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The effect of lactation on the transport of serum-derived IgA into bile of sheep

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Summary. The effect of lactation on the selective transport of IgA from serum into bile and the relationship between volume of milk production and IgA recovery in bile was examined in sheep following intravenous injection of radiolabelled dimeric IgA.

Bile:plasma radioactivity ratios for lactating and non-lactating sheep were $6{\cdot}20 \pm 1{\cdot}50$ and $1{\cdot}97 \pm 0{\cdot}35$, respectively, and in lactating sheep the milk:plasma ratio was $8{\cdot}20 \pm 0{\cdot}81$. When biliary flow rates and milk yield were taken into account, 7·3% and 42.0% of the radiolabelled IgA present in plasma at 1 min after injection was recovered in bile and milk, respectively, of low milk yielding ewes, whereas in high milk yielding ewes, recoveries were 4·0% and 66·0%, respectively.

The data suggest that during lactation there is an overall increase in transport of IgA into bile, possibly due to the effect of lactogenic hormones on secretory epithelia, but that there may be competition between hepatocytes and mammary epithelial cells for available serum-derived IgA, depending on the secretory activity of the gland.

INTRODUCTION

In rodents, polymeric IgA (pIgA) is selectively transported from blood into bile by a mechanism involving the combination of IgA with secretory component (SC) expressed on hepatocytes, and transport of the pIgA-SC complex via endocytic vesicles through the hepatocytes into the bile canaliculae (Vaerman *et al.*, 1982).

Although previous evidence for this phenomenon in ruminants has been conflicting (Hall, Gyure & Payne, 1980; Hall *et al.*, 1981; Fukumoto & Brandon, 1982; Vaerman *et al.*, 1982), it has recently been demonstrated in this laboratory that homologous dimeric IgA is selectively transported into ovine bile (Scicchitano, Husband & Cripps, 1984). In view of the findings of Sheldrake *et al.* (1984) that, during lacation, IgA is also selectively transported into ovine milk, it was of interest to determine whether, in lactating animals, the mammary gland and biliary tree compete for available serum IgA. Alternatively, in view of the data of Wira & Sandoe (1980), Sullivan, Underdown & Wira (1983) and Weisz-Carrington *et al.* (1978) that SC expression, glandular epithelium development and the subsequent transport of IgA are influenced by circulating levels of lactogenic hormones, during lactation biliary transport of IgA may be increased compared with non-lactating animals.

The accumulation of radiolabelled dimeric IgA was observed simultaneously in bile and milk of lactating sheep following its intravenous injection to determine the effect of lactation upon the transport of IgA into bile, to describe the kinetics of transport under these circumstances, and to quantitate the relative importance of transport at these two sites.

Abbreviations: pIgA, polymeric IgA; SC, secretory component; TCA, trichloroacetic acid; $T_{1\ min}$, 1 min after injection.

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MATERIALS AND METHODS

Animals

Five adult Merino ewes which had lambed approximately 10 days earlier were used. All ewes had lambs suckling, but two of the ewes were additionally milked out twice daily to encourage higher milk production. For the duration of the sampling period, lambs were removed from these two ewes and hand milking was continued so that accurate milk yields could be determined. Yields of 54 and 56 ml/hr, respectively, were recorded for these two ewes. In order to maintain milk yields at physiological levels in the remaining three, ewes, lambs were allowed to suckle; it was not possible to obtain accurate milk yields from these sheep.

In addition, another four ewes which were not lactating were used to obtain measurements of biliary flow rate.

All animals were maintained in metabolic cages throughout the duration of the experiment and were fed hay and water *ad libitum*.

Immunoglobulin preparation and radioactive labelling

Dimeric non-secretory IgA was prepared from intestinal lymph and labelled with either ^{125}I or ^{131}I (obtained as sodium iodide; New England Nuclear, Boston, MA) as previously described (Sheldrake *et al.*, 1984).

