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In vivo experiments involving secretory component in the rat hepatic transfer of polymeric IgA from blood into bile

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Accepted for publication 8 December 1980

Summary. Human or rat purified secretory IgA (sIgA) injected intravenously (i.v.) into rats is transferred to bile much less (seven to twenty-four times) than human or rat polymeric IgA (pIgA) devoid of secretory component (SC). A polymeric Fcα (pFcα) fragment of a human IgAl myeloma protein, obtained by IgA-protease digestion, bound *in vitro* to rat SC and was actively transferred *in vivo* into bile, in contrast to the corresponding Fabα. The IgA recovered in bile was not degraded, as judged by sedimentation in density gradients. Purified rabbit IgG anti-rat SC antibody was also efficiently transported *in vivo* into bile, about forty times more than normal rabbit IgG. The biliary transport of anti-SC antibody could be reduced and retarded by the simultaneous i.v. injection of purified rat SC or human pIgA. The transfer of rat ^{125}I-pIgA into bile was also significantly reduced and retarded by the concomitant i.v. injection of purified rat or human SC. Moreover, i.v. injection of purified rat or human SC induced a marked and prolonged decrease of the sIgA level in bile. Rat SC was more effective than human SC in this respect. All these *in vivo* experiments confirm the *in vitro* findings of Orlans, Peppard, Fry, Hinton & Mullock, (1979) and Socken, Jeejeebhoy, Bazin & Underdown, (1979) showing that SC is the IgA-receptor on the hepatocyte membrane for the transfer of pIgA from rat plasma into bile.

Abbreviations: SC, secretory component; sIgA, secretory IgA; FSC, free secretory component; pIgA, polymeric IgA; pFcα, polymeric fragment of IgA; i.v., intravenous(ly); c.p.m., counts per minute; PBS, phosphate buffered saline, pH 7·2.

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0019-2805/81/0600-0261$02.00

INTRODUCTION

High levels of secretory IgA (sIgA) and free secretory component (FSC) are found in rat bile (Lemaître-Coelho, Jackson & Vaerman, 1977) suggesting that rat liver transports IgA from the blood into bile. Active transfer of polymeric IgA (pIgA) from the circulation into bile was demonstrated *in vivo* in normal rats with cannulated bile ducts (Orlans, Peppard, Reynolds & Hall, 1978; Vaerman & Lemaître-Coelho, 1979) and in liver perfusion experiments (Jackson, Lemaître-Coelho, Vaerman, Bazin & Beckers, 1978) and it was confirmed by bile duct obstruction (Lemaître-Coelho, Jackson & Vaerman, 1978a) and liver damage (Kaartinen, 1978). The transferred IgA appears in bile in the form of sIgA (Jackson *et al.*, 1978). This transfer occurs through hepatocytes as demonstrated by electron microscopic autoradiography (Birbeck, Cartwright, Hall, Orlans & Peppard, 1979). Recently, Orlans, Peppard, Fry, Hinton & Mullock (1979) and Socken, Jeejeebhoy, Bazin & Underdown (1979)

showed that rat FSC behaved as the receptor for pIgA on *in vitro* isolated and cultured rat hepatocytes.

In the gut also, pIgA is transferred from the *lamina propria* into the intestinal lumen through the columnar epithelial cells (Brandtzaeg, 1974; Nagura, Nakane & Brown, 1979), by binding to FSC that behaves as a membrane receptor specific for pIgA or IgM (Brandtzaeg, 1978; Crago, Kulhavy, Prince & Mestecky, 1978).

Does this mechanism also apply to the *in vivo* hepatic transfer of IgA? In liver perfusion experiments, rat ^{125}I-sIgA was poorly transferred into bile as compared with pIgA devoid of secretory component (SC); moreover, purified rabbit IgG anti-rat-SC antibodies were also transferred into bile to a small but significant extent (Fisher, Nagy, Bazin & Underdown, 1979). These data suggested that rat FSC was involved in this transfer. In addition, *in vitro* cultured isolated rat hepatocytes synthesize and secrete FSC (Socken *et al.*, 1979; Zevenbergen, May, Wanson & Vaerman, 1980).

