Immunology 1984 **53** 121

Biliary transport of serum IgA in sheep

R. SCICCHITANO, A. J. HUSBAND & A. W. CRIPPS* *Faculty of Medicine, The University of Newcastle, New South Wales, and *Hunter Immunology Unit, Royal Newcastle Hospital, Newcastle, New South Wales, Australia*

Accepted for publication 13 April 1984

Summary. The existence of a hepato-biliary pump for IgA has been studied in sheep by measuring appearance of radioactivity in bile after simultaneous i.v. injection of IgG2 and homologous dimeric IgA each labelled with either ^{125}I or ^{131}I. These experiments confirm that in sheep, as in rodents, IgA is the predominant immunoglobulin in bile and is selectively transported into bile from serum relative to IgG2. In addition, there is evidence for local production of IgA in bile presumably by plasma cells within the liver and biliary tree.

INTRODUCTION

Since the first demonstration that rat bile contains secretory IgA (Lemaître-Coelho, Jackson & Vaerman, 1977) and that polymeric IgA is rapidly and selectively transferred from blood to bile (Jackson *et al.*, 1978; Orlans *et al.*, 1978), much effort has been devoted to elucidating the mechanism and relevance of this secretion process. The cells involved appear to be the hepatocytes (Birbeck *et al.*, 1979) which bind polymeric IgA by secretory component (SC) expressed on the sinusoidal membrane (Orlans *et al.*, 1979; Socken *et al.*, 1979) and the SC-IgA complex is transported by endocytic vesicles through the cytoplasm and discharged into the canaliculae (Renston *et al.*, 1980). Although original studies were concerned with plasmacytoma-derived IgA, it is now known that polyclonal IgA with defined antibody activity as well as antigen complexed with IgA are also transported into bile by similar mechanisms (Reynolds *et al.*, 1980; Hall *et al.*, 1979; Lemaître-Coelho *et al.*, 1981; Russell, Brown & Mestecky, 1981, 1982).

All of this work was undertaken in rodents, in particular, rats, which have the ability to transport homologous and heterologous polymeric IgA (Peppard *et al.*, 1981; Lemaître-Coehlo *et al.*, 1981) and also in rabbits (Hall *et al.*, 1981; Delacroix *et al.*, 1982). Whether this mechanism exists in larger mammals has been a controversial issue (Hall, Gyure & Payne, 1980; Hall *et al.*, 1981; Fukumoto & Brandon, 1982; Vaerman *et al.*, 1982).

In sheep, circulating IgA is in the dimeric form (Vaerman, 1970) and plasma concentrations are low despite the fact that the intestinal mucosa produces large amounts of IgA, and much of this reaches the circulation via the intestinal lymphatic duct (Quin, Husband & Lascelles, 1975). Because the secretory immune system in ruminants is analogous to that in rodents in many respects (Lascelles & McDowell, 1974) it is possible that the hepato-biliary transport of IgA plays an important role in ruminant secretory immunity.

In this study the kinetics and magnitude of the

Abbreviations: SC, secretory component; PBS, phosphate-buffered (pH 7·4) saline; TCA, trichloroacetic acid; RIA, radioimmunoassay.

Correspondence: Dr A. J. Husband, Faculty of Medicine, The University of Newcastle, NSW 2308, Australia.

transfer from the circulation into bile of radiolabelled ovine dimeric IgA has been compared with that of IgG2 in sheep and estimates of local production have been obtained for these immunoglobulins.

MATERIALS AND METHODS

Animals

Six adult cross-bred wethers were used and were housed in metabolic cages for the duration of the experiment. They were offered lucerne hay and water *ad libitum*.

Preparation of immunoglobulins and antisera

Ovine IgG2 was prepared from sheep serum by Sephadex DEAE-A50 (Pharmacia, Uppsala, Sweden) ion exchange chromatography as previously described (Brandon, Watson & Lascelles, 1971). Dimeric IgA was prepared from ovine intestinal lymph as follows: lipoprotein was precipitated by adding 0·02 ml of 10% (w/v) dextran sulphate-500 (Pharmacia), and 0·1 ml of 1 M $CaCl_2$ per ml of intestinal lymph. The precipitate was removed by centrifugation at 3000 r.p.m. for 10 min at 4°, and the supernatant concentrated five-fold by ultrafiltration using an XM50 filter (Amicon, Lexington, U.S.A.). The concentrated lymph was fractionated on a 2·5 × 80 cm Sephacryl S300 (Pharmacia) column. The IgA-rich fractions were pooled, concentrated and applied to a Sepharose-4B (Pharmacia) column (0·9 × 8·0 cm) to which rabbit anti-ovine IgA (α-chain specific) antiserum had been coupled. Bound IgA was eluted with 0·1 M glycine-HCl buffer (pH 2·0) containing 0·5 M NaCl.

