Selective Transport of Polymeric Immunoglobulin A in Bile

QUANTITATIVE RELATIONSHIPS OF MONOMERIC AND POLYMERIC IMMUNOGLOBULIN A, IMMUNOGLOBULIN M, AND OTHER PROTEINS IN SERUM, BILE, AND SALIVA

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ABSTRACT In 17 adults, serum, hepatic bile, and saliva samples were analyzed for their sedimentation profile of IgA and secretory component (SC), and for their concentrations of albumin, orosomucoid, transferrin, IgG, IgA, α_2-macroglobulin (α_2M), IgM, and SC. Polymeric IgA(p-IgA) averaged 13% (50–700 μg/ml) of total IgA in serum, 70% (43–88%) in bile, and 93% (74–98%) in saliva. Most of the p-IgA in bile sedimented with SC, which also occurred free (8–44%), and with IgM. In bile, albumin (155–1,485 μg/ml) was the predominant protein, followed by IgG (32–480 μg/ml), and total IgA (37–209 μg/ml). In saliva, p-IgA (72–902 μg/ml) predominated, followed by albumin (16–385 μg/ml) and IgG (9–178 μg/ml). Secretion-to-serum albumin-relative concentration ratios (S/S-ARCR = 1 for albumin) in bile averaged 22 for p-IgA, 1.91 for IgM, 1.28 for monomeric IgA (m-IgA), 0.70 for IgG, and 0.57 for α_2M, indicating for p-IgA, IgM, and to a lesser extent for m-IgA, a selective excretion into bile. In saliva, a 16-fold greater selective excretion of p-IgA (mean S/S-ARCR = 354) was found. Labeled m- and p-IgA were injected intravenously into five patients. Specific activities indicated that for p-IgA 50% was serum derived in bile, as compared with 2% in saliva, and to 85% for m-IgA in bile. In the patient with the highest excretion of ^{125}I-p-IgA in bile, only 2.8% of the injected dose was recovered in bile within 24 h after injection. Compared with rats and rabbits, the serum-to-bile transport of p-IgA in humans is much smaller.

INTRODUCTION

Transepithelial transfer of polymeric J chain-containing IgA and IgM into secretions is well documented in man and animals. At several mucosal sites secretory component (SC)[1], an epithelial cell surface glycoprotein, acts as a specific receptor and initiates an endocytotic vesicular transport to lumen of polymeric IgA (p-IgA) and IgM synthesized by submucosal plasmacytes (1–10). Recently, it has been shown that p-IgA is also actively transported from serum into bile by the hepatocyte of the rat and other mammals (11–15). Suggested roles for this transport are reinforcement of intestinal immunity (16, 17) and clearance of p-IgA antibodies and immune complexes from blood (18–21).

In man, the concentration, source, and significance of biliary IgA are controversial. The presence of IgA was first demonstrated in gallbladder bile (22, 23). In hepatic bile, Dive and Heremans (24, 25) measured secretion-to-serum concentration ratios for several proteins and showed their passive, molecular weight-dependent transport from serum. For IgA, found at

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Received for publication 8 October 1981 and in revised form 15 February 1982.

[1] *Abbreviations used in this paper*: Alb, albumin; CS, coefficient of sedimentation; d-IgA, dimeric IgA; FSC, free SC; IN, immunonephelometry; IRMA, immunoradiometric assay; m-IgA, monomeric IgA; α_2M, α_2-macroglobulin; PBS, phosphate-buffered saline; p-IgA, polymeric IgA; SC, secretory component; SDGU, sucrose density gradient ultracentrifugation; sIgA, secretory IgA; S/S-ARCR, secretion-to-serum albumin-relative concentration ratio; S/S-SAR, secretion-to-serum specific activity ratio.

similar concentrations as IgG, they suggested the existence of an additional local origin. Recently, Nagura et al. (26) also measured the concentration of IgG, IgA, and IgM in hepatic bile, but not in serum, and found predominantly 11S sIgA in bile with virtually undetectable IgG and IgM. They presented elegant immunohistochemical evidence for a SC-mediated endocytotic transport of p-IgA across the biliary epithelium, and like others (27), could not demonstrate SC on and in hepatocytes, despite positive results of another group (28).

In the present article, we measured the concentrations of eight serum proteins in hepatic bile, saliva, and serum, considering separately monomeric (m-IgA) and p-IgA. To evaluate the selectivity of excretions of IgA or of other proteins in secretions, all protein concentrations in bile and saliva were related to their corresponding serum concentrations. However, secretions display large variations in protein concentrations both between different individuals and, in the same individual, between different secretions. Thus, to allow quantitative comparisons between different individuals and between bile and saliva, the individual secretion-to-serum concentration ratios for each protein were "normalized" by expressing results relatively to albumin (Alb), which, in bile (24, 29), and saliva (25, 30), is entirely derived from serum. Thus, results were expressed as Alb-relative secretion-to-serum concentration ratios (S/S-ARCR).

$$\text{S/S-ARCR} = \frac{\text{(secretion-to-serum concentration ratio) protein}}{\text{(secretion-to-serum concentration ratio) Alb}}.$$

In addition, the proportion of the selective excretion of IgA in bile and saliva, which derived from a serum-to-secretion transport, was quantitated in five patients after intravenous injection of polyclonal ^{125}I-p-IgA and/or ^{131}I-m-IgA by calculation of their secretion-to-serum specific activity ratios (S/S-SAR).

METHODS

Patients and samples. Bile was collected, after overnight fasting, from the upper common bile duct of 17 patients: (*a*) in 7 patients, during endoscopic cholangiography for investigation of cryptogenic abdominal pain; (*b*) in 9 patients, with T or cystic tube biliary drainage at 7–12 d after cholecystectomy; and (*c*) in 1 patient with 21 d of total biliary drainage (transhepatic catheter) before surgery for complete tumor obstruction of the common bile duct. The latter was demonstrated by injection of contrast agent in the upper duct, before collection of bile, and at the time of surgery.

Serum samples in all, and unstimulated whole mixed saliva in 14 of the 17 patients were obtained simultaneously.

Routine liver function tests were normal in all patients except for a slightly elevated (2.2 mg/100 ml) level of bilirubin in the patient with total biliary drainage, who had no liver metastases demonstrated by angiography and laparotomy.