The present report deals with *in vivo* experiments showing that SC is involved in the hepatic transfer of IgA, confirming the data of Orlans *et al.* (1979) and Socken *et al.* (1979).

MATERIALS AND METHODS

Animals

Male OFA rats (180–220 g; 6–8 weeks old) and Wistar rats (200–300 g; 8–12 weeks old) were used throughout the experiments. They received UAR-A03 pellets (U.A.R., Villemoisson-sur-Orge, France) and tap water *ad libitum*.

Cannulation of rat bile duct

This procedure has been described previously (Lemaître-Coelho *et al.*, 1977).

Antisera

Goat antisera to anti-rat-α-chains or anti-rat-SC have been described earlier in detail (Vaerman, Heremans, Bazin & Beckers, 1975; Lemaître-Coelho *et al.*, 1977; Jackson *et al.* 1978): they reacted only with α-chains of IgA from normal rat serum, monoclonal rat IgA and rat sIgA, or with rat FSC and sIgA, respectively. Rabbit anti-rat-FSC was obtained by eight injections of rat bile FSC purified following Acosta-Altamirano, Barranco-Acosta, Van Roost & Vaerman (1980). This antiserum was adsorbed with normal rat serum and rat monoclonal IgA which were insolubilized by coupling to Sepharose 4B as described by Cambiaso, Goffinet, Vaerman & Heremans (1975). Goat anti-human-α-chains was raised by multiple (over twenty) injections (subcutaneous and intradermal) of α-chains purified from a monoclonal human IgA; it was adsorbed by percolating it through columns of insolubilized rat monoclonal IgA and human IgG. Goat anti-rabbit-Fcγ was obtained by injecting Fcγ purified from papain-digested (Porter, 1959) rabbit IgG on Protein A-Sepharose CL-4B, as described by Goding (1976); it was adsorbed by passage through insolubilized rabbit Fab.

Purified proteins

Human monoclonal pIgA was obtained by gel filtrations and preparative electrophoresis on blocks of Pevikon as described earlier (Vaerman & Lemaître-Coelho, 1979). Fcα and Fabα were obtained from a pIgAl by digestion with IgA-protease (Mehta, Plaut, Calvanico & Tomasi, 1973) and gel filtration of the digest on Ultrogel AcA 34; the undigested pIgA was eluted in a first peak, followed by the polymeric Fcα (pFcα) fragment in the second peak and much later by the Fabα fragment in the third peak. Human sIgA and FSC were purified from milk (Kobayashi, 1971), with additional purification of FSC by affinity chromatography on a column of human IgA-Sepharose (Acosta-Altamirano *et al.*, 1980). Rat monoclonal pIgA was obtained by repeated gel filtrations of serum or ascites of tumour-bearing rats (IR-699 and IR-22) on Ultrogel AcA 22 followed by preparative electrophoresis on Pevikon (Vaerman, 1970) of the pIgA-fractions; rat sIgA and FSC were isolated from rat bile, as described elsewhere (Acosta-Altamirano *et al.*, 1980). Normal rabbit IgG was obtained by affinity chromatography on Protein A-Sepharose CL-4B (Goding, 1976). Purified rabbit IgG anti-rat-SC was obtained by passing 5 ml aliquots of rabbit anti-rat-FSC antiserum through a small (1·5 ml) column of insolubilized rat FSC; the antibodies were eluted at 4° with 5 ml of 3 M ammonium thiocyanate, pH 6·8, dialysed at 4° against phosphate-buffered saline (PBS) and concentrated to 1 ml by vacuum ultrafiltration before analysis.

Quantitation of proteins

Purified IgG, IgA and sIgA were quantified by absorption at 280 nm using extinction coefficients of 13·8 for IgG and of 13·4 for IgA (Heremans, 1974). IgA in rat bile was measured by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965).

Iodination of proteins

The chloramine T method (McConahey & Dixon, 1966) was followed. One milligram of protein was labelled with 1 mCi of carrier-free ^{125}I. Unbound iodine was removed, after the addition of 4 mg of bovine serum albumin, by passing the labelled sample through a 1 × 5 cm column of AG1-X8 (Biorad, Richmond) in PBS. Over 95% of the sample radioactivity was precipitable with 10% trichloroacetic acid.