Antisera to ovine immunoglobulins were raised in rabbits using the method of Watson, Brandon & Lascelles (1972) and were rendered heavy chain-specific by affinity chromatography. The specificity of all reagents was tested by double diffusion and immunoelectrophoresis in agarose. The dimeric nature of the IgA was confirmed by gel filtration chromatography, described elsewhere (Scicchitano, Husband & Cripps, 1984).

Radiolabelling of immunoglobulins

IgA and IgG2 were labelled with ^{125}I and ^{131}I (obtained as sodium iodide; New England Nuclear, Boston, U.S.A.) following the chloramine-T method of Bolton (1977) with the following modification. For labelling with ^{131}I, borate buffer (pH 8·0) was used instead of phosphate-buffered (pH 7·4) saline (PBS). This resulted in higher levels of labelling as judged by 20% w/v trichloroacetic acid (TCA) precipitation. Labelled preparations were chromatographed on Sephadex G25 (Pharmacia) to separate protein-bound from free iodine.

Characterization of labelled immunoglobulin

Radiolabelled preparations were characterized by adding 100 μl of labelled material to 2·0 ml of normal sheep serum and fractionating on Sephacryl S300 (Pharmacia). Radioactivity was shown to be associated with the appropriate region of the normal sheep serum optical density (280 nm) profile (Scicchitano *et al.*, 1984).

Radioimmunoassay

The concentration of ovine immunoglobulins and albumin in plasma and lymph were measured by radioimmunoassay (RIA) described by Cripps *et al.* (1983) in which the specificity was modified for sheep (Cripps *et al.*, 1984). Briefly, the assay was performed by adding to polypropylene tubes a dilution of 100 μl of the sample to be assayed, 100 μl of appropriate antiserum at an optimal dilution and 100 μl of the respective radiolabelled protein at a standard concentration. After mixing, the tubes were incubated at 4° overnight, then 100 μl of goat anti-rabbit IgG immunobeads (Bio-Rad Laboratories, Richmond, U.S.A.) were added. The tubes were mixed, incubated at room temperature for 4 hr then 1 ml of cold PBS was added. The tubes were mixed again and the immunobeads pelleted by centrifugation at 800 *g* for 15 min. This procedure was repeated, the supernatants were aspirated and the radioactivity of the pelleted beads counted. Serial dilutions of a standard serum of known immunoglobulin and albumin content were assayed to construct standard curves. All assays were performed in duplicate.

Experimental procedure

The bile ducts of six sheep were cannulated under thiopentone and halothane general anaesthesia. A right subcostal incision was made, the liver retracted and the cystic duct ligated. A longitudinal incision (approximately 2 cm) was made in the well of the common bile duct above the level of the pancreatic duct, and a silicon rubber 'T' tube inserted into the duct. The proximal arm of the 'T' tube was placed in the hepatic duct and the distal arm positioned to prevent contamination by pancreatic secretions. The incision in the bile duct was sutured and the tube

exteriorized through the skin incision. Animals were given parental antibiotics for 3 days and the bile cannula flushed once daily with physiological saline to prevent bile salt precipitation. The exteriorised portion of the cannula was occluded except at sampling times, so that bile flow into the duodenum was maintained at normal levels.

Animals were allowed to recover from surgery for 24 hr and were then injected intravenously with radiolabelled IgA and IgG2. Six sheep were used in these experiments; two were injected with ^{125}I-IgA alone, two with ^{125}I-IgA and ^{131}I-IgG2, and two with ^{125}I-IgG2 and ^{131}I-IgA.

After injection of radiolabelled immunoglobulin, paired samples of plasma and bile were taken over a period of 48 hr. Bile was collected over a period of 20 min, and blood was withdrawn into heparinized tubes from an indwelling jugular venous catheter during the middle of the bile collection period. Both blood and bile were centrifuged at 3000 r.p.m. for 5 min and the supernatants stored at $-20°$ until analysed.

Radioactivity was measured in duplicate aliquots of 500 μl of bile and plasma using an LKB Wallac 1270 gamma counter. Counts for ^{125}I were adjusted for carryover of ^{131}I counts. The proportion of radioactivity associated with protein in bile and plasma was obtained in four of the six sheep by precipitation with 20% TCA of 100 μl of plasma or 500 μl of bile.

Bile samples taken before injection of label were tested for the presence of immunoglobulin by double diffusion and immunoelectrophoresis in agarose using monospecific rabbit antisera to ovine immunoglobulins.

Samples of bile taken 1 hr after injection were fractionated by gel filtration chromatography on Sepharose 6B (Pharmacia), using 0·01 M Tris HCl buffer (pH 8·0) in 0·15 M NaCl. Normal ovine serum or colostrum was added to the bile samples before fractionation to provide a reference profile. Radioactivity was measured on 500 μl aliquots of each fraction.