Measurement of protein concentrations. Serum, bile, and saliva samples were frozen at −20°C immediately after collection. Bile samples were checked for blood by the benzidine test. Alb, orosomucoid, transferrin, IgG, IgA, α_2-macroglobulin (α_2M), IgM, and SC concentrations were measured by immunonephelometry (IN) (31) in serum, except for α_2M, orosomucoid, and SC which, like all proteins in secretions, were measured by immunoradiometric assays (IRMA), all performed as described for IgA (32). For each assay, seven standards were obtained from a pool of 1,000 blood donors, also used for IN. Standard ranges used, with coefficients of variation <8%, were, respectively, 3–100 ng/ml (Alb), 2–60 ng/ml (orosomucoid), 4–200 ng/ml (transferrin), 3–80 ng/ml (IgG), 4–100 ng/ml (IgA), 5–500 ng/ml (α_2M), 3–140 ng/ml (IgM), and 10–400 ng/ml (SC). Purified 11S sIgA (33) was used as standard in the SC assay, which measured almost equally (molar basis) both free SC (FSC) and immunoglobulin-bound SC. The specificity of each IRMA was demonstrated with purified monoclonal immunoglobulins, or fractions of bile and serum after chromatography on Ultrogel AcA 22 (LKB Products, Bromma, Sweden). All samples were assayed at five serial twofold dilutions in 20% horse or goat serum in phosphate-buffered saline, pH 7.4, containing 1 g/liter NaN_3 (PBS), each in duplicate, and results within the standard range were always closely parallel to the standard curves.

Sucrose density gradient ultracentrifugation (SDGU)

All samples were first passed through a Millipore filter (Millipore Corp., Bedford, MA) of 0.22 μm. For each patient the serum (3 μl) and secretions (50 μl), diluted to 0.3 ml in PBS, were layered onto isokinetic (34) sucrose gradients (12 ml of 5–21% sucrose in PBS) and ultracentrifuged together for 16 h at 34,000 rpm (bottom rate centrifugal force, 195,000 *g*) or at 38,000 rpm in one case. A modified ISCO (Instrumentation Specialties Company, Lincoln, NE) gradient-former, model 570, was used to simultaneously form six sucrose gradients, which were ultracentrifuged in a swinging bucket SW-41 Ti rotor using a Beckman Spinco L2-65B ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). Gradients were eluted from the bottom of the tubes in 31 fractions of 0.4 ml in a modified ISCO gradient-fractionator, model 640, with continuous monitoring of absorption at 280 nm. IgM and SC for secretions, and IgA for all samples, were measured by IRMA in each fraction diluted at least 10 times in PBS with 20% horse serum. The influence of sucrose concentration on IRMA was shown to be insignificant at such dilutions. In isotopic studies, the concentrated secretions (0.3 ml) were ultracentrifuged together with serum and aliquots of the injected labeled m- and d-IgA mixed with cold 7S and 10.5S markers (monoclonal IgA) (35). The radioactivity was counted in each fraction for 10 min. The 7S position was located by the optical density peaks of the serum IgG or of the cold IgA marker. The other coefficients of sedimentation (CS) were derived from the $\omega^2 t$ conditions of the run. The percentages of m- and p-IgA or of free and bound SC were measured by planimetry.

Corrections for influence of size heterogeneity in IgA assays. Highly purified (35) polyclonal m- and d-IgA, milk sIgA and m-, d- and tetrameric monoclonal IgA of two different patients with myeloma, were used to compare the behaviour of polymers and monomers in IRMA and IN. When monomers were used as standard, correction factors

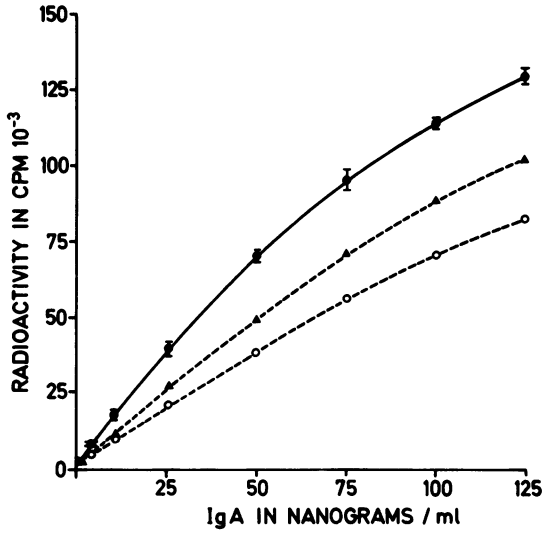

FIGURE 1 IRMA of polyclonal m- (●), d- (▲), and sIgA (○).

for polymer results (concentration measured by optical density divided by the immunoassay-measured concentration) in IRMA (32) were 1.5 for d-IgA, and 2.0 for sIgA (Fig. 1). In IN (36), they were 1.0 for d-IgA, and 1.2 for sIgA. The reproducibility of these factors by IRMA (32), and the similarity between results obtained on serum by IRMA (32) and IN (36) was demonstrated. In the present study, all polymers measured by IRMA in bile were multiplied by a mean factor of 2.0, and polymers measured by IRMA in SDGU-fractions of serum by a factor of 1.5 for the 10.5S peak or 2.0 for the 12.5S peak.

Labeled polyclonal m- and d-IgA. Polyclonal m- and d-IgA, prepared from serum of a patient with alcoholic cirrhosis (35), were labeled with ^{131}I and ^{125}I, respectively. Our criteria of purity and biological activity of the m- and d-IgA were as follows: (*a*) absence of precipitation when gel diffused, at 8 mg/ml, against 16 different monospecific antisera against non-IgA serum proteins (35); (*b*) single IgA precipitin line upon immunoelectrophoresis developed with antiwhole-human-serum (35); (*c*) CS of 10.5S for >95% of d-IgA and of 7S for >95% of m-IgA, by SDGU (35); (*d*) in vitro binding of ^{125}I-labeled FSC to the d-, but not the m-IgA (37); (*e*) release of free J chain after reduction-alkylation (38) of the d-, but not of the m-IgA; and (*f*) active biliary transfer of the d-IgA (>45% of the intravenously injected dose in 3 h) into rats (n = 4) (39) and rabbits (40) (n = 2).

