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Secretory IgA and Antibodies to *Escherichia coli* in Porcine Colostrum and Milk and their Significance in the Alimentary Tract of the Young Pig

P. Porter, D. E. Noakes and W. D. Allen

Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford

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Summary. Specific antisera prepared in rabbits against porcine immunoglobulins have been used in the measurement of IgG, IgA and IgM in porcine colostrum and milk throughout the first weeks of lactation. The immunoglobulins account for more than 60 per cent of the colostral whey protein and approximately 80 per cent of the immunoglobulin is IgG. During the first 2–3 days of lactation IgG and IgM fall to approximately one-tenth of the original level but IgA shows only a two- to three-fold decrease and becomes the predominant immunoglobulin in sow milk.

Antibodies to *Escherichia coli* 0141 and 08 antigens were predominantly associated with IgA although IgM is an important antibody in colostrum. Immunofluorescent studies of IgA in mammary tissue provide some evidence for local synthesis.

The passage of sow milk IgA through the alimentary tract was studied in young pigs with re-entrant fistulae prepared in the small intestine. The observations are discussed in relation to the function of IgA as an antibody providing protection in the alimentary tract.

INTRODUCTION

Under normal conditions piglets cease to absorb immunoglobulins from the colostrum after the first 24–36 hours of life (Lecce and Morgan, 1962). The animal does not synthesize any appreciable level of antibody before the age of 3–4 weeks (Brown, Speer, Quinn, Hays and Catron, 1961; Miller, Harman, Ullrey, Schmidt, Luecke and Hoeffer, 1962) and passively acquired antibody falls to very low levels during the first 2–3 weeks of life (Sharpe, 1965; Arbuckle, 1968). It has often been suggested that antibodies in milk play an important role in the protection of the young animal against *Enterobacteria* but no attempt has been made to characterize the relevant antibody in porcine milk or to demonstrate that it survives digestion and plays its role in the alimentary tract.

In human colostrum IgA plays an important antibacterial role (Adinolphi, Glynn, Lindsay and Milne, 1966) and it is the predominant colostral immunoglobulin (De Muralt, Gugler and Boulet, 1960). In porcine colostrum, however, IgA is a minor component and although it contains antibody to *Escherichia coli*, antibody is also associated with IgM (Porter, 1969). Further, whilst rapid changes in the protein composition of porcine colostrum during the early part of lactation have been described (Karlsson, 1964;

Morgan and Leece, 1964) nothing is known of the changes in specific immunoglobulins; although a comparatively constant level of *E. coli* antibody has been demonstrated throughout lactation (Arbuckle, 1968).

In the present paper the levels of immunoglobulins and *E. coli* antibodies were measured in colostrum and milk throughout lactation and its passage through the alimentary tract was studied in fistulated animals. The antibodies in milk were characterized as being predominantly IgA.

MATERIALS AND METHODS

Gel filtration chromatography

Chromatography was carried out in Sephadex G-200 columns (45×2·5 cm) and also on thin layer plates using techniques described by Porter, Porter and Shanberge (1967).

Ion exchange chromatography

Anion exchange chromatography was carried out by the method of Augustin and Hayward (1960) on diethylaminoethyl (DEAE) cellulose using a column 30×2·0 cm.

Micro-electrophoresis

Disc electrophoresis was carried out in polyacrylamide gels (Orstein and Davies, 1964). Protein fractions were examined by immunoelectrophoresis using antisera produced in New Zealand White rabbits (Porter, 1964).

Quantitative estimation of immunoglobulins

Immunoglobulin levels were estimated by the radial immunodiffusion technique of Mancini, Carbonara and Heremans (1965). The isolation of specific porcine immunoglobulins and the preparation of rabbit antisera specific for IgG, IgA and IgM have been described (Porter, 1969).

Analytical ultracentrifugation

Ultracentrifugal analysis was carried out in a Beckman model E centrifuge equipped with a phase plate Schlieren diaphragm. Sedimentation coefficients were measured at 20° and 59,780 rev/min.

Bacterial antibody tests

Haemagglutination and anti-globulin haemagglutination tests were used as described by Buxton and Thomlinson (1961). Sheep red cells were modified with lipopolysaccharides from a haemolytic strain of *E. coli* (serotype 0141 : K85a, c(B)iH4) and a non-haemolytic strain of *E. coli* (serotype 08 : H) prepared by the method of Westphal, Luderitz and Bister (1952).

The effect of treating chromatographic fractions of milk with anti-IgA-globulin serum was investigated by adding 1 volume of the specific anti-IgA-globulin serum to 3 volumes of each fraction.