Data analysis

Comparison of radioactivity counts. To enable direct comparisons between the radioactive counts from different sheep, all c.p.m. data were expressed as a percentage of c.p.m. in plasma at 15 min ($\%T_{0\cdot25}$ c.p.m.) for each isotope.

Selective indices. The selectivity of transfer of IgA relative to IgG2 from plasma into bile was computed from radioactivity ratios using a modification of the selective index ratio described by Brandon *et al.* (1971):

$$\text{Selective index} = \frac{\text{c.p.m. IgA bile}}{\text{c.p.m. IgA plasma}} \times \frac{\text{c.p.m. IgG2 plasma}}{\text{c.p.m. IgG2 bile}}$$

Any selective index value >1 indicates selective transport of IgA relative to IgG2.

An alternative method for calculation of selective indexed based on immunoglobulin and albumin concentration ratios was also used:

$$\text{Selective index} = \frac{\text{mg/ml Ig bile}}{\text{mg/ml albumin bile}} \times \frac{\text{mg/ml albumin plasma}}{\text{mg/ml Ig plasma}}$$

Any value >1 indicates selective transport of immunoglobulin relative to albumin.

Specific activities. The specific activity of IgA and IgG2 was determined in plasma and bile at 12, 18, 24, 36 and 48 hr after injection as the ratio of the radioactive count and the respective immunoglobulin concentration, expressed as $\%T_{0\cdot25}$ c.p.m./mg. Bile:plasma specific activity ratios were computed for each sheep for IgA and IgG2. Specific activity ratios <1 indicate local antibody production.

Statistical significance of the difference between means was determined with the use of Student's *t*-test.

RESULTS

Double diffusion analysis of normal bile against monospecific ovine anti-immunoglobulin sera demonstrated the presence of all immunoglobulin isotypes in ovine bile including IgA. The concentration of immunoglobulins and albumin in plasma and bile are shown in Table 1. While levels of albumin, IgG1, IgG2 and IgM in bile are all much less than in plasma the level of IgA in bile is about twice the plasma concentration.

Radioactivity associated with IgA and IgG2 in bile and plasma, expressed as a percentage of the activity in plasma at 15 min after injection ($T_{0\cdot25}$) is shown in Fig. 1. Plasma radioactivity associated with IgA rapidly decreased, so that at 3 hr only $53\cdot2 \pm 5\cdot8\%$ (mean $\pm$ SE for six sheep) of the counts present at $T_{0\cdot25}$ remained in the circulation. IgA activity in bile rapidly increased and the peak mean value recorded at 1 hr was $151\cdot82 \pm 30\cdot48\%$ of the $T_{0\cdot25}$ plasma value, significantly greater than the corresponding activity in plasma ($P < 0\cdot05$).

Although some IgG2-associated radioactivity appeared in bile, the peak radioactivity at 1 hr was only $35\cdot3 \pm 2\cdot0\%$ of the $T_{0\cdot25}$ plasma activity for IgG2

Table 1. Concentration of immunoglobulins and albumin in plasma and bile of sheep

	IgG1	IgG2	IgM	IgA	Albumin
Plasma	16·35±2·11	6·80±0·73	2·41±0·23	0·56±0·18	23·90±2·53
Bile	0·05±0·01	0·04±0·01	0·20±0·14	0·93±0·43	0·08±0·03

Values are mean mg/ml±standard error of measurements from six animals (10 samples per animal).

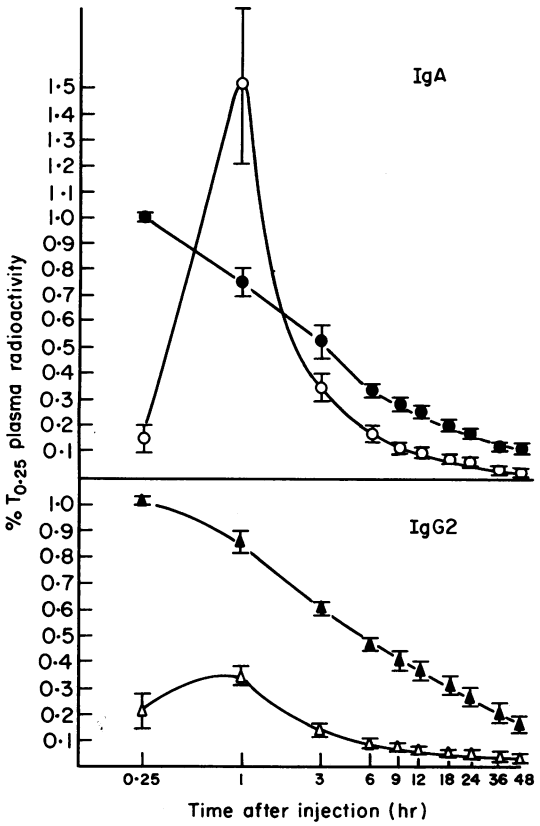

Figure 1. Radioactivity associated with IgA in plasma (●——●) and bile (○——○) and with IgG2 in plasma (▲——▲) and bile (△——△) expressed as a percentage of the activity at 15 min (%$T_{0·25}$) in plasma. Plotted points represent means of data from six sheep for IgA and four sheep for IgG2. Vertical bars represent standard errors.

and levels remained well below that of plasma at all times.