Radioactive sample assay and gel filtration

Samples of bile, milk and plasma were counted in duplicate for 60s using a volume of 0·5 ml. The proportion of protein-bound radioactivity in bile, milk and plasma was determined by precipitation with trichloracetic acid (TCA) 20% w/v using 100 μl of sample.

Samples of bile and plasma taken 1 hr after injection, and milk taken 3 hr after injection, were fractionated by gel filtration chromatography on Sepharose 6B (Pharmacia Fine Chemicals AB, Uppsala, Sweden), eluting with 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 in 500-μl aliquots of each fraction. These elutions indicated that the bulk of radioactivity in plasma and bile was associated with the appropriate portion of the gel filtration profile as previously shown (Scicchitano *et al.*, 1984). For milk, there was a second peak of radioactivity associated with small molecular weight material.

Surgical procedures

The bile ducts of all sheep were cannulated under halothane-N_2O general anaesthesia using the technique described by Scicchitano *et al.* (1984). Briefly, the cystic duct was ligated to isolate the gall bladder and a T-tube, with an internal diameter of 2 mm, was inserted into the common bile duct allowing bile to flow continuously into the duodenum, but enabling bile samples to be obtained when required.

For the four ewes used to determine total biliary flow rate, the cystic duct was similarly ligated and, in two sheep, a biliary shunt was inserted with an external sampling tap, while the remaining two sheep a single tube inserted into the common bile duct was exteriorized and the duct ligated below the point of cannulation.

Experimental design

Lactating ewes with bile ducts cannulated by T-tube were reunited with their lambs and allowed a period of 3–4 days to recover from surgery. They were then injected i.v. with 5 ug of radiolabelled IgA in a volume of 2·0 ml. After injection of the radiolabelled immunoglobulin, samples of bile, milk and plasma were collected at intervals of 1, 5, 10, 15, 20, 30, 45 and 60 min, and 3, 6, 9, 12, 18, 24 and 48 hr. Blood was withdrawn into heparinized tubes, centrifuged at 1500 *g* and the supernatants collected for analysis.

In the two ewes which were additionally hand-milked, lambs were removed at the time of injection of label. In order to facilitate milk collection, 10 IU oxytocin (Sintocinon, Sandoz (Aust.) Sydney, Australia) were injected intravenously into all ewes prior to sampling, and the bulk of the milk in the udder removed from the hand-milked ewes, and 10-ml aliquots taken from the remaining three suckled ewes. Bile was collected via the exteriorized arm of the biliary T-tube for a period of 1–2 min, after which the collecting arm was occluded to restore the flow of bile to the duodenum.

For the sheep used to assess total biliary output, two sheep with only a single cannula were drained continuously over a 3-day period, and bile flow rates were measured. For the two sheep with biliary shunts, bile was collected for a 3 hr period each day for 3 days, and flow rates recorded. Bile flow to the duodenum was restored after each sampling period. In addition, for the five lactating ewes, biliary flow rates for the

exteriorized arm of the T-tube were also recorded by sampling over a 1–3 hr period at the end of the experiment.

Data analysis

An analysis of variance was used to determine any significant changes in the proportion of TCA precipitable activity in bile, milk or plasma throughout the period of sample collection.

In order to enable direct comparisons of radioactivity counts among sheep, all radioactivity data were expressed as a percentage of the radioactivity (c.p.m.) in plasma at 1 min post-injection ($T_{1\ min}$). Means $\pm$SE were calculated using this transformed data. For the five lactating sheep, comparisons were made between the three suckled ewes and the two hand-milked ewes on this basis using Student's *t*-test. Ratios of total radioactivity (c.p.m.) for bile:plasma and milk:plasma were determined for all sheep, and the mean $\pm$SE determined for each time period. These data were computed to enable comparison with the data from non-lactating sheep of Scicchitano *et al.* (1984). An estimate of the total recovery of labelled IgA in bile or milk as a percentage of the amount available in plasma 1 min after injection ($T_{1\ min}$) was obtained in the following manner:

$$\% \text{ recovery} = \frac{(\text{total c.p.m./ml between 1 min and 48 hr}) \times (\text{volume of milk or bile secreted})}{(\text{plasma } (T_{1\ min}) \text{ c.p.m./ml}) \times (\text{plasma volume})} \times \frac{100}{1}.$$