Density gradient ultracentrifugations

This was performed in a Beckman Spinco L65-B ultracentrifuge with the SW41-Ti rotor. Isokinetic (Johns & Stanworth, 1976) sucrose gradients (12 ml) of 5%–34% sucrose were run for 15–18 hr at 36,000 r.p.m. at 20°. Samples of 0·2–0·3 ml were applied on top of the gradient. The gradient was recovered in 30 fractions of 0·4 ml, being monitored during elution by absorption at 280 nm.

RESULTS

Biliary transfer of rat ^{125}I-labelled serum pIgA or bile sIgA

Rats were cannulated and injected intravenously (i.v.) with rat monoclonal ^{125}I-pIgA or with polyclonal ^{125}I-sIgA isolated from rat bile to compare their transfer in bile. Bile was collected over a period of 3 hr and the radioactivity recovered in bile during this time was expressed as the percentage of the injected dose. Results, listed in Table 1, indicate that rat serum pIgA is transferred about seven times better than rat bile sIgA.

Transfer of unlabelled or ^{125}I-labelled human serum pIgA or milk sIgA

In similar experiments, both labelled and unlabelled proteins were injected i.v. The doses of the unlabelled proteins were 5·0–11·3 mg whereas for labelled proteins the doses ranged between 5 and 40 μg. Large doses were used to assess the capacity of the liver transfer system. Recoveries in 3 hr bile for unlabelled human proteins were measured by radial immunodiffusion using anti-human-α-chain antiserum. For human proteins also, the recoveries of sIgA were much lower than those of serum pIgA in both small and large doses (Table 1). When large doses (6 mg) of human pIgA were i.v. injected, the recovery in bile within 3 hr was not smaller than when 5–40 μg of the same pIgA were injected, indicating that the liver pIgA-transfer system is far from being saturated in normal conditions and has a high capacity to transfer additional pIgA.

Polymeric Fcα of human monoclonal IgA binds rat FSC and is actively transferred into rat bile

Digestion of the human pIgA1 with IgA protease was effective and the polymeric Fcα fragment, isolated by gel filtration (see Materials and Methods), was able to combine *in vitro* with added rat bile FSC. These data are illustrated by immunoelectrophoreses in Fig. 1. When the isolated pFcα and Fabα were labelled and

Table 1. Comparative efficiencies of the hepatic transfer in bile of i.v.-injected human and rat serum pIgA, sIgA and polymeric Fc_α and Fab_α from a human monoclonal pIgA1

Protein intravenously injected	Dosage	Number of rats	Recovery* in bile after 3 hr Mean ± SD (range)	Difference
Rat monoclonal polymeric ^{125}I-IgA	2·5–75 μg	8	42† ± 9·6 (26–51)	
^{125}I-sIgA from rat bile	2·5–30 μg	6	6·2 ± 2·3 (3·1–9·2)	7 X
Human monoclonal polymeric ^{125}I-IgA	5–40 μg	12	41 ± 7·0 (31–53)	
^{125}I-sIgA from human milk	15 μg	3	3·2 ± 0·9 (2·3–4·0)	13 X
Unlabelled human monoclonal polymeric IgA	6 mg	9	49‡ ± 10 (34–68)	
Unlabelled sIgA from human milk	5–11·3 mg	6	2·0 ± 0·8 (1·3–3·5)	24 X
Human monoclonal polymeric ^{125}I-Fcα	5–50 μg	3	48 ± 2·6 (45–50)	
Human monoclonal ^{125}I-Fabα	50 μg	3	2·4 ± 0·8 (1·6–3·1)	20 X

* Recovery of i.v.-injected protein expressed as percentage of the injected dose.
† Recovery of i.v.-injected protein measured by counting the radioactivity in bile.
‡ Recovery of i.v.-injected protein measured by radial immunodiffusion in bile.