The TCA precipitability of counts associated with IgA and IgG2 respectively in bile and plasma at varying times after injection are shown in Fig. 2. While plasma activities were always between 80 and 95% TCA-precipitable, percent protein-bound activities in bile were always lower than plasma (50–70% TCA-precipitable at 1 hr, declining to around 30% by the last sample collection at 48 hr). It is not clear whether the lower TCA precipitability in bile resulted from selective transport of degraded label or release of label after transport.

In view of the findings of Fukumoto & Brandon (1982) regarding catabolism of immunoglobulins in the liver in sheep (see discussion section) the latter possibility seems most likely and therefore all subsequent calculations are based on total c.p.m. recovered. In any case since the percentage of TCA-precipitable counts for IgG2 and IgA are similar within the plasma or bile compartments it is unlikely that this method of calculations would prejudice the outcome with respect to comparisons between the two immunoglobulins.

Gel filtration analysis of bile samples taken 1 hr after injection substantiated the findings with regard to TCA precipitability in that radioactivity associated with the isotope used to label IgA eluted in two peaks, one corresponding with intact dimeric IgA and the other corresponding with smaller molecular weight fragments (Fig. 3). Double diffusion analysis revealed no α-chain determinants in fractions associated with the second radioactivity peak. Radioactivity associated with IgG2 was too low in bile to allow detection after gel filtration.

Calculation of recovery of injected dose of labelled IgA in bile was based on a flow rate of 200 ml/hr for sheep bile published previously (Fukumoto & Brandon, 1982) since direct estimates of flow rate were not possible because of the need to use a 'T' tube cannula to maintain animals in normal physiologic state. On this basis, calculations of cumulative recovery of labelled IgA in bile in two sheep (Fig. 4) indicate that 40% of the injected dose was recovered over the 48 hr collection period. Approximtely 36% of the total

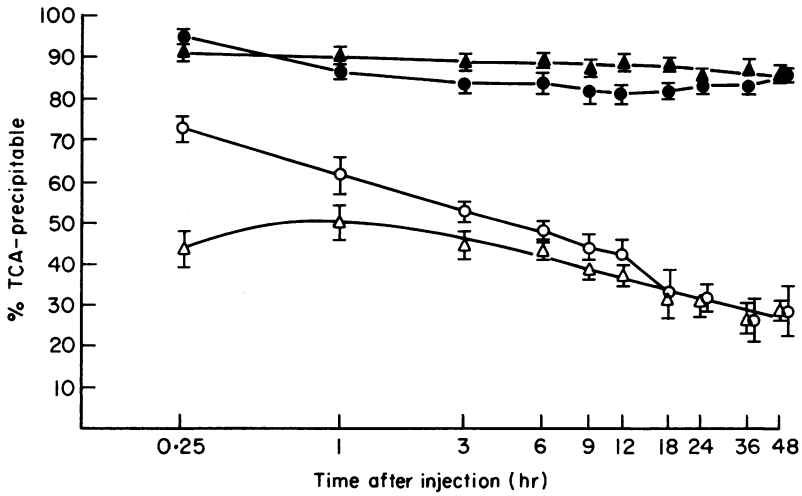

Figure 2. Percentage of TCA precipitable counts for IgA in plasma (●——●) and bile (○——○) and for IgG2 in plasma (▲——▲) and bile (△——△). Plotted points represent means of data from four sheep. Vertical bars represent standard errors.