The c.p.m./ml recovered between 1 min and 48 hr post-injection was calculated as the area under the curve that resulted when a plot of c.p.m./ml (% plasma $T_{1\ min}$) for milk or bile against time was constructed. Plasma volume was assumed to be 1500 ml (Dukes, 1955).

RESULTS

Radioactivity associated with IgA as a percentage of the activity in plasma 1 min after injection ($T_{1\ min}$) for milk, bile and plasma for the naturally suckled and hand-milked groups, respectively, are presented in Fig. 1. There was no significant difference between the two plasma curves, so plasma data from all five sheep was pooled. Plasma radioactivity rapidly decreased so that, by 1 hr, only $36 \pm 2\%$ of counts present at 1 min remained. Radioactivity in bile appeared at 5 min and rapidly increased to a peak of $128 \pm 8\%$ ($T_{1\ min}$) at 45 min for the hand-milked group, and to $293 \pm 54\%$ ($T_{1\ min}$) at 1 hr for the suckled group. These values were not significantly different ($0{\cdot}05 < P < 0{\cdot}10$). Bile radioactivity decreased to a level similar to that of plasma by 3 hr post-injection. In milk, radioactivity first appeared between 10 and 15 min post-injection, and in both groups peaked at $144 \pm 55\%$ and $185 \pm 62\%$ ($T_{1\ min}$) for hand-milked and suckled ewes, respectively, at 3 hr post-injection, remaining greater than the corresponding plasma values for the duration of the experiment.

The mean $\pm$SE for bile:plasma and milk:plasma ratios for all lactating sheep were compared with the bile:plasma ratios for non-lactating sheep, obtained from previously published data (Scicchitano *et al.*, 1984) (Fig. 2). The bile:plasma ratios for lactating sheep were significantly higher ($P < 0{\cdot}05$) than the same ratios for non-lactating sheep. The results indicate that the selective transport of IgA into bile is three-fold greater in lactating ewes than in non-lactating ewes.

TCA precipitable counts as a percentage of the total radioactivity for plasma and milk collected between 1 and 24 hr post-injection were $85 \pm 0{\cdot}3\%$ and $32 \pm 3\%$ (mean $\pm$SE), respectively. For bile, the level decreased from $80 \pm 8\%$ at 30 min after injection to $48 \pm 5\%$ at 12 hr post-injection.

In order to allow estimates of relative recoveries of labelled IgA to be made between bile and milk, it was necessary to take into account the biliary and milk flow rates for the suckled and hand-milked ewes. There was no significant difference between biliary flow rates obtained by the different sampling methods and a mean value for the nine sheep of 26 ± 3 ml/hr was determined.

For the two ewes which, in addition to being suckled, were completely milked out by hand, and for which total milk yield throughout the 48 hr period was available, a mean yield of 55 ± 1 ml/hr was obtained. For the remaining three ewes suckling 10-day-old lambs, a milk yield of 25 ml/hr was assumed (based on the data of Fulkerson & McDowell, 1974a, b) and an estimated recovery of labelled IgA in milk calculated on this basis.

The results in Fig. 3 show that, for the suckled group, 42% of available labelled IgA present in plasma at 1 min after injection was removed via milk, while 7·3% was removed via bile. For the hand-milked group, 66% was removed via milk and only 4·0% via bile.