Radioiodination was performed with chloramine T (41). Free label was separated by gel filtration on disposable Sephadex G-50 columns, prewashed with sterile human serum albumin in PBS. The labeled proteins were passed through a Millipore (0.22 μm) filter also prewashed with human serum albumin. Pyrogenicity of the labeled proteins was tested by injecting rabbits intravenously with 10 times the doses injected into humans. The yield of labeling varied between 48 and 67%, and <4% of the radioactivity was soluble in TCA (100 g/liter).

Isotopic studies

Isotopic studies were performed in four of the patients with cystic or T tube drainage (patients 2–5) and in the patient 1 with total biliary drainage. Patients gave informed consent and the protocol was approved by the Ethical Committees of the Cliniques Universitaires St-Luc, Brussels, and the Royal Postgraduate Medical School, London. Labeled samples (3–10 μCi of each ^{125}I-d-IgA and/or ^{131}I-m-IgA) were injected intravenously within 24 h after labeling. The specific activity of m- and d-IgA in secretions and serum was calculated as follows:

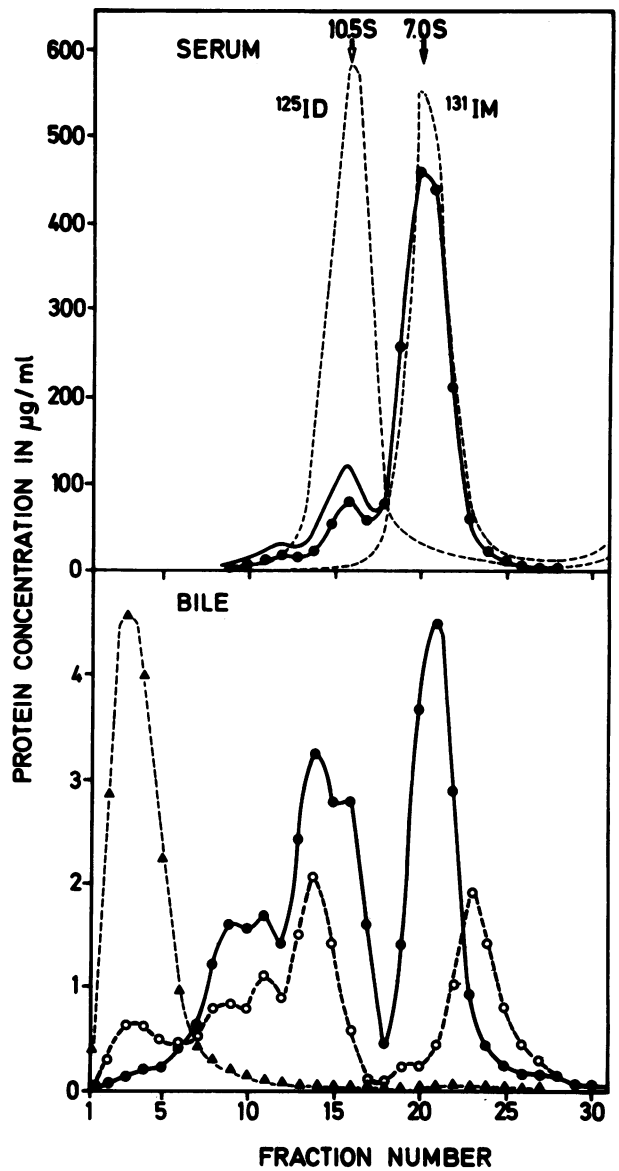

FIGURE 2 Simultaneous ultracentrifugation of serum and bile of one patient. In serum, the profile of IgA measured by IRMA (●), and the correction for size influence of polymers (——) on IRMA, are compared with the sedimentation patterns of ^{125}I-d-IgA and ^{131}I-m-IgA internal markers (– – –). In bile, profiles of IgA (●), SC (○), and IgM (▲) were also measured by IRMA.

Bile. Collection of bile was started 14 h after intravenous injection and lasted 12–14 h. Concentrations of m- and p-IgA were measured by IRMA and SDGU on the collections. Radioactivities were measured on 100 ml of the collections after 20-fold concentration by vacuum ultrafiltration in dialysis membranes (Visking, size $1^{8}/_{32}$ in., Medicell International Ltd., London) whereby most of the low molecular weight label was removed. Membrane-bound counts per minute were included in this measurement. The percentage of anti-α-precipitable radioactivity was measured by mixing an aliquot (0.3–1.0 ml) of the concentrated samples (>2,000 counts of either isotope per 3 min) with a slight excess (1–2 ml) of goat anti-α-chain, or anti-γ-chain as control, incubating 3 h at 37°C, adding 8 ml of sheep antigoat IgG (antibody excess) and further incubating for 2–4 h at room temperature and overnight at 4°C. After centrifugation, the precipitates were washed with 4 ml of PBS and counted enough time to accumulate 1,000 counts in the controls. In patient 3, specific activity was also measured in four subsequent bile collections.

Saliva. Two equal volumes of saliva, collected at the beginning and at the end of bile collection, were pooled. IgA concentrations were measured on this pool, whereas nondialyzed and anti-α-precipitable radioactivities were measured after 20-fold concentration, as for bile, but on 10 ml of the saliva pools.

Serum. The concentrations of m- and p-IgA in serum were measured on a sample taken before the intravenous injection; these concentrations were assumed to be constant. Serial samples (2 ml) were obtained after the intravenous injection, counted (3 min) and their anti-α-precipitable radioactivities measured to construct a specific activity-disappearance curve. The serum specific activity of m- and d-IgA to be compared with those in bile and saliva were derived from that curve at mid-time of the bile collection period. The serum anti-α-precipitable radioactivities were obtained by direct immunoprecipitation (2 ml of anti-α/0.25 ml of serum), using anti-α_1-antitrypsin as control.

Anti-SC-precipitable radioactivity was measured in bile in the same way as anti-α-precipitable radioactivity, using anti-SC instead of anti-α-antiserum.