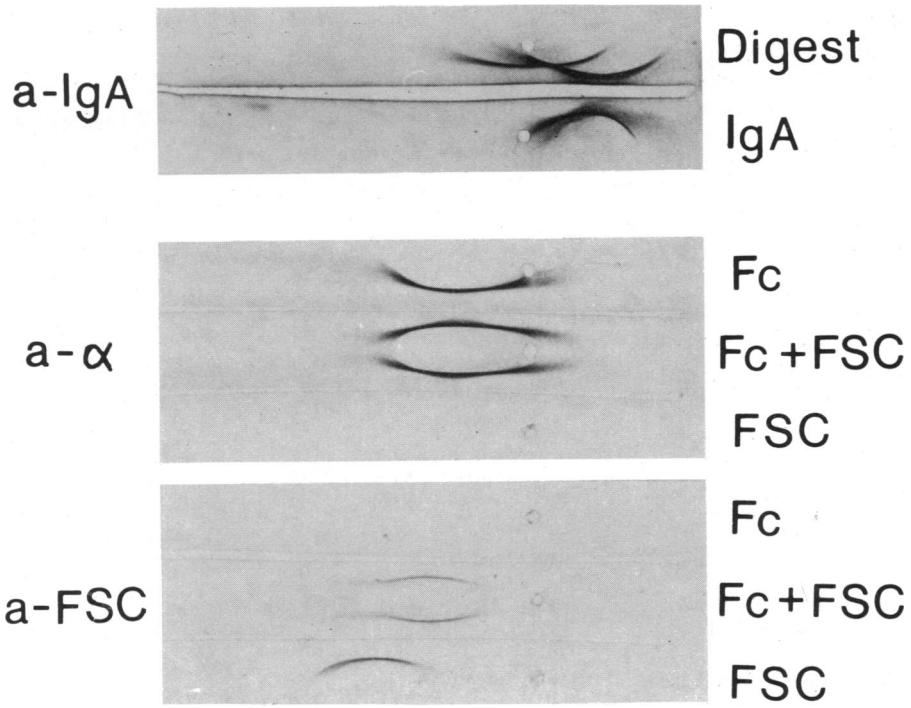

FIGURE 1. Immunoelectrophoreses of a human polymeric monoclonal IgA1 and of its IgA-protease digest, revealed with unadsorbed anti-human IgA antiserum. Note the appearance of a fast Fcα and a slow Fabα in the digest. The two lower plates illustrate the binding of rat bile FSC by the human Fcα fragment (Fc). Anti-α is anti-human α-chain antiserum and anti-FSC is anti-rat FSC antiserum. There is displacement of most of the FSC by the pFcα in the mixture Fc+FSC. Anode to the left.

injected i.v., the recovered radioactivity in 3 hr bile was twenty times larger for pFcα than for Fabα (Table 1).

Molecular size of the radioactive material recovered in bile

Density gradient ultracentrifugations were performed on the labelled proteins and on the bile collected between 45 and 60 min after i.v. injections (not shown). The size distribution of the radioactivity in bile was similar to that of the injected monoclonal pIgA, indicating the absence of degradation products in bile. However, with labelled human sIgA, large amounts of low molecular weight (<40,000) radioactive material were found in bile, accounting for 50% of the total counts per minute (c.p.m.) in the sample collected between 2 hr 45 min and 3 hr (not shown). Recovery based only on c.p.m. (Table 1) was then overestimated. There was also a small amount of low molecular weight radioactivity in the bile of rats injected with labelled rat sIgA. The nature of these low molecular weight radioactive materials was not further investigated.

Biliary transfer of purified rabbit IgG antibodies against rat FSC

The electrophoretic and immunoelectrophoretic analyses of normal rabbit IgG and of rabbit IgG anti-rat-SC antibody are shown in Fig. 2. The two IgG samples were very similar except for their antibody activity. The ^{125}I-IgG anti-rat SC antibody was actively transferred into bile, about forty times better than the normal ^{125}I-IgG (Table 2). Figure 3 illustrates the kinetics of the transfer of the i.v. injected anti-rat-FSC antibody into bile. The transfer into bile of IgG anti-rat-FSC antibody was partly inhibited by adding purified rat bile FSC (83% inhibition) or human monoclonal pIgA (44% inhibition) from 5 to 10 min before i.v. injection, as listed in Table 2.