Immunofluorescent histochemistry

Rabbit antisera to pig immunoglobulins IgG, IgA and IgM were conjugated with fluorescein isothyocyanate (FITC) using the technique of Goldstein, Slizys and Chase (1961).

Blocks of tissue were rapidly frozen in isopentane cooled with liquid nitrogen and stored in liquid nitrogen until required. Cryostat sections fixed in alcohol were incubated with the conjugated reagents.

The specificity of the reaction was controlled by: (a) blocking with unconjugated antiserum prior to incubating with conjugated reagent, (b) absorbing the conjugated antiserum with the specific immunoglobulin prior to staining and (c) the use of non-immune rabbit serum.

The stained sections were examined by dark ground microscopy on a Reichert Zetopan microscope using an HBO 200 light source, a U.G. 1 exciter filter and a G.G. 13 plus Wratten folie 2B barrier filter.

Milking methods

Sow milk for feeding fistulated piglets was obtained from donor sows by manual expression of milk. A flow of milk was stimulated by the intravenous injection of 5 units of Posterior Pituitary Extract (Parke Davis Ltd, London).

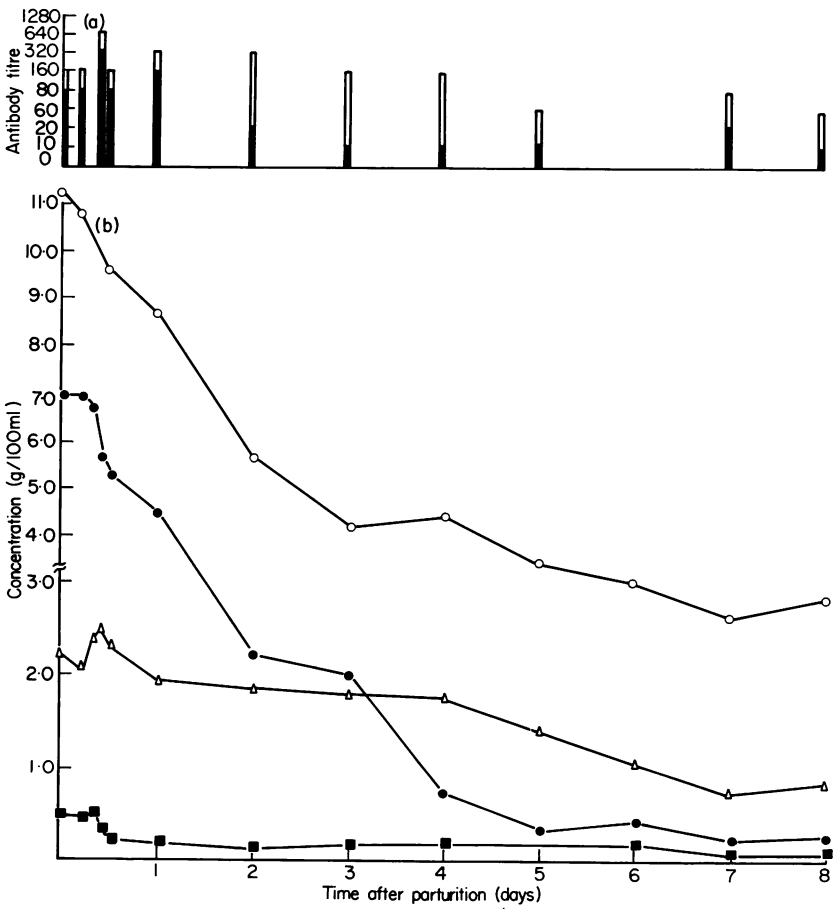

FIG. 1. Variation in immunoglobulin levels (b) and *E. coli* 0141 antibodies (a) in porcine colostrum and milk whey during the 1st week of lactation. Solid columns, *E. coli* 0141 haemagglutination; open columns, *E. coli* 0141 anti-globulin haemagglutination. ○, Total protein; ●, IgG; △, IgA; ■, IgM.

Anaesthesia and preparation of re-entrant fistulae

Single re-entrant fistulae were prepared in four Large White × Wessex pigs 7–28 days of age. Anaesthesia was induced by intravenous injection of metho-hexitone sodium ('Brietal', Elanco Ltd, London) 12 mg/kg. Following endotracheal or nasal intubation anaesthesia was maintained with mixtures of cyclopropane and oxygen.

Re-entrant fistulae were prepared by the method described by Markowitz (1954) using Perspex cannulae of $\frac{3}{8}$ in. internal diameter, which were exteriorized through the body wall in the region of the right flank. Continuity of flow was re-established by connecting the two elbow pieces together with flexible polyvinyl tubing (Harrison and Hill, 1962).