Fractional catabolic rates were calculated over 4.5 d according to Matthews (42) and compared with those obtained in eight healthy volunteers injected with the same labeled m- and d-IgA.

In patient 1, bile was also collected immediately after intravenous injection, allowing to measure the total amount of anti-α-precipitable radioactivity excreted in 24 h.

Statistical analysis

Means and standard deviations relating to protein concentrations were obtained from their log values and expressed as the antilog values. S/S-ARCR were compared using the nonparametric Wilcoxon rank test for paired values (43).

RESULTS

Size distribution of IgA and SC in serum and secretions. In serum (Fig. 2), IgA was eluted from SDGU in a major 7S peak and one or two minor peaks of polymer, with CS of 10–10.5S and 12–13S, which represented 5–24% (mean = 13%) of the total serum IgA.

In bile (Fig. 2), the IgA profile displayed a less important 7S peak, and two or three peaks of polymers with CS of ~11–11.5S, 13S, and 15S, which together represented 43–88.5% (mean = 70%) of the total bile IgA. To each of these IgA peaks corresponded a peak of SC. An additional, often poorly resolved 10–10.5S IgA peak was found in most biles without corresponding SC peak. FSC with a CS of 4.6S was found in all biles (8–44% of total SC; mean = 29%), as well as small 18–19S SC peak that corresponded to the IgM peak.

In saliva (not shown), IgA and SC SDGU profiles were similar to those in bile, but with a much smaller 7S IgA peak, no 10–10.5S IgA peak, and in several cases, traces of anti-α-chain reacting material of smaller CS than 7S. Polymers represented 74–98% (mean = 93%) of the total IgA.

Protein concentration. Means and ranges of protein concentrations in serum, bile, and saliva are listed in Table I.

In bile, Alb was the predominant protein. The absolute concentrations of IgG and IgA in bile were sim-

TABLE I
*Protein Concentrations in Serum, Bile, and Saliva**

	Alb	Orosomucoid	Transferrin	IgG	m-IgA	p-IgA	α_2M	IgM
Serum	35,915‡	1,030	2,651	11,226	1,618	249	2,088	1,095
(n = 17)	27,000–41,000§	520–1,900	1,810–3,528	7,400–17,380	430–2,810	50–700	1,230–5,040	300–1,720
Bile	405	23.2	36.3	88.8	23	54.1	13.5	19.6
(n = 17)	155–1,485	6–66.4	11.4–160	32–480	11.8–66.7	25.3–146	2.7–100	2.2–60
Saliva	85.7	6.2	8.7	41.6	13.2	193	5.2	6.4
(n = 14)	15.8–385	1.4–50	2.8–83	8.7–178	3.2–33	72–902	0.4–37	1.1–73

* Concentration is expressed in micrograms per milliliter.
‡ Antilog of mean of log values.
§ Range.

ilar. Mean concentrations of bile proteins ranged between 0.64 and 2.3% of their corresponding mean serum values, except for p-IgA (22%).

In saliva, IgA was the predominant protein. The mean concentrations of other proteins were lower in saliva than in bile, ranging from 0.25 to 0.83% of their corresponding mean serum values, except for p-IgA (77.5%); in four cases, the p-IgA concentration was higher in saliva than in serum.

The mean concentrations of total SC (immunoglobulin-bound SC and FSC, in moles $\cdot 10^{-5}$/ml), were 2.8 (0.75–8.0) in serum, 12.1 (4.1–44) in bile, and 58 (26–222) in saliva.

S/S-ARCR. Mean S/S-ARCR±SD for the different proteins studied in bile and saliva are presented in Fig. 3.

In bile, S/S-ARCR for some proteins [orosomucoid(mean = 2.08) > Alb(mean = 1) ($P < 0.001$) > IgG(mean = 0.70) ($P < 0.005$) > α_2M(mean = 0.57) ($P > 0.1$)] were inversely related to their molecular weights, suggesting a predominantly passive mechanism for their secretion in bile, as reported before (24). However, this relation was not found for several other proteins when compared with proteins of similar or smaller size: greater S/S-ARCR were found for transferrin (mean = 1.21) than for Alb(mean = 1) (*P*