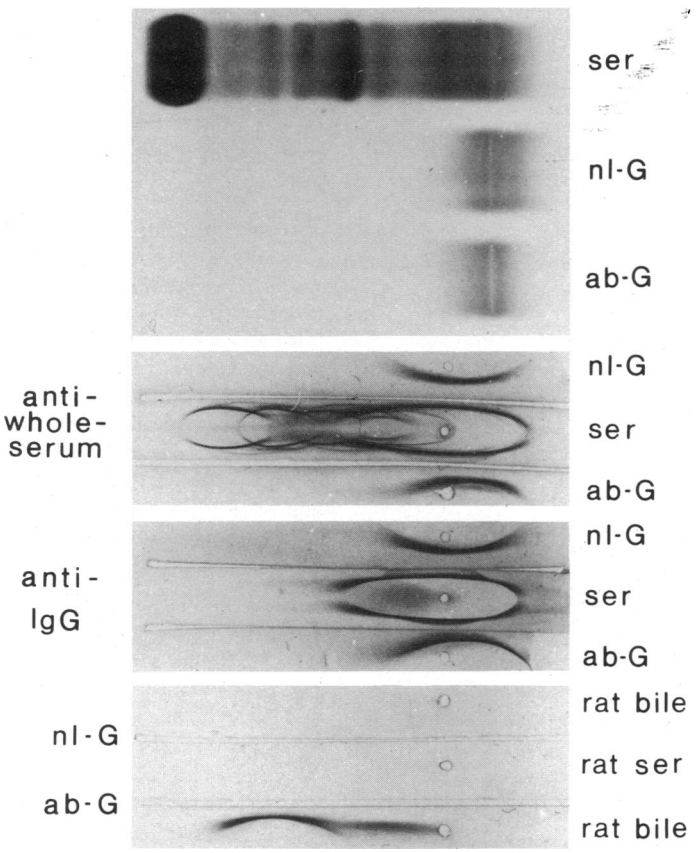

Figure 2. Agarose gel electrophoresis and immun-electrophoreses of normal rabbit serum (ser), normal rabbit IgG (nl-G), purified rabbit IgG anti-rat-SC antibody (ab-G) developed with anti-whole-rabbit serum or anti-rabbit IgG. The specific anti-rat SC antibody activity of the purified antibody is illustrated in the lower plate. The antibody reacts with both FSC and sIgA from rat bile. Anode to the left.

Table 2. Comparative efficiencies of the hepatic transfer in bile of i.v.-injected rabbit ^{125}I-IgG anti-rat FSC antibodies, alone or mixed with unlabelled rat FSC or human pIgA, and of normal rabbit IgG

i.v.-Injected protein(s)	Number of rats	Recovery* in bile after 3 hr Mean ± SD (range)
Rabbit ^{125}I-IgG anti-rat FSC antibody (20–250 μg)	5	24 ± 1·8 (22–26)
Normal rabbit ^{125}I-IgG (12·5–250 μg)	5	0·6 ± 0·1 (0·4–0·8)
Rabbit ^{125}I-IgG anti-rat FSC (25 μg) mixed† with cold rat bile FSC (0·3–0·5 mg)	7	6·2 ± 3·5 (3·3–11)
Rabbit ^{125}I-IgG anti-rat FSC (25 μg) mixed† with cold human pIgA (10 mg)	4	13 ± 4·3 (8·9–17)

* Recovery as percentage of the injected dose.
† Mixtures at room temperature from 5 to 10 min before i.v. injection.

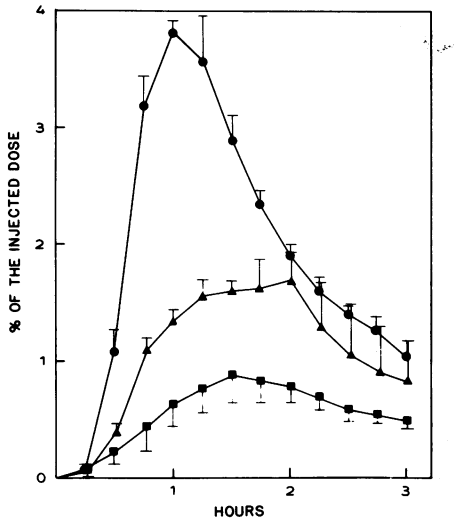

Figure 3. Kinetics of the hepatic transfer of rabbit ^{125}I-IgG anti-rat-SC antibody (●—● $n=4$) and its alteration by simultaneous injection of rat FSC (■—■ $n=6$) or human polymeric IgA (▲—▲ $n=4$). Each point is given as the mean ± SE.