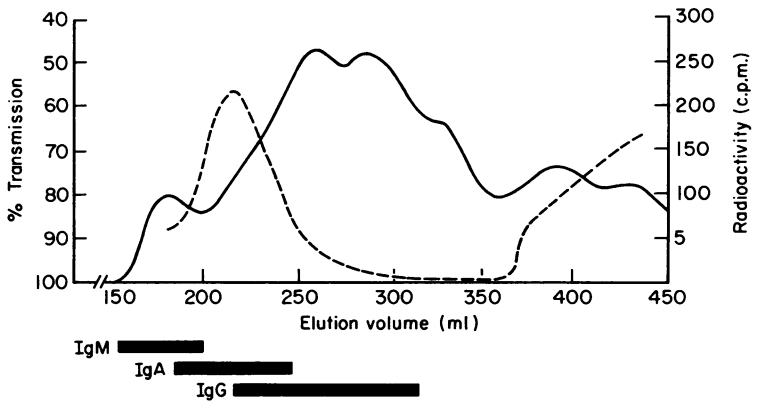

Figure 3. Elution profile of 100 μl of a bile sample (taken 1 hr after intravenous injection of radiolabelled IgA) mixed with 2·0 ml of normal sheep serum eluted on Sepharose 6B. The *solid line* represents a typical optical density profile (% transmission at 280 nm), and the *broken line* represents the profile of radioactivity associated with IgA. *Solid bars* underneath the graph represent immunoglobulin distribution in the profile. Column dimensions, 2·5 × 80 cm; buffer, 0·01 M Tris HCl (pH 8·0) in 0·15 M NaCl; flow rate 20 ml/hr.

counts recovered appeared in bile within 6 hr of injection and 50% of the total recovery occurred within 12 hr.

An estimate of the selectivity of IgA transport relative to IgG2 was obtained from radioactivity ratios using a modification of the selective index ratio previously described by Brandon *et al.* (1971). The change in this ratio with time after injection of label is shown in Table 2. A selective index value > 1 indicates selective transport of IgA relative to IgG2. The selective index increased rapidly following injection of IgA to reach a peak at 1 hr (mean value of 4·21 ± 0·18), coinciding with peak radioactivity, and thereafter decreased, although it remained greater than unity throughout the sampling period. These findings indicate the selective transport of IgA relative to IgG2.

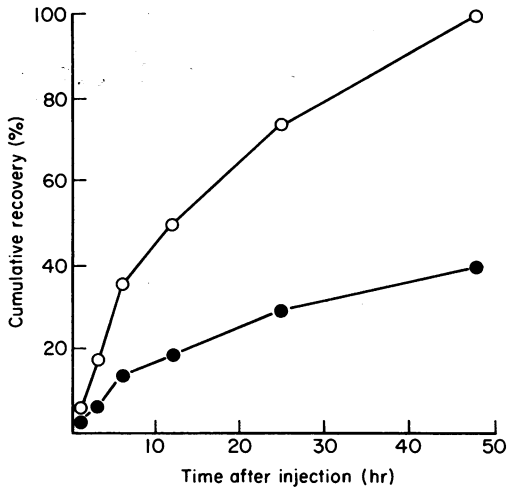

Figure 4. Cumulative recovery of radioactivity associated with IgA in bile expressed as % injected dose (●——●) and as % of total bile recovery (○——○). Calculations based on estimated flow rate of 200 ml/hr (Fukumoto & Brandon, 1982). Plotted points represent mean of data from two sheep.

Table 2. Selectivity of transport of IgA into bile relative to IgG2 by calculation of selective index based on radioactivity ratios

Time after injection (hr)	Selective index*
0·25	0·90±0·17
1	4·21±0·18
3	2·77±0·39
6	2·63±0·50
9	2·55±0·55
12	2·30±0·50
18	2·17±0·39
24	2·04±0·37
36	2·01±0·31
48	1·85±0·33
Mean±SE of all observations	2·34±0·27

$$\text{* Selective index} = \frac{\text{c.p.m. IgA bile}}{\text{c.p.m. IgG2 bile}} \times \frac{\text{c.p.m. IgG2 plasma}}{\text{c.p.m. IgA plasma}}$$

Values are mean±SE of observations from four sheep. Values > 1 indicate selective transport.

An alternative method for demonstrating this selectivity is by calculation of immunoglobulin:albumin concentration ratios for bile and plasma. These calculations for each immunoglobulin (Table 3) indicated that whereas there was no selectivity for IgG1 and IgG2 transport, there was significant selectivity for IgM and dramatic selectivity for IgA.

Specific activities (c.p.m./mg) were calculated after equilibration of IgA and IgG2 between plasma and bile. The ratio of specific activity in bile compared to plasma provides an estimate of local production.

Table 3. Selectivity of transport of immunoglobulins into bile relative to albumin by calculation of selective index based on concentration ratios*

IgG1	IgG2	IgM	IgA
1·05±0·32	1·05±0·32	6·53±1·41	450·30±184·20

$$\text{* Selective index} = \frac{\text{mg/ml Ig bile}}{\text{mg/ml albumin bile}} \times \frac{\text{mg/ml albumin plasma}}{\text{mg/ml Ig plasma}}$$

Values are mean±SE of observations from six sheep. Values > 1 represent selective transport.