In three pigs re-entrant fistulae were prepared in the duodenum 3–6 cm from the pyloric sphincter and between the openings of the biliary and pancreatic ducts. Fistulae were

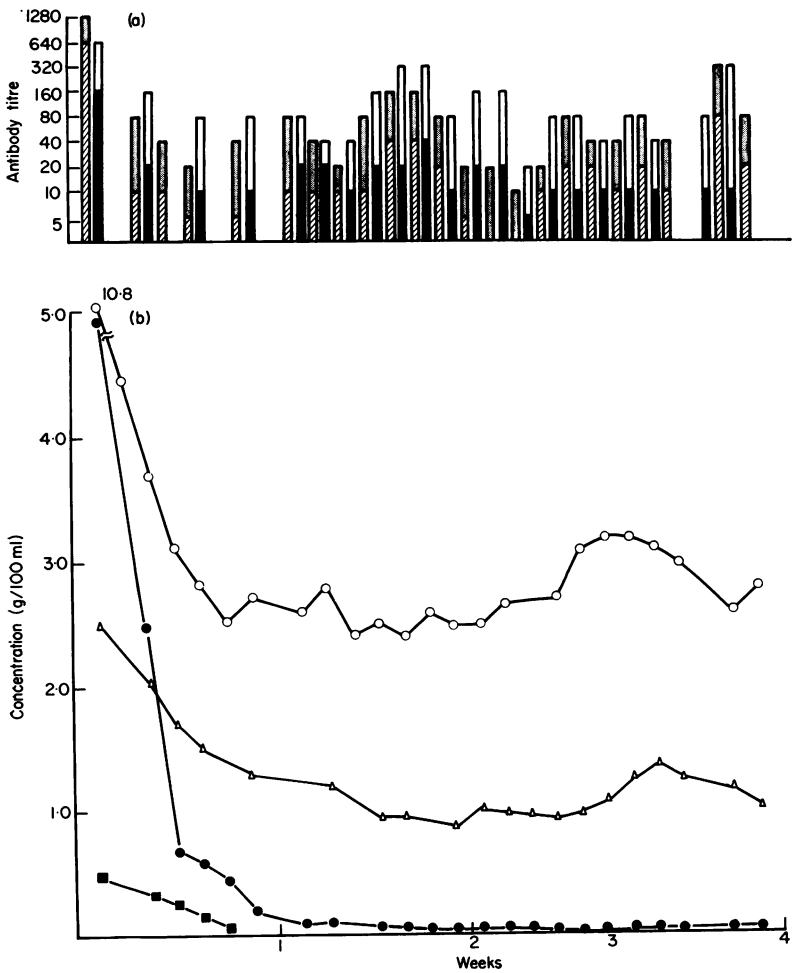

FIG. 2. Variation in immunoglobulin levels (b) and *E. coli* 0141 and 08 antibodies (a) porcine colostrum and milk whey during the first 4 weeks of lactation. Hatched columns, *E. coli* 08 haemagglutination; stippled columns, *E. coli* 08 anti-globulin haemagglutination; solid columns, *E. coli* 0141 haemagglutination; open columns, *E. coli* 0141 anti-globulin haemagglutination. ○, Total protein, ●, IgG; △, IgA; ■, IgM.

prepared in the jejunum approximately 4 m from the pylorus and 9 m from the ileo-caecal junction.

Method of sampling and measurement of the rate of flow

Samples of digesta were obtained and the rate of flow of digesta was measured by disconnecting the piece of flexible polyvinyl tubing and attaching lengths of light weight tubing to the elbow pieces. Digesta flowed from the cranial cannula and were collected in a graduated measuring cylinder. Aliquots of 50 ml were collected, and after sampling, the remainder was returned immediately to the duodenum through the caudal cannula.

RESULTS

VARIATION IN MILK IMMUNOGLOBULIN LEVELS AND ANTIBODIES TO *E. coli* DURING LACTATION

A detailed study of changes in immunoglobulin levels in porcine milk whey in the 1st week of lactation is presented in Fig. 1. Immunoglobulin levels in colostrum were high in the first 24 hours which is the period of intestinal absorption in the young pig. Immunoglobulin IgG was the predominant component which accounted for as much as 80 per cent of the total γ-globulin and this is the main immunoglobulin absorbed by the neonatal piglet. The immunoglobulin levels fell rapidly during the first 2 days of lactation and over the first 4-day period IgG and IgM fell to concentrations approximately one-tenth of those found in the first colostrum secreted. The decline in IgA was not so marked and only diminished to approximately one-third of its original level. After 3 days IgA became the predominant immunoglobulin in sow's milk and this accounted for as much as 70 per cent of the total immunoglobulins and 30 per cent of the whey protein.