Density gradient ultracentrifugations of bile from rats i.v. injected with labelled anti-rat FSC showed little radioactive material of the size of an IgG, with labelled peaks heavier and lighter than the starting antibody; but less than 8% of the c.p.m. corresponded to materials with molecular weights smaller than 40,000 (not shown).

Inhibition of transfer of pIgA into bile by i.v. injected FSC

Three groups of rats were injected i.v. with one ml of PBS containing 2 mg of purified rat bile FSC, or 2 mg of bovine serum albumin (BSA), or no protein, respectively. Their bile ducts were cannulated 45 min after the injection and the concentration of endogenous sIgA in bile collected for 1 hr was measured by radial immunodiffusion (Table 3, upper part). Whereas the serum IgA levels of the three groups of rats were nearly identical (not shown), the level of sIgA in bile of rats injected with FSC was about 4·2 times lower than that of rats injected with BSA or saline.

Three other groups of rat were cannulated and i.v. injected with one ml of PBS containing rat monoclonal ^{125}I-pIgA, either alone or mixed with cold rat bile FSC (2 mg) or human milk FSC (5 mg) from 5 to 10 min before i.v. injection. After admixture of rat or human FSC, the labelled rat pIgA was only transferred 24% and 37% of the control value, respectively (Table 3, lower part). The kinetics of appearance of the ^{125}I-pIgA in bile of these three groups are illustrated in Fig. 4. Both rat and human FSC, not only reduced this

Table 3. i.v.-Administered FSC inhibits the biliary secretion of endogenous pIgA and of exogenous ^{125}I-pIgA

i.v.-Injected protein(s) (volume: 1 ml)	Endogenous sIgA level in bile* (mg/ml)	Recovery of exogenous ^{125}I-pIgA in bile after 3 hr (% of injected dose)
Rat FSC (2 mg)	0·23 ± 0·13 (0·18–0·38)† $n=5$	
BSA (2 mg)	0·94 ± 0·09 (0·83–1·03) $n=5$	
None	0·97 ± 0·33 (0·75–1·55) $n=5$	
Rat ^{125}I-pIgA (10–60 μg)		49 ± 2·8 (45·0–51·0) $n=5$
Rat FSC (2 mg) mixed‡ with rat ^{125}I-pIgA (10–60 μg)		12 ± 4·9 (4·4–15·0) $n=4$
Human FSC (5 mg) mixed‡ with rat ^{125}I-pIgA (10–60 μg)		18 ± 1·9 (17·0–21·0) $n=4$

* Bile collected for 1 hr, starting 45 min after i.v. injection of protein.
† Mean ± SD (range).
‡ Mixture at room temperature from 5 to 10 min before i.v. injection.

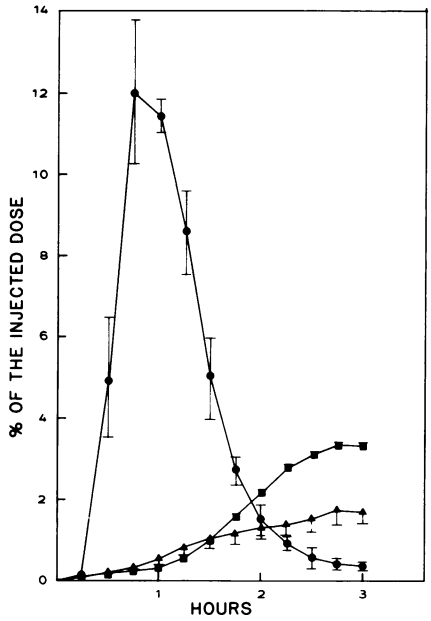

Figure 4. Kinetics of the hepatic transfer of radiolabelled polymeric rat monoclonal IgA (●—●, $n = 5$) and its alteration by the simultaneous injection of rat (▲—▲, $n = 4$) or human (■—■, $n = 4$) FSC. Each point is the mean ± SE.

transfer, but also slowed it down because the radioactivity in bile did not yet decline after 3 hr.