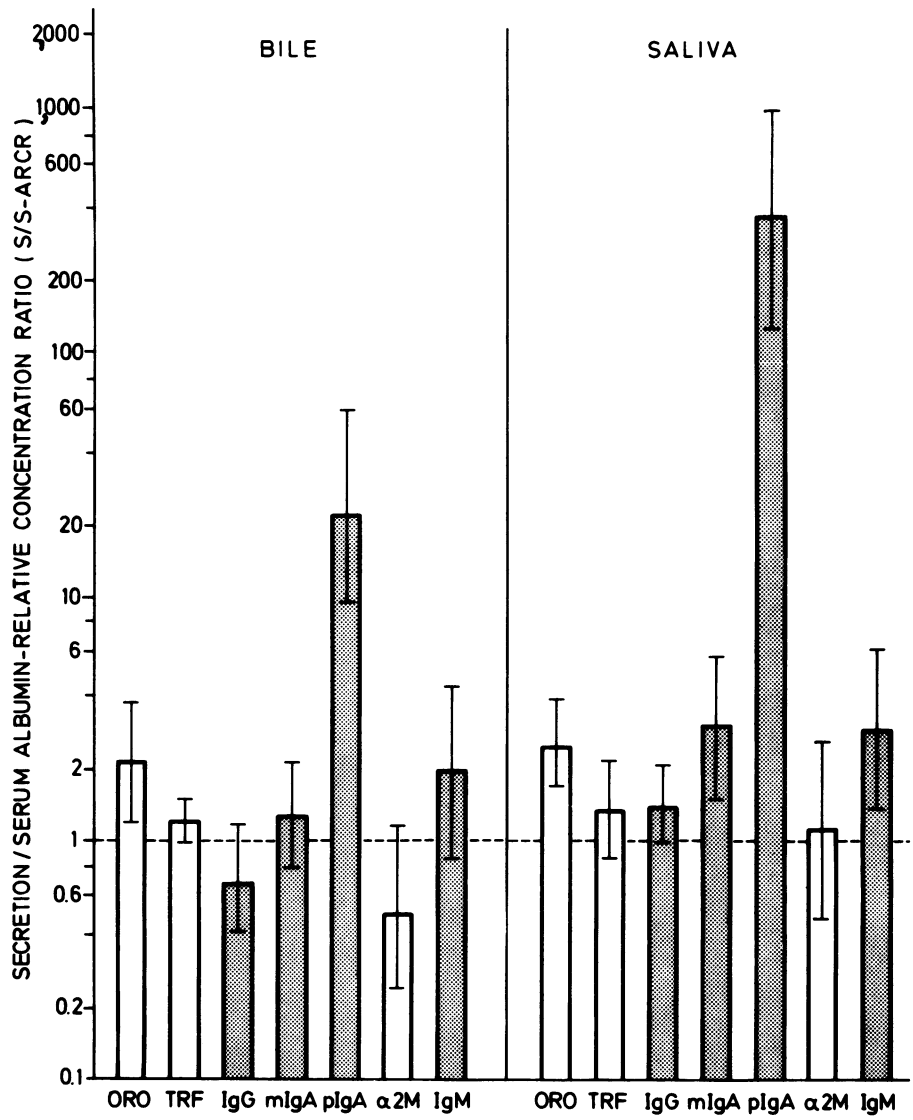

FIGURE 3 S/S-ARCR (mean±SD) of various proteins in bile and saliva. Immunoglobulins are represented by shaded columns. The horizontal dotted line represents the S/S-ARCR of Alb, taken as unit. ORO, orosomucoid; TRF, transferrin.

< 0.005), for m-IgA(mean = 1.28) than for IgG(mean = 0.70) ($P < 0.001$), for IgM(mean = 1.91, range: 0.3–7.5) than for α_2M(mean = 0.57) ($P < 0.005$), and for p-IgA(mean = 22, range 4.6–103) than for all other proteins ($P < 0.001$). Mean S/S-ARCR for p-IgA in the 7 cases of endoscopic collection of bile was 27.5 (5.3–103), as compared with 18.0 (4.6–86) in the 10 other cases.

In saliva, S/S-ARCR of m-IgA(mean = 2.98) and IgM (mean = 2.90) were higher, respectively, than those of IgG and α_2M ($P < 0.005$). Also, p-IgA had a S/S-ARCR (mean = 354, range: 46–2,394) much higher than that of all other proteins, and 16 times that of p-IgA in bile.

Fractional catabolic rates of m- and d-IgA. Homogeneity in size of both ^{131}I-m-IgA and ^{125}I-d-IgA is illustrated in Fig. 2. In serum of the five patients, specific activity of ^{125}I-d-IgA disappeared nearly twice as fast as that of ^{131}I-m-IgA, as illustrated for patient 3 (Fig. 4). Mean fractional catabolic rates were 53% (38–59%) for d-IgA and 29% (24–35%) for m-IgA, as compared with 43% (34–58%) and 25% (15–35%), respectively, in eight healthy adults. In patient 1, undergoing complete biliary drainage and with the highest S/S-ARCR for p-IgA, the biliary anti-α-precipitable radioactivity recovered over the first 24 h after injection (volume of bile = 485 ml) was 2.8% of the injected dose for d-IgA, and 0.11% for m-IgA.

Size distribution of radioactivity in concentrated bile and saliva. In bile, the ^{125}I-d-IgA peak shifted from a CS of 10.5S toward a peak with larger CS, which was largely superposed with the profile of p-IgA (Fig. 5); ^{131}I-m-IgA in bile was largely superposable to the 7S IgA peak as illustrated for patient 5 (Fig. 6).

In saliva, most of the radioactivity of both m- and d-IgA was found with CS below 7S (Figs. 5 and 6).

S/S-SAR of m- and d-IgA. The radioactivities, their anti-α-precipitable percentages, the total IgA amounts, and the S/S-SAR are listed for ^{125}I-d-IgA in Table II, and for ^{131}I-m-IgA in Table III. The percentages of anti-α-precipitable ^{125}I-d-IgA radioactivities, which were also specifically precipitable with anti-SC-antiserum, were respectively 76, 64, 49, and 53% for patients 1–4, whereas they were not significant for ^{131}I-m-IgA. The evolution of the specific activity in bile was comparable to that in serum as illustrated in Fig. 4 for patient 3 in whom five collections of bile were obtained after injection.

Individual total S/S-ARCR of IgG, m-IgA, and p-IgA for the four injected patients are listed in Table IV together with their corresponding serum-derived S/S-ARCR (S/S-ARCR × S/S-SAR) of m- and p-IgA, and their means±SD are illustrated in Fig. 7. For p-IgA in bile, the serum-derived S/S-ARCR (mean = 4.9) represented 51% of the total S/S-ARCR (mean = 9.6),

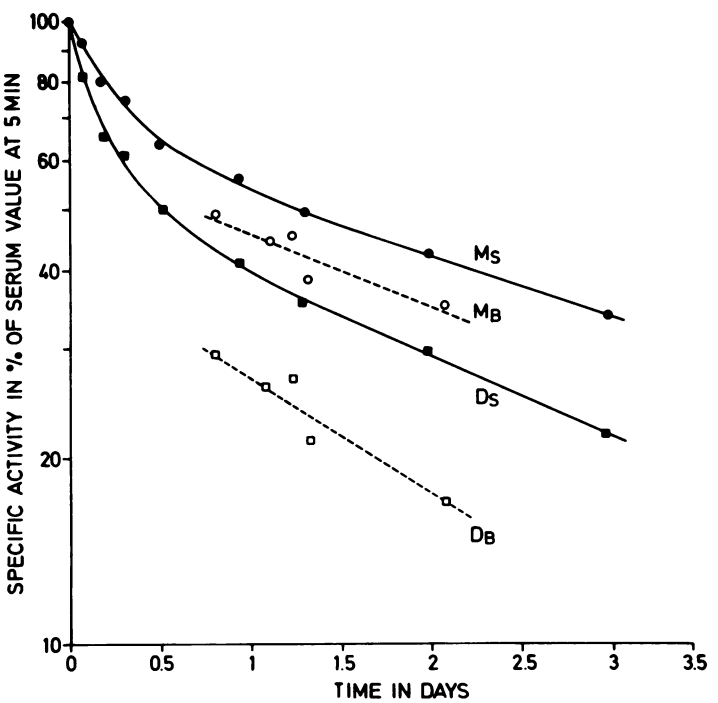

FIGURE 4 Specific activities of labeled m-IgA (circles) and d-IgA (squares) in serum (——) and bile (- - -) of patient 3, related to time after their intravenous injection.