Calculations from six sheep yielded a mean (±standard error) of 0·59± 0·18 for IgA and 80·89±21·58 for IgG2. This indicates that approximately 60% of the IgA in bile is plasma-derived, the remainder presumably arising by local production. Specific activity ratios for IgG2 greater than unity indicate that the IgG2 in bile was wholly plasma-derived.

DISCUSSION

In this study the selective transport of homologous dimeric IgA, but not IgG2, from plasma to bile has been demonstrated in sheep. Following the intravenous injection of radiolabelled IgA, radioactivity rapidly appeared in bile, reaching a peak at 1 hr at which time it exceeded that of plasma in all animals. Samples were not taken between 15 min and 1 hr after injection, however, in the course of other experiments in this laboratory where samples were taken every 5 min for the first hour, radioactivity appeared in bile within 10 min and always reached a peak between 45 and 60 min. Gel filtration (Fig. 3) and TCA precipitability analysis (Fig. 2) revealed that at 1 hr after

injection a high proportion of radioactivity was associated with intact dimeric IgA. In contrast, there did not appear to be selective transport of IgG2 from plasma to bile and at no time did the radioactivity in bile exceed that of plasma.

The low proportion of TCA-precipitable counts in bile in the later collections was of some concern although there are several explanations to account for this. By 48 hr the total radioactivity in bile was very low so that the accuracy of estimations of the percentage of TCA-precipitable counts may have been compromised. It is also documented that in sheep the liver is a site of immunoglobulin catabolism (Fukumoto & Brandon, 1982) and much of the non-protein-bound activity probably resulted from degradation of protein after transport from the circulation, especially in view of the consistently high level of protein-bound activity in plasma (Fig. 2).

The results indicate that IgA is the predominant immunoglobulin in sheep bile, levels being approximately twice that of plasma, and this is in contrast to data of Fukumoto & Brandon (1982) who were unable to detect significant amounts of IgA (or IgM) in bile, although Vaerman *et al.* (1982) and Orlans *et al.* (1983) detected IgA in sheep bile in higher concentrations than in plasma, and calculated bile: plasma concentrations ratios which were consistent with the concentrations reported here (Table 1). A selective index based on relative concentration ratios (Table 3), obtained by dividing the bile:plasma ratio for each protein by the same ratio for albumin, provides further evidence that IgA, and possibly IgM, are selectively transported into sheep bile. In rabbits, where active transport of IgA into bile is well documented, a similar index for IgA and IgM has been reported (Delacroix *et al.*, 1982). The evidence for selective transport of IgM arising from the concentration ratios is not suprising in view of the ability of human IgM to bind SC (Brandtzaeg, 1975) and the reported transport of IgM in rat bile by Peppard, Jackson & Hall (1983). A selective index based on radioactivity ratios in plasma and bile (Table 2) confirmed the selective transport of IgA relative to IgG2 and this index was highest when radioactivity in bile was highest at 1 hr after injection.

Following equilibration of IgA and IgG2 in plasma and bile it was possible to estimate their local production in bile by calculation of specific activity ratios. Specific activity ratios in bile for IgG2 were greater than unity, suggesting the absence of local production of this immunoglobulin. This is consistent with data presented by Fukumoto & Brandon (1982). On the other hand, only 60% of IgA in bile was serum derived, the remainder arising presumably from local production. Although in normal sheep there is an abundance of IgA-containing cells in the wall of the gall bladder (Scicchitano & Husband, unpublished), this was not the source of locally produced IgA here because the cystic duct was ligated in all cannulated sheep. Therefore production of IgA must have occurred in the liver or common bile duct. Immunofluorescence studies of liver indicate the presence of only occasional IgA-containing cells, but this low number may be sufficient to account for the extent of local production because of the bulk of the liver.

There is conflicting evidence regarding the existence of a hepato-biliary pump for IgA in ruminants. Hall *et al.* (1980) injected sheep with labelled heterologous (human and rat) polymeric IgA and found no evidence of transport into bile, and indeed were unable to detect any radioactivity in bile. Beh (reported by Vaerman *et al.*, 1982) injected labelled human polymeric IgA into two sheep and was able to demonstrate the appearance of radioactivity in bile and this activity exceeded that in plasma at 1 and 2 hr. However, these workers suggested that most, if not all, of this activity as associated with low ($< 40{,}000$) molecular weight protein which they attributed to degradation of labelled protein by the liver. In the present study, homologous dimeric IgA was used and by gel filtration it was demonstrated that a substantial proportion of the radioactivity was associated with intact dimeric IgA (Fig. 3). Furthermore, most of the radioactivity was protein bound as demonstrated by the high TCA precipitability at 1 hr (Fig. 2). Following gel filtration, the radioactivity which was not associated with dimeric IgA eluted well after the void volume and may represent label which had become detached from IgA after transport into bile. In any case ovine α-chains could not be detected in fractions associated with the second radioactivity peak of the elution profiles using double diffusion analysis.