Finally, the changes in endogenous sIgA levels in successive bile samples of three further groups of rats i.v. injected with rat FSC (2 mg), human FSC (5 mg) or PBS, respectively, are shown in Fig. 5. Both human and rat FSC injections strongly reduced the output of endogenous sIgA in bile, as shown by the large decrease in sIgA levels in successive 15 min samples of bile. Bile flow was not altered in any of these groups.

DISCUSSION

In both rat and human IgA, the presence of SC in the molecules of pIgA i.v. injected strongly decreased its transfer into bile. This confirms earlier data (Fisher *et al.*, 1979) obtained by liver perfusion.

Since in man SC is bound to pFcα (Seligman, Mihaesco, Hurez, Mihaesco, Preud'homme & Rambaud, 1969; Garcia Pardo, Lamm, Plaut & Frangione, 1979), it was interesting to verify if the fragment responsible for binding to FSC would be transferred as well as the whole molecule. The data of Table 1 confirmed that the pFcα was transferred very well, and not the Fabα.

Density gradient ultracentrifugation showed that the molecular size of the radioactive material recovered in bile was similar to that of the injected protein; only in the case of sIgA did some degradation take place.

Normal IgG is virtually not transferred into bile (Jackson *et al.*, 1978; Orlans *et al.*, 1978). However, Fisher *et al.* (1979) reported that, in two liver per-

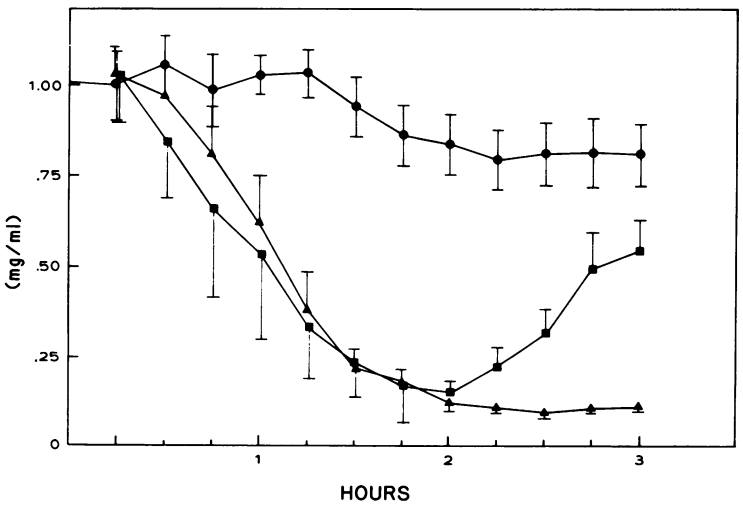

Figure 5. Kinetics of IgA levels in bile of control rats (●—●, $n = 5$) and in bile of rats injected with rat (▲—▲, $n = 4$) or human (■—■, $n = 4$) FSC. Each point is the mean ± SE.

fusions, IgG anti-rat FSC antibody was slightly transferred (3·2% as compared with 24% for pIgA). Here, in five *in vivo* experiments, we obtained a much better transfer of IgG anti-rat SC antibody (24% as compared with 42% for pIgA). This difference is even more striking because, in theory, our IgG could be removed from the blood at other places than the liver. A stronger affinity of our anti-SC antibodies or/and less denaturation could explain our larger transfer. Also, the *in vivo* situation might favour higher recoveries in bile as compared with a perfusion system.

The heterogeneity in molecular size of the radioactive material in bile of rats injected with rabbit ^{125}I-IgG anti-rat SC could be explained by soluble immune complexes (FSC with IgG anti-FSC) of various sizes, including possible $F(ab')_2$ fragments. Further experiments are in progress to compare the transfer of Fab and $F(ab')_2$ fragments with the intact IgG anti-SC antibodies.