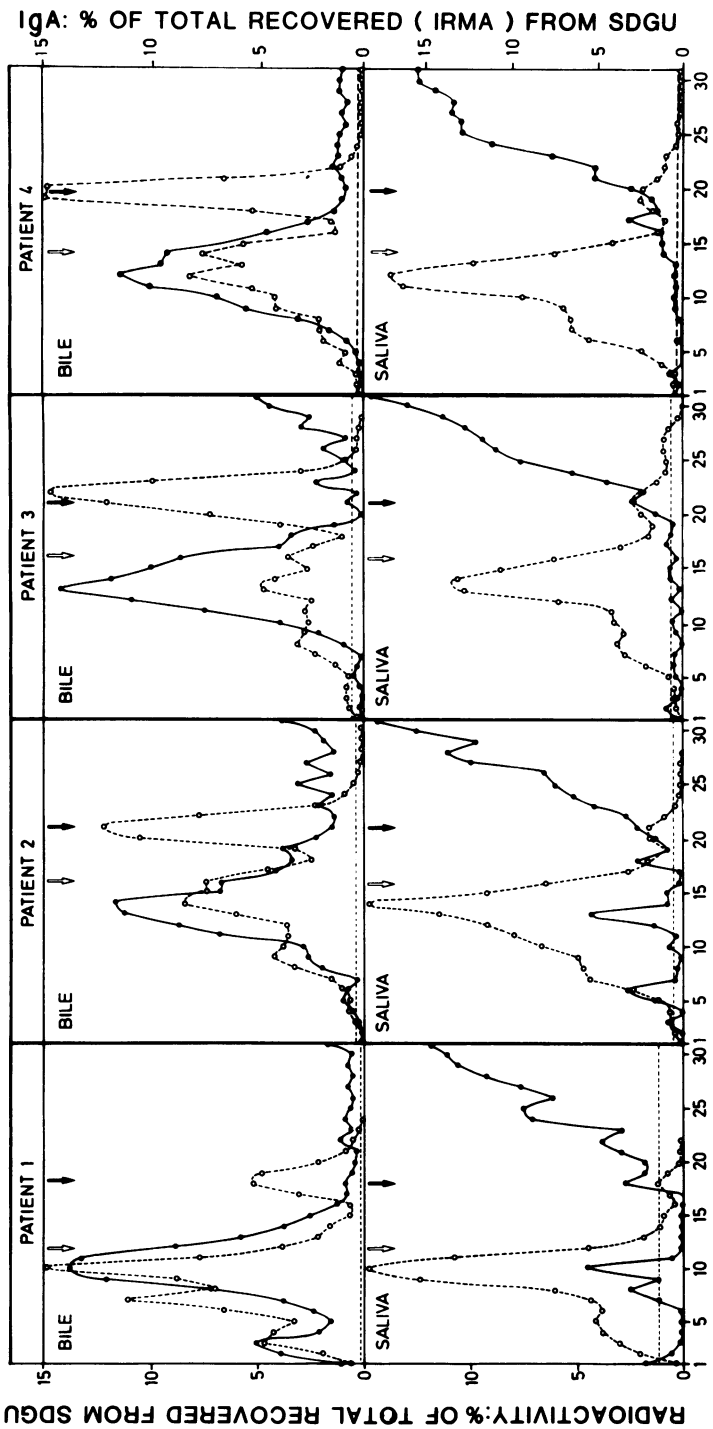

FIGURE 5 Simultaneous SDGU-profiles of ^{125}I-d-IgA (●) and cold IgA (IRMA) (○) in bile and saliva of patients 1–4. Horizontal dashed lines represent 2 SD above background. The highest numbers of counts per 10 min above background for biles were 2,620 (patient 1), 1,085 (patient 2), 1,146 (patient 3), and 1,340 (patient 4); for saliva they were 340, 822, 1,412 and 1,622 for patients 1–4, respectively. Open arrow: position of d-IgA; filled arrow: position of m-IgA.

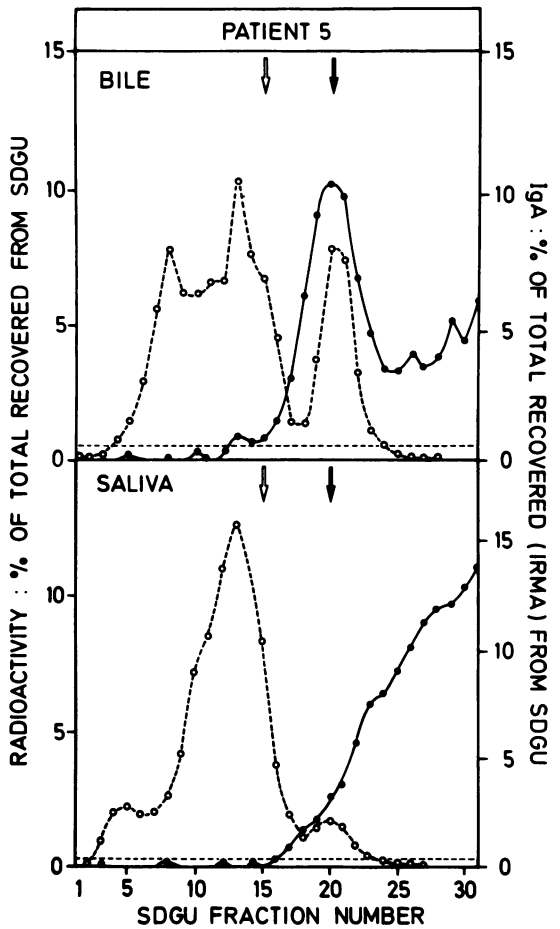

FIGURE 6 Simultaneous SDGU-profiles of ^{131}I-m-IgA (●) and cold IgA (IRMA) (O) in bile and saliva of patient 5. Horizontal dashed lines represent 2 SD above background. The highest numbers of counts per 10 min above background for bile was 1,204, and for saliva 1,900. Open arrow: position of d-IgA; filled arrow: position of m-IgA.

whereas in saliva, it (mean = 4.4) represented only 1.6% of total S/S-ARCR (mean = 269). For m-IgA in bile, the serum-derived S/S-ARCR (mean = 0.84) represented 87% of total S/S-ARCR (mean = 0.97), whereas in saliva, it (mean = 1.6: average of only three out of five significant values) represented 36% of the total S/S-ARCR (mean = 4.5).

