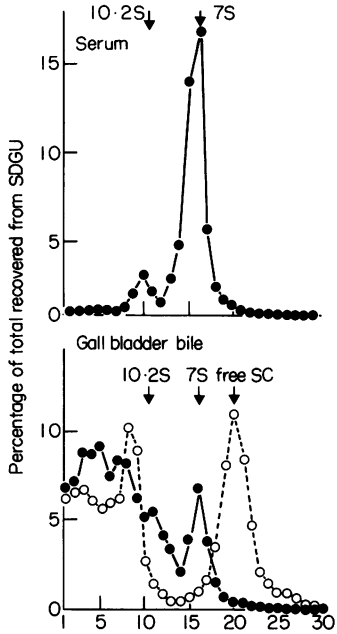

Fig. 1. Simultaneous Sucrose Density Gradient Ultracentrifugation (SDGU) of serum and gall bladder bile of one patient. Concentrations of IgA and SC were measured by IRMA; values were corrected for size influence of polymers on IRMA. Immunoglobulin A (●); Secretory component (○).

Table 2. Protein concentrations (μg/ml) in serum and gall bladder bile as compared to hepatic bile

	Albumin (μg/ml)	IgG (μg/ml)	m-IgA (μg/ml)	p-IgA (μg/ml)	IgM (μg/ml)	Secretory component* (moles × 10^{-5}/ml)
Serum	36,875†	8,734	1,544	255	1,107	2·2
($n=6$)	(32,340–42,840)‡	(10,920–7,600)	(1,010–3,200)	(190–500)	(950–1500)	(1–4·1)
Gall-bladder	52	12	19	73	46	34·0
Bile ($n=6$)	(18–235)	(5–23)	(7–83)	(24–168)	(12–112)	(16–69)
Hepatic bile§	405	89	23	54	20	12·1
($n=17$)	(155–1,485)	(32–480)	(12–67)	(25–146)	(2–60)	(4–44)

* Secretory component includes free and/or Ig bound SC.
† Antilog of the mean of log values.
‡ Range.
§ Values previously obtained with the same assays and standards (Delacroix *et al.* 1982b).

Relative (to albumin) coefficients of excretion (RCE) of proteins in gall bladder bile

Mean RCE of the different proteins in gall bladder bile as compared to those of hepatic bile are shown on Fig. 2. Interindividual differences in plasma protein concentrations may affect the bile protein concentrations, simply by plasma seepage. RCE are relative to albumin and correct the data for these differences. Using this expression of the results, comparison between gall bladder and hepatic bile showed that the former was enriched with the four Ig studied. The enrichment of gall bladder bile was small for IgG (RCE increased from 0·7 to 0·99). The enrichment with m-IgA (RCE increased from 1·28 to 8·6) was five times greater than with IgG although both immunoglobulins have the same molecular weight. For p-IgA (RCE = 196) and IgM (RCE = 31), RCE values in gall

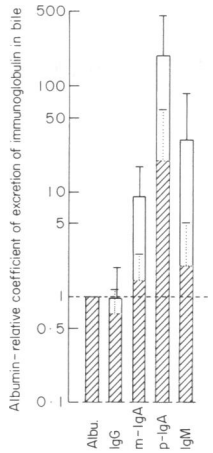

Fig. 2. Relative-to-albumin coefficients of excretion (RCE; mean + s.d.) of immunoglobulins in gall bladder and hepatic bile. The horizontal dashed line represents the RCE of albumin (albu), taken as unit. Gall bladder bile (□); hepatic bile (■).

bladder bile were markedly increased as compared to those in hepatic bile (RCE = 22 and 1·9, respectively) indicating that the enrichment of the gall bladder bile with these two polymeric Ig was respectively 6·5, and 11·5 times greater than with IgG.

DISCUSSION

The present study allows us to further characterize the parameters of secretion of IgA in human bile and highlights two poorly known aspects of this still controversial question. On one hand, the concentrations of plasma proteins in gall bladder bile were markedly different from those previously measured in the same conditions in hepatic bile (Delacroix *et al.*, 1982b). The concentrations of albumin and IgG were eight times lower whereas those of m-IgA appeared to be similar in both fluids, and those of p-IgA, IgM and especially SC were increased by a factor of 1·3 to 2·8. Using the RCE, we showed that, regardless of the absolute protein concentrations in bile which may have been decreased following the spontaneous coprecipitation of proteins and mucins in the gall bladder, m-IgA, p-IgA and IgM in gall bladder bile were much more selectively secreted relative to albumin or IgG than previously observed in hepatic bile. In fact, the RCE presently calculated for m-IgA, p-IgA and IgM in gall bladder bile were quite similar to those previously recorded for the same Ig in external secretions derived from mucosal or glandular organs populated by a large number of IgA and IgM plasma cells and covered by an epithelium expressing SC at its basolateral surface (jejunal wall, salivary gland, breast and lacrymal glands) (Delacroix & Vaerman, 1983).