The transfer of IgG anti-rat SC antibody was partly inhibited by addition of rat FSC or human pIgA, before the i.v. injection (Table 2 and Fig. 3). On a molar basis, the FSC was in twenty-five to forty-fold molar excess, whereas the pIgA was in about 120-fold molar excess to the anti-SC antibody. Why could IgG anti-SC antibody, in presence of SC excess, still be transferred into bile if the transfer implies binding to FSC on rat hepatocytes? A possible explanation is that the complexes of IgG anti-FSC antibody with FSC excess, once in blood, could dissociate by a dilution effect and/or by removal of free FSC by catabolism and/or binding to plasma pIgA. Human pIgA, in large molar excess, also decreased the transfer of the IgG anti-FSC. A competition between pIgA and anti-SC antibody for the SC on the hepatocyte membrane could explain it, although the affinity of human pIgA for rat FSC of around 10^7 M^{-1} (Socken & Underdown, 1978), is probably lower than that of the anti-SC antibody. In both cases, the kinetics of the antibody transfer were slower, suggesting that a competition was taking place.

In the model with FSC present on hepatocytes as receptor for pIgA transport, one could predict that the i.v. injection of a large amount of the receptor itself would decrease the transfer of both endogenous and exogenous pIgA. This prediction was indeed verified (Table 3; Figs 4 and 5). Transfer of exogenous pIgA in bile was delayed and reduced four times after simultaneous injection of rat FSC, and 2·7 times after human FSC injection. Furthermore, i.v. injections of rat or human FSC (Fig. 5) induced a subsequent fall of the endogenous sIgA concentration in bile; a difference appeared in bile after 2 h between the rats injected with human and those injected with rat FSC. The latter maintained a low IgA level in bile (7·5-fold lower than controls) from 2 to 3 hr after injection, whereas the former displayed minimal IgA levels at 2 hr and increasing levels thereafter. This again suggests that, despite the larger dose administered, the human FSC was less efficient in impairing this transfer. It is suggested that the mechanism of this transfer inhibition could be as follows: FSC injected combines immediately with the majority of the circulating pIgA, forming sIgA, which is poorly transferred. The excess FSC, if able to circulate for enough time in the plasma, could continue to form sIgA by combining with additional pIgA which continuously arrives in blood from the thoracic duct lymph (Vaerman, André, Bazin & Heremans, 1973) and perhaps other sources as well (Nakamura, Roy & Mäkelä, 1973). The result would be a prolonged decrease of IgA transfer into bile.

Our *in vivo* data are compatible with the model of hepatic transfer of IgA according to which rat FSC is a receptor for pIgA, on the sinusoidal membrane of rat hepatocytes. After binding of pIgA to this receptor the complex could be endocytosed, the vesicles could migrate towards the biliary secretory pole and be discharged in the bile canaliculus (Mullock, Hinton, Dobrota, Peppard & Orlans, 1979; Renston, Jones, Christiansen, Hradek & Underdown, 1980), in a way very similar to that described for the translocation of IgA through differentiated human colon cancer cells (Nagura *et al.*, 1979).

It was recently demonstrated that rat SC is the IgA-receptor on *in vitro* cultured hepatocytes (Orlans *et al.*, 1979; Socken *et al.*, 1979; Limet, Schneider, Trouet & Vaerman, 1981). Altogether, the *in vitro* results and the data of our *in vivo* experiments, are complementary and give a strong molecular basis for the proposed model of transfer of IgA from blood to bile. Antibody activity in bile sIgA has been well demonstrated (Lemaître-Coelho, Jackson & Vaerman, 1978b; Hall, Orlans, Reynolds, Dean, Peppard, Gyure & Hobbs, 1979). It remains to be established if this antibody transfer is of any functional value for the rat local intestinal immunity and if such transfer occurs in different species with the same efficiency.

ACKNOWLEDGMENTS

The authors are indebted to Dr H. Bazin (UCL,

IMEX, B-1200 Brussels) for his generous supply of rat myeloma sera. The technical assistance of Mrs Naze De Mets, A. De Ryck-Langendries and Mr E. Van Roost was highly appreciated.

This work was supported by Grant No. 3.4504.70 from the Fonds de la Recherche Scientifique Médicale, Brussels. I.L.-C. is Chargée de Recherches from the F.N.R.S., Brussels. G.A.-A. is a fellow of CONACYT, Mexico and C.B.-A. a scholar of the Belgian government.

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