It should also be emphasized that the design of the experiment provided a control for non-selective transport of degradation products, by simultaneous injection of IgA and IgG2, there being no selective transport mechanism for the latter immunoglobulin. If the appearance of radioactivity in bile had been due to transport of degradation products from plasma to bile, or to catabolism of denatured or abnormal immunoglobulins by the liver, there might have been no difference in behaviour between the two immunolo-

gulins. Further, the results were the same whether IgA was labelled with ^{131}I or ^{125}I.

Hall *et al.* (1981) did study the transfer of homologous ovine IgA from plasma to bile although results from only one sheep were reported and the molecular weight of the injected IgA was not determined. Radioactivity was detected in bile within 1 hr, and the kinetics of radioactivity recovery were identical to those reported here, but only 5% of the injected activity was recovered from bile within 8 hr. In the present experiments it was not possible to directly determine the proportion of the injected activity recovered in bile because of the presence of a 'T' tube cannula enabling sampling of only an aliquot of the total bile output. This was considered necessary to maintain physiologic conditions. But, based on flow rates reported by Fukumoto & Brandon (1982) of 200 ml/hr in sheep with a similar shunt, it was estimated that up to 20% of the activity was recovered in bile in the first 6 hr, a figure comparable with that reported in rats (Hall *et al.*, 1981; Lemaître-Coelho *et al.*, 1981), although in those experiments much greater quantities of IgA were injected than were used in the current experiment and the amount of labelled IgA available for transport by hepatocytes may be dose-dependent.

The experiments reported in this paper provide both direct and indirect evidence for the selective transport of IgA from plasma to bile in sheep indicating that in ruminants, as in rodents, this is an important component of the secretory immune system. This is not surprising in view of the similarities in other respects between the rodent and ruminant secretory immune processes. These findings are also teleologically consistent, since a substantial amount of dimeric IgA enters the circulation from the intestine (Quin *et al.*, 1975) and respiratory tract (Scicchitano *et al.*, 1984) of sheep, yet, as in other species, plasma IgA concentrations remain low; and ligation of the common bile duct in sheep leads to a rapid elevation of plasma IgA levels (Fukumoto & Brandon, 1982).

ACKNOWLEDGMENTS

The authors acknowledge the skilled technical assistance of Ms N. Kiem and the cooperation of the C.B. Alexander Agricultural College in making sheep available for this experiment. This project was supported by grants from the Wool Research Trust Fund of the Australian Wool Corporation and by the National Health and Medical Research Council. R.S. was supported by the NH & MRC as a Research Associate in Allergic Diseases.

REFERENCES

BIRBECK M.S.C., CARTWRIGHT P., HALL J.G., ORLANS E. & PEPPARD J. (1979) The transport by hepatocytes of immunoglobulin A from blood to bile visualised by autoradiography and electron microscopy. *Immunology*, **37**, 477.

BOLTON A.E. (1977) *Radioiodination Techniques*. Review 18, Radiochemical Centre, Amersham.

BRANDON M.R., WATSON D.L. & LASCELLES A.K. (1971) The mechanism of transfer of immunoglobulin into mammary secretion of cows. *Aust. J. exp. Biol. med. Sci.* **49**, 613.

BRANDTZAEG P. (1975) Human secretory immunoglobulin M. An immunochemical and immunohistochemical study. *Immunology*, **29**, 559.

CRIPPS A.W., VIRGIN R.J., LEWINS E.G. & CLANCY R.L. (1983) Radioimmunoassay of human IgG, IgA and IgM. *J. immunol. Meth.* **57**, 185.

CRIPPS A.W., HUSBAND A.J., SCICCHITANO, R. & SHELDRAKE R.F. (1984) Quantitation of ovine IgG1, IgG2, IgA, IgM and albumin by radioimmunoassay. *Vet. Immunol. Immunopath.* (in press).

DELACROIX D.L., DENEF A.M., ACOSTA G.A., MONTGOMERY P.C. & VAERMAN J.P. (1982) Immunoglobulins in rabbit hepatic bile: Selective secretion of IgA and IgM and active plasma-to-bile transfer of polymeric IgA. *Scand. J. Immunol.* **16**, 343.

FUKUMOTO T. & BRANDON M.R. (1982) Importance of the liver in immunoglobulin catabolism. *Res. vet. Sci.* **32**, 62.

HALL J.G., GYURE L.A. & PAYNE A.W.R. (1980) Comparative aspects of the transport of immunoglobulin A from blood to bile. *Immunology*, **41**, 899.

HALL J.G., GYURE L.A., PAYNED A.W.R. & ANDREW E. (1981) Comparative aspects of secretory immunity: the transport of heterologous IgA from blood to bile in experimental animals. In: *The Mucosal Immune System* (ed. F. J. Bourne), p. 31. Martinus Nijhoff, London.