Two mechanisms can be proposed to account for the present observations either the existence of (a) a selective resorption of small molecular weight bile proteins such as albumin and IgG during storage of bile in the gall bladder or (b) a local production of IgA and IgM within the gall bladder wall, followed by their passive or active transport to the lumen via the epithelium. In the second mechanism, despite the known phenomenon of water resorption in the gall bladder and the local production of Ig, all the concentrations of plasma proteins in gall bladder bile would apparently be low due to the coprecipitation of proteins and mucins during storage of the viscous bile. This phenomenon of coprecipitation well known for saliva, appeared to be macroscopically major for gall bladder bile.

The first hypothesis i.e. a molecular size dependent resorption of proteins during storage of bile in the gall bladder was proposed by John, Mullock & Hinton (1983). While studying guinea-pig bile, these authors also found that the concentration of albumin in gall bladder as compared to hepatic fluid was lowered by a factor of 10 fold whereas the concentrations of haptoglobin and p-IgA were

only five times lower or equal respectively. The present study does not favour the hypothesis of John *et al.* (1983). In comparing the parameters of secretion of m-IgA and IgG in gall bladder versus hepatic bile we found an enrichment of gall bladder bile with m-IgA relative to albumin, approximately five times greater than with IgG. Since both Ig have the same molecular size and should be resorbed at a similar extent, the difference observed between IgG and m-IgA and the general agreement on the fact that the proteins are not resorbed during bile storage in the gall bladder (Davenport, 1973; Wheeler, 1975) indicate that m-IgA is added to the bile while stored in the gall bladder. This hypothesis agrees with the previous report of Green & Fox (1972) on the distribution of Ig containing cells in the normal and inflamed human gall bladder. These authors have shown that 3,000 to 22,000 IgA containing cells are present per mm^3 of normal to moderately inflamed gall bladder mucosa with an IgA/IgM ratio of 1·7/1 to 4·6/1 and an IgA/IgG ratio of 4·3/1 to 7·4/1. This pattern of distribution, close to that observed in the intestinal lamina propria (Crabbe *et al.*, 1970), supports the concept that m-IgA produced locally in the gall bladder wall and passively transported to the lumen will progressively enrich the hepatic bile during its storage in the gall bladder; this enrichment increases in case of long lasting storage such as overnight storage. Polymeric-IgA on the other hand would not only be produced locally in the gall bladder wall but also actively transported across epithelial cells by a SC-dependent transepithelial transport mechanism. Two lines of evidence support the reality of such a mechanism. First, SC has been observed in epithelial cells of the gall bladder in both humans (Tourville *et al.*, 1969) and guinea pigs (Lemaître-Coelho, Naccache Corbic & Vaerman, 1978b) two species in which SC staining among hepatobiliary tissues is restricted to the biliary epithelium (Delacroix *et al.*, 1983b). Evidence that biliary epithelial cells synthesize SC and transport p-IgA associated to SC in cytoplasmic vesicles has been provided by Nagura *et al.* (1981). Second, the greatest protein enrichment presently observed in the gall bladder bile compared to hepatic bile was found for SC. The concentration of this glycoprotein was increased by three times while that of albumin was decreased by eight times. In addition, the proportion of SC found in free form in gall bladder versus hepatic bile was also increased by 1·5 times allowing us to calculate a 36-fold enrichment of gall bladder bile with free SC relative to the smaller-in-size albumin, thus arguing against a phenomenon of selective resorption of small proteins. This enrichment of gall bladder bile with free SC indirectly demonstrates that the receptor of p-IgA is indeed synthetised by gall bladder epithelial cells. There was also an important enrichment of the gall bladder bile in IgM. This Ig is probably also produced in significant amounts in the gall bladder wall and like p-IgA actively transported by SC. IgM containing cells in the gall bladder wall not only account for 33% of mucosal Ig containing cells but are also present in substantial number in the gall bladder muscle coat where they represent the predominant type of Ig containing cells (Green & Fox, 1972).

Taken together, the results of the present study indicated that a molecular size-affected resorption of plasma proteins from gall bladder bile is unlikely to occur and that the real concentrations of plasma proteins in gall bladder bile are underestimated due to their coprecipitation with mucins during storage. The analysis of proteins released from the precipitate indicated that coprecipitation of albumin was lower than that of IgA and SC. Therefore, the present results demonstrate that while stored in the gall bladder, hepatic bile becomes enriched with p-IgA, most likely produced locally under the biliary epithelium and transported in association with SC. The factor of enrichment averages 6·5 or 10 depending on the protein used as reference, either albumin or IgG. In their study on human hepatic bile, Delacroix *et al.* (1982b) calculated that 0·8 mg/kg/day of p-IgA are delivered from bile to the intestine, 0·4 mg/kg/day being derived from plasma, another 0·4 mg/kg/day being derived from local synthesis by plasma cells surrounding the biliary tree (Nagura *et al.*, 1981). The present study suggests that 5 mg/kg/day of p-IgA would be added to bile during storage in the gall bladder which should therefore be regarded as the predominant source of human bile IgA, balancing to some extent the small rate of plasma to bile transport of p-IgA due to the lack of SC-expression by human hepatocytes.

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