DISCUSSION

Our data demonstrate a selective transport of p-IgA in bile from both serum and local origin. Correcting for the molecular weight-dependent passive diffusion in bile of serum proteins by the use of S/S-ARCR, the amount of p-IgA excreted in bile is ~31 times that of the smaller IgG. Our isotopic studies show that serum p-IgA contributes to about half of this selective excretion in bile, whereas virtually all the Alb and IgG in bile were shown to derive from serum (24, 25, 29). The other half of the p-IgA in bile is likely to represent the local contribution of IgA plasmacytes present in portal tracts and biliary submucosa (25–27). For m-IgA, the S/S-ARCR and isotope data do not support selective transport from serum and show that there is only ~12% of m-IgA in bile that is not serum derived.

IgM displayed a fourfold higher S/S-ARCR than the smaller α_2M in favor of its selective excretion in bile. We cannot state whether this derives from serum or from local production. Local IgM plasmacytes probably contribute, because IgM, but not IgG, has been found associated to bile duct cells (26).

Our total IgA concentrations in bile are consistently lower than those reported by Nagura et al. (26) and are, in contrast to their results, similar to the levels of IgG. Technical differences related to the size heterogeneity influence upon their immunoassay, and to the sensitivity of their radial immunodiffusion assays for IgG and IgM in bile, may explain these discrepancies. However, the absence of simultaneous serum study by this group makes their results difficult to compare with ours.

In saliva, our S/S-ARCR results show a pattern broadly similar to bile, but with a much greater se-

TABLE II
Calculations of S/S-SAR of ^{125}I-p-IgA*

Patient No.	Serum (2 ml) Counts per 3 min	Serum (2 ml) Anti-α-chain precipitable counts	Serum (2 ml) p-IgA	Bile (100 ml) Counts‡ per 3 min	Bile (100 ml) Anti-α-chain precipitable counts	Bile (100 ml) p-IgA	Bile (100 ml) S/S-SAR*	Saliva (10 ml) Counts‡ per 3 min	Saliva (10 ml) Anti-α-chain precipitable counts	Saliva (10 ml) p-IgA	Saliva (10 ml) S/S-SAR*
		%	mg		%	mg			%	mg	
1	6,587	92	0.38	92,736	89	14.2	0.37	6,722	7.7	1.34	0.024
2	17,723	91	0.92	40,828	61	2.8	0.51	16,291	6.2	1.58	0.037
3	18,223	90	0.78	64,304	76	3.4	0.69	14,187	1.1	1.13	0.006
4	16,171	87	0.55	79,025	77	4.6	0.52	18,317	8.0	4.32	0.013

* $\text{S/S-SAR} = \left(\frac{\text{counts} \times \text{percentage of anti-}\alpha\text{-precipitability}}{\text{milligrams of IgA}}\right)$ in secretion $/ \left(\frac{\text{counts} \times \text{percentage of anti-}\alpha\text{-precipitability}}{\text{milligrams of IgA}}\right)$ in serum

‡ Nonultrafiltrated radioactivity after 20-fold concentration by ultrafiltration; it represented 76±14% of total bile radioactivity, and 18±12% of total saliva radioactivity.

TABLE III
Calculations of S/S-SAR of ^{131}I-m-IgA*

Patient no.	Serum (2 ml)			Bile (100 ml)				Saliva (10 ml)			
	Counts per 3 min	Anti-α precipitable counts	m-IgA	Counts‡ per 3 min	Anti-α precipitable counts	m-IgA	S/S-SAR*	Counts‡ per 3 min	Anti-α precipitable counts	m-IgA	S/S-SAR*
		%	mg		%	mg			%	mg	
1	9,186	93	5.62	4,675	59	1.8	1.00	9,364	NS§	0.07	—
2	16,200	91	3.40	13,872	44	1.7	0.83	7,268	5.0	0.33	0.25
3	26,489	92	4.94	27,994	76	4.8	0.90	8,911	NS	0.24	—
4	29,350	86	4.48	27,962	72	4.9	0.73	7,742	16.2	0.29	0.77
5	30,839	91	5.18	26,547	55	2.9	0.93	23,615	1.1	0.24	0.22

* S/S-SAR: see explanation in Table II.

‡ Nonultrafiltrated radioactivity after 20-fold concentration; it represented 52±18% of total bile radioactivity and 16±4% of total saliva radioactivity.

§ NS: <200 counts above the 1,000 counts of the control.

lective excretion of p-IgA, ~16 times that in bile. Our isotopic studies, however, indicate that only 1.6% of total salivary p-IgA originated from serum, as compared with 50% in bile, pointing to a much greater contribution of local synthesis of IgA. Such a serum-to-saliva transport of p-IgA, although subject to large errors due to a low percentage of anti-α-precipitable counts, is nevertheless in agreement with results on serum and saliva of IgA myeloma patients (44), and on canine saliva using intravenously injected labeled p-IgA (25, 30). It is probable that proteins other than IgA and IgM also reach secretions by mechanisms different from simple molecular weight-dependent passive transfer from serum, as suggested by our S/S-ARCR results for transferrin in bile and saliva or for IgG and α_2M in saliva.

Since the daily output of hepatic bile and of saliva are ~1 liter, roughly 28 mg of p-IgA will be transported from serum into external secretion for bile as compared with 3 mg for saliva. The daily total contribution to intestinal IgA should be of ~77 mg of IgA for bile as compared with 206 mg for saliva.