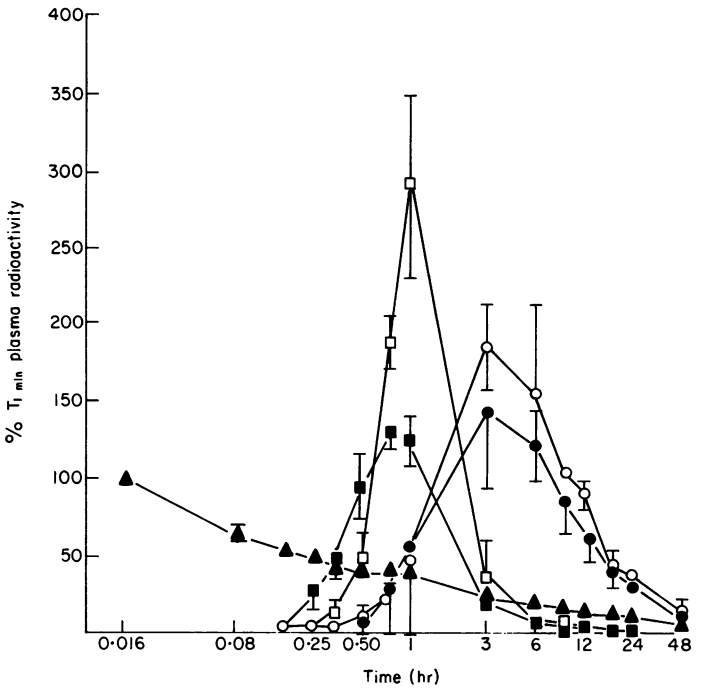

Figure 1. The effect of mammary activity on the transport of radiolabelled IgA from plasma to milk and bile in lactating sheep. Plotted points represent the mean percentage of the plasma radioactivity at 1 min after injection ($T_{1\ min}$): (▲) plasma IgA (all ewes); (○) milk IgA of suckled ewes; (●) milk IgA of hand milked ewes; (□) biliary IgA of suckled ewes; (■) biliary IgA of hand milked ewes.

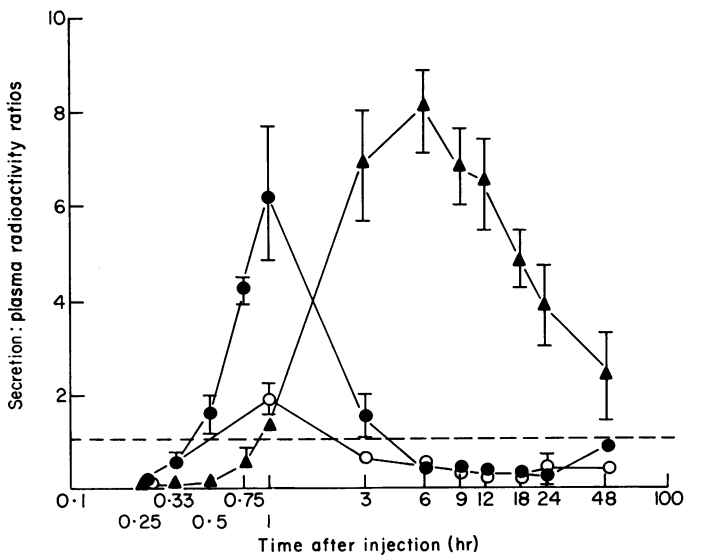

Figure 2. The radioactivity ratios of milk to plasma and bile to plasma in lactating sheep, and bile to plasma in non-lactating sheep following the intravenous injection of radiolabelled IgA: (▲) milk; (●) bile from lactating sheep; (○) bile from non-lactating sheep.