HALL J., ORLANS E., REYNOLDS J., DEAN C., PEPPARD J., GYURE L. & HOBBS S. (1979) Occurrence of specific antibodies of the IgA class in the bile of rats. *Int. Archs Allergy appl. Immun.* **59**, 75.

JACKSON G.D.F., LEMAÎTRE-COELHO I., VAERMAN J.P., BAZIN H. & BECKERS S. (1978) Rapid disappearance from serum of intravenously injected rat myeloma IgA and its secretion into bile. *Eur. J. Immunol.* **8**, 123.

LASCELLES A.K. & MCDOWELL G.H. (1974) Localized humoral immunity with particular reference to ruminants. *Transplant. Rev.* **19**, 170.

LEMAÎTRE-COELHO I., ALTAMIRANO G.A., BARRANCO-ACOSTA C., MEYKENS R. & VAERMAN J.P. (1981) *In vivo* experiments involving secretory components in the rat hepatic transfer of polymeric IgA from blood into bile. *Immunology*, **43**, 261.

LEMAÎTRE-COELHO I., JACKSON G.D.F. & VAERMAN J.P. (1977) Rat bile as a convenient source of secretory IgA and free secretory component. *Eur. J. Immunol.* **7**, 588.

ORLANS E., PEPPARD J., FRY J.F., HINTON R.H. & MULLOCK B.M. (1979) Secretory component as the receptor for polymeric IgA on rat hepatocytes. *J. exp. Med.* **150,** 1577.

ORLANS E., PEPPARD J.V., PAYNE A.W.R., FITZHARRIS B.M., MULLOCK B.M., HINTON R.H. & HALL J.G. (1983) Comparative aspects of the hepato-biliary transport of IgA. *Ann. N.Y. Acad. Sci.* **409,** 411.

ORLANS E., PEPPARD J., REYNOLDS J. & HALL J. (1978) Rapid active transport of immunoglobulin A from blood to bile. *J. exp. Med.* **147,** 588.

PEPPARD J.V., JACKSON L.E. & HALL J.G. (1983) The occurrence of secretory IgM in the bile of rats. *Clin. exp. Immunol.* **53,** 623.

PEPPARD J., ORLANS E., PAYNE A.W.R. & ANDREW E. (1981) The elimination of circulating complexes containing polymeric IgA by excretion in the bile. *Immunology*, **42,** 83.

QUIN J.W., HUSBAND A.J. & LASCELLES A.K. (1975) The origin of immunoglobulins in the intestinal lymph of sheep. *Aust. J. exp. Biol. med. Sci* **53,** 205.

RENSTON R.H., JONES A.L., CHRISTIANSEN W.D. & HRADEK G.T. (1980) Evidence for vesicular transport mechanism in hepatocytes for biliary secretion of immunoglobulin A. *Science*, **208,** 1276.

REYNOLDS J., GYURE L., ANDREW E. & HALL J.G. (1980) Studies of the transport of polyclonal IgA antibody from blood to bile in rats. *Immunology*, **39,** 463.

RUSSELL M.W., BROWN T.A. & MESTECKY J. (1981) Role of serum IgA. Hepatobiliary transport of circulating antigen. *J. exp. Med.* **153,** 968.

RUSSELL M.W., BROWN T.A. & MESTECKY J. (1982) Preferential transport of IgA and IgA-immune complexes to bile compared with other external secretions. Molec. Immunol. **19,** 677.

SCICCHITANO R., HUSBAND A.J. & CRIPPS A.W. (1984) Immunoglobulin-containing cells and the origin of immunoglobulins in the respiratory tract of sheep. *Immunology*, **52,** 529.

SOCKEN D.J., JEEJEEBHOY K.H., BAZIN H. & UNDERDOWN B.J. (1979) Identification of secretory component as an IgA receptor on rat hepatocytes. *J. exp. Med.* **50,** 1538.

VAERMAN J.P. (1970) Studies on IgA immunoglobulins in man and animals. *Thesis*, Sintal-Louvain.

VAERMAN J.P., LEMAÎTRE-COELHO J.M., LINNET J.N. & DELACROIX D.L. (1982) Hepatic transfer of polymeric IgA from plasma to bile in rats and other mammals: a survey. In: *Recent Advances in Mucosal Immunity* (eds W. Strober, L. Å. Hanson and K. W. Sell), p. 233. Raven Press, New York.

WATSON D.L., BRANDON M.R. & LASCELLES A.K. (1972) Concentrations of immunoglobulins in mammory secretions of ruminants during involution, with particular reference to selective transfer of IgG_1. *Aust. J. exp. Biol. med. Sci.* **50,** 535.