The serum-derived p-IgA in bile was shown, by immunoprecipitation and by the shift of the ^{125}I-d-IgA toward a greater CS in bile, to be significantly associated with SC. The studies by SDGU on the 17 bile samples, however, regularly documented a significant proportion of serum-type 10–10.5S IgA not associated with SC, in contrast to saliva. We also identified FSC in bile in substantial amounts, as well as SC sedimenting together with IgM. The finding of FSC in normal bile as well as in bile of an IgA-deficient subject (45) is not surprising, as FSC is found in most exocrine secretions. The presence in bile of d-IgA unassociated with SC can be partly explained by passive transfer, as for other proteins, but could also reflect some spontaneous dissociation of noncovalently d-IgA-bound SC. Indeed, it is not known whether all p-IgA transported via SC-binding reaches the bile as covalent SC-p-IgA complex, despite the presence in rat liver of an enzyme enhancing covalent binding of human SC to human p-IgA and IgM (46).

Nagura et al. (26) provided immunohistochemical evidence for a SC-mediated vesicular transport of IgA through bile duct epithelial cells in man as shown earlier for rats (47). However, in contrast to Nagura et al. (26) and others (27), another group (28) found intrahepatocyte SC and IgA, using amplified immunoperoxidase techniques but surprisingly, they found less SC and IgA staining in bile duct cells. In addition, a particular relationship between SC and hepatocytes was suggested by the rapid fall of the high serum SC concentration observed in patients with acute hepatitis when fulminant massive parenchymal necrosis occurred (48). It seems likely that SC-mediated endocytotic transepithelial transfer of IgA through bile ducts explains the accumulation of locally synthesized IgA in bile. A similar transfer could also occur for IgM, which in man, in contrast to rats, has a higher noncovalent affinity for SC than does p-IgA (49, 50) and is also SC-transported in exocrine secretions (10, 51). Regarding the serum-to-bile selective transport of p-IgA, our data do not allow to distinguish between a selective transport mediated by both hepatocytes and bile duct cells, or by the latter only as suggested by Nagura et al. (26). However, our data show a much smaller transfer of p-IgA from serum to bile than in rats and rabbits, in which hepatocyte synthesis of SC has been demonstrated (52–54). After intravenous injection into rats and rabbits of the same iodine-labeled

TABLE IV

S/S-ARCR for Total and Serum-derived m- and p-IgA in Bile and Saliva, Compared with Their S/S-ARCR for IgG

Patient No.	S/S-ARCR in bile					S/S-ARCR in saliva				
		m-IgA		p-IgA			m-IgA		p-IgA	
	IgG	Total	Serum-derived*	Total	Serum-derived*	IgG	Total	Serum-derived*	Total	Serum-derived*
1	0.15	0.46	0.46	50.90	18.83	0.97	1.59	—	436	10.46
2	0.49	0.91	0.76	5.30	2.70	2.34	8.58	2.14	150	5.55
3	1.02	1.58	1.42	7.00	4.83	0.38	12.00	—	359	2.15
4	0.82	0.61	0.44	4.60	2.39	0.73	1.89	1.45	225	2.90
5	0.92	2.10	1.95	—	—	1.52	6.20	1.36	—	—

* Serum-derived S/S-ARCR obtained by multiplying each total S/S-ARCR by its corresponding S/S-SAR to be found in Tables II and III, respectively, for p-IgA and m-IgA.

d-IgA preparations, >45% of the injected dose appeared in the bile of these animals within 3 h as compared with 2.8% in man over 24 h, in our patient 1, and with 0.53% over 8 h as reported by others (55). The absolute concentration of p-IgA is 5–30 times larger in bile than in serum for rats (56, 57) and rabbits (40), whereas in man, levels of p-IgA in bile are about one-fourth of those in serum. S/S-ARCR values for p-IgA in hepatic bile of rats (58) and rabbits (40) are between 200 and 1,000 as compared with about 22 in humans, and in rats virtually all of the bile IgA is serum-derived. It is tempting to relate this striking quantitative difference to the presence of easily detectable SC in rat and rabbit, but not in human hepatocytes.

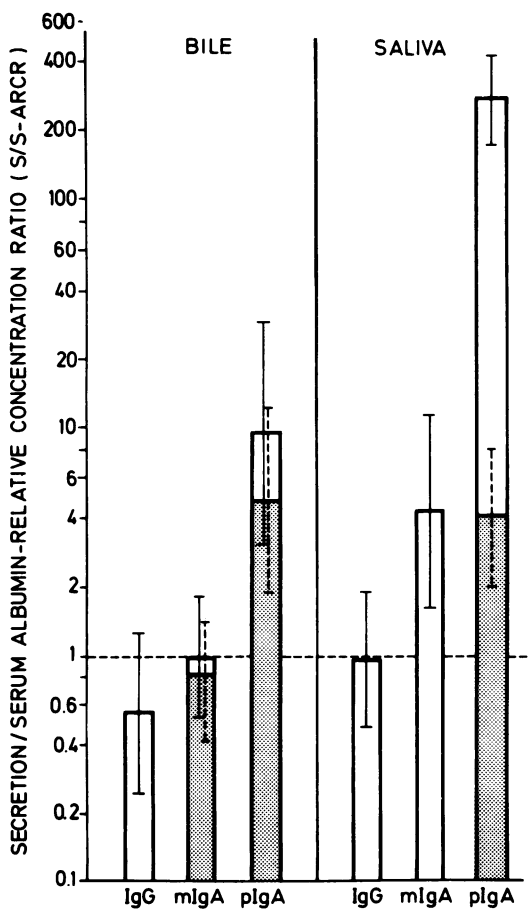

FIGURE 7 S/S-ARCR (mean±SD) of IgG and IgA for bile and saliva of five patients injected with labeled IgA. For m- and p-IgA, the shaded area represents the S/S-ARCR calculated only from their serum-derived fractions. The horizontal dashed line represents the S/S-ARCR of Alb, taken as unit.

In conclusion, selective transport of p-IgA in human bile occurs from both serum and nonserum (local) origin. When compared with the rat and rabbit, it plays a much less significant role in the clearance of circulating IgA and in the contribution to sIgA in intestinal secretions.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Mrs. J. P. Dehennin, R. Meykens, and Mme J. Naze-De Mets.

This work was supported by grants 4.4504.70 and 220992 from the Fonds de la Recherche Scientifique Médicale, Brussels, and by the Wellcome Trust.

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