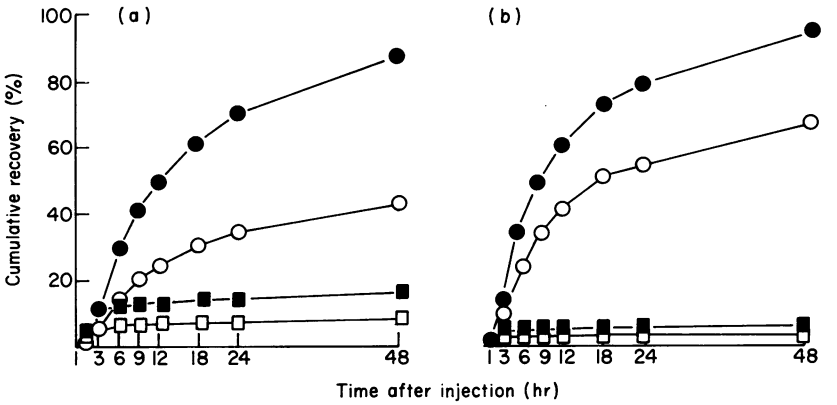

Figure 3. The cumulative recovery of radiolabelled IgA in bile and milk of (a) suckled and (b) hand-milked ewes following its intravenous injection, calculated as a proportion of the radioactivity injected for milk (○) and bile (□), or as a proportion of the total radioactivity recovered for milk (●) and bile (■).

DISCUSSION

The data in this report confirm previous observations that IgA is selectively transported into milk (Sheldrake *et al.*, 1984) and bile (Scicchitano *et al.*, 1984) as shown by the rapid accumulation of radiolabelled IgA in milk and bile after its intravenous injection (Fig. 1) and the high secretion:plasma radioactivity ratios (Fig. 2).

All data have been calculated on the basis of total recovered radioactivity and have not been corrected for TCA precipitability. TCA precipitable counts in plasma remained high at all times, averaging $85 \pm 0{\cdot}3\%$ over the period 1–24 hr after injection. The lower protein-bound activity in bile was considered to reflect loss of label after transport, particularly in view of the findings of Fukumoto & Brandon (1982) with regard to immunoglobulin catabolism in the sheep liver. Similarly, the low TCA precipitability of counts in milk was considered to reflect loss of label after transport as discussed previously (Sheldrake *et al.*, 1984).

Comparisons were made between lactating ewes with respect to the effect of milk yield on bile transport of IgA. One group of ewes was hand-milked and suckled in an attempt to increase milk production on the basis of the documented effects of supplementary milking on ruminant milk yield (Dodd & Griffin, 1977). The other group was only suckled. Based on data of Fulkerson & McDowell (1974a, b), lactating ewes at a similar stage of lactation yield about 25 ml/hr, whereas the two hand-milked ewes in this experiment yielded a mean of 55 ± 1 ml/hr. However, with respect to total recoveries over the sampling period, the results in Fig. 3 indicate that, whereas for low producing ewes 42% of radioactivity in plasma at 1 min was recovered in milk by 48 hr post-injection, in the high milk yield group 66% was recovered.

The data could be explained on the basis of increased competition at the expense of hepatocytes for serum-derived IgA by mammary epithelial SC in sheep producing larger volumes of milk, presumably due to the positive correlation between milk yield and glandular epithelial surface area (Cowie, 1977; Knight, Docherty & Peaker, 1984).

The findings are in apparent conflict with those of Dahlgren *et al.* (1981) and Russell, Brown & Mestecky (1982) who were unable to detect selective transport of serum derived IgA into mammary secretion, although Halsey *et al.* (1982) did find selective transport of IgA into milk of lactating mice. This apparent paradox may be resolved on the basis of the inverse relationship between the transport of serum-derived IgA and the extent of local production of IgA by plasmacytes demonstrated by Sheldrake *et al.* (1984). The lactating ovine mammary gland contains relatively few IgA plasma cells (Lee & Lascelles, 1969) and consequently there is little local production of IgA. In the rodent lactating mammary gland which contains considerable numbers of IgA plasma cells (Lee, Ladds & Watson, 1978; Weisz-Carrington, Roux & Lamm, 1977) it is likely that competition for SC between locally produced IgA and serum-derived IgA accounts for the relative lack of transport of serum IgA in this species. The data of Halsey *et al.* (1982) support this

hypothesis in that, at the beginning of lactation, when there are fewer IgA plasma cells in the gland (Weisz-Carrington *et al.*, 1977), all IgA in milk was serum-derived, whereas at 8 days *postpartum*, by which time the IgA plasma cell population is substantially increased (Weisz-Carrington *et al.*, 1977), only 23% of IgA was serum-derived.

It should also be noted that there was a 2 hr time lag between bile and milk, with respect to peak radioactivity. This is probably due to the anatomical difference between the hepato-biliary and mammary transport systems, as suggested by Russell *et al.* (1982). In the liver, the blood-borne IgA has only to pass through the fenestrated sinusoidal endothelium before being transported by SC through the hepatocyte into the bile canaliculi. In the mammary gland, the IgA must pass from blood into the interstitial spaces via the capillary endothelium prior to being transported through the mammary alveolar epithelium.

The proportion of radioactivity transported from plasma to bile in these studies represented only 4·0% and 7·3% of the plasma ($T_{1\ min}$) value for high and low milk yielding ewes, respectively, based on a mean measured flow rate of 26 ml/hr. These figures are substantially less than the estimate of 47% of injected dose recovered in bile reported by Scicchitano *et al.* (1984), but the discrepancy is explained on the basis of different biliary flow rates used. Scicchitano *et al.* (1984) based their calculations on a flow rate of 200 ml/hr reported by Fukumoto & Brandon (1982). However, these authors did not ligate the cystic duct and only sampled over a 5 min period to calculate hourly flow rates, and it is possible that their flow rates were artificially elevated due to contraction of the gall bladder or a build-up of pressure in the biliary tree resulting from a partial blockage in the distal end of the shunt. The findings of this study, with respect to biliary recovery of IgA based on our measured flow rates, are now consistent with a previous report by Hall *et al.* (1981) in which only 5% of injected dose was recovered in an 11 hr period after intravenous injection of radiolabelled homologous ovine IgA.

Since the data presented here suggest an inverse correlation between milk production and biliary transport of IgA, indicating competition between the two sites for available serum IgA, it was of interest to compare biliary transport of IgA in lactating and non-lactating ewes. However, the data in Fig. 2 indicate that the bile: plasma ratio for lactating ewes at 1 hr after injection of radioactive material was significantly greater than for non-lactating ewes ($6·2 \pm 1·5$ for lacting ewes, compared to $1·97 \pm 0·35$ for non-lactating ewes). Similar findings have been reported in rats (Kleinman *et al.*, 1983). This could reflect changes in hormone status during lactation, since it has been demonstrated (Wira & Sandoe, 1980; Sullivan & Wira, 1983; Sullivan *et al.*, 1983) that levels of SC and IgA in uterine secretions are affected by changes in oestrogen levels. More significant are the findings of Weisz-Carrington *et al.* (1978) who showed that the lactogenic hormones oestrogen, progesterone and prolactin enhanced the development of the mammary gland epithelium and intraepithelial IgA. In contrast, testosterone which antagonizes lactation had an inhibitory effect on the secretory immune system. These findings, and those of the present study, suggest a strong hormonal influence on the secretory IgA mechanism, mediated perhaps by increasing the epithelial surface area or by stimulating SC synthesis. It would be of interest to determine whether similar changes occur at other mucosal sites in response to the hormonal changes associated with lactation, which may explain the increased biliary transport of IgA during lactation.

In this study, we have demonstrated selective transport of ovine IgA into bile and milk of lactating ewes, and the data suggest that, during lactation, biliary IgA transport is inversely related to the level of milk production, possibly due to competition between the epithelial sites for serum-derived IgA. On the other hand, selective transport of IgA into bile is significantly greater in lactating ewes than non-lactating ewes, although the kinetics of transport are similar. This may reflect the effect of lactogenic hormones on secretory epithelia.

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