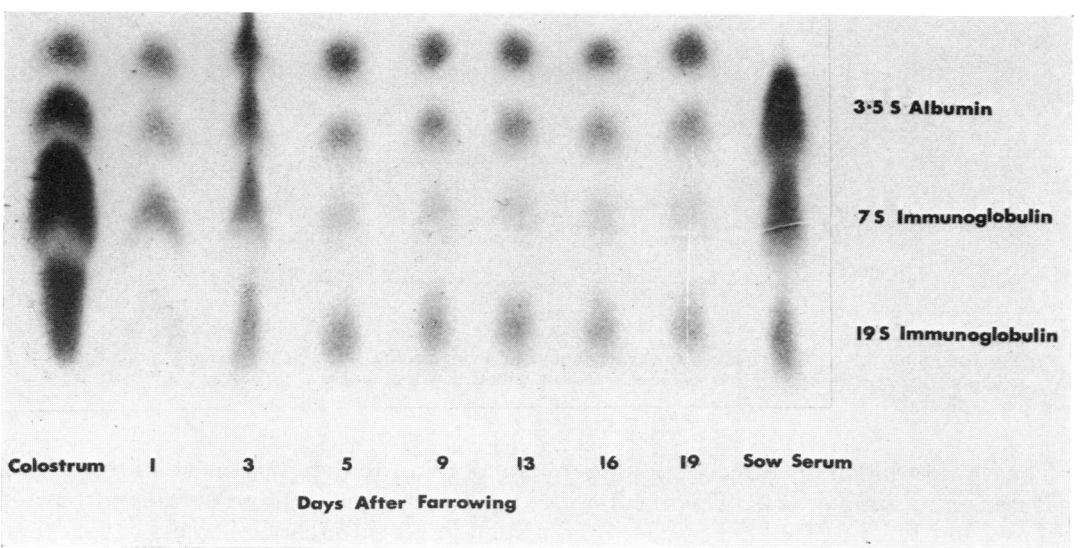

FIG. 3. Thin layer gel filtration studies on Sephadex G-200 of protein changes in colostrum and milk whey throughout the first 4 weeks of lactation.

The *E. coli* antibody levels did not follow a pattern comparable to that of the total immunoglobulins. The amount of antibody assayed in the indirect test fell rapidly from the 1st day of lactation but the proportion of incomplete antibody detected by the anti-globulin haemagglutination test increased.

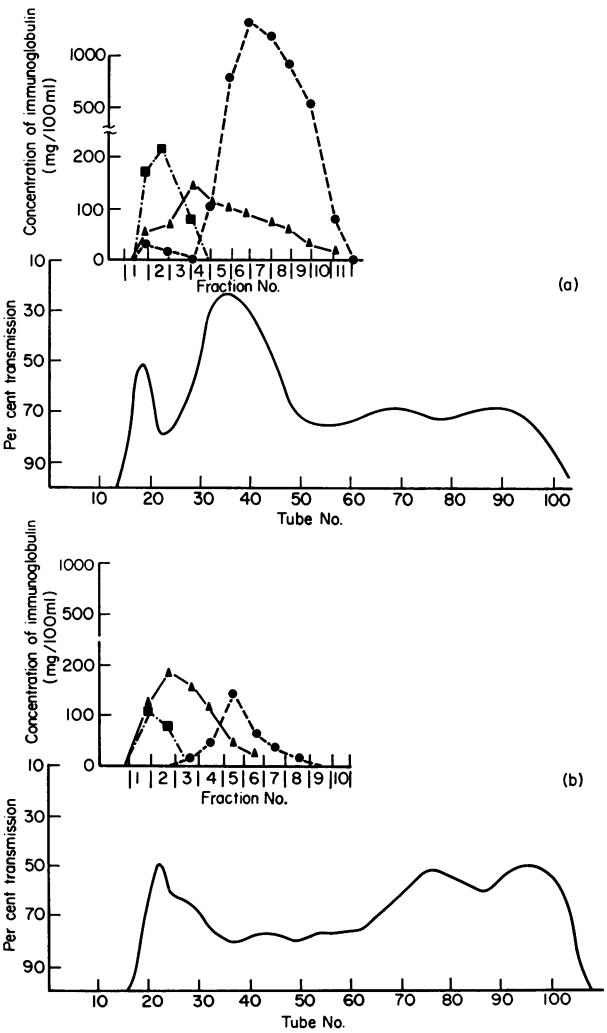

FIG. 4. Gel filtration of porcine colostral whey and milk whey on Sephadex G-200 giving pooling data and showing elution of immunoglobulins. (a) Sow colostral whey; (b) sow milk whey. ▲, IgA; ●, IgG; ■, IgM.

The changes in *E. coli* antibodies and immunoglobulins were studied over a longer period of lactation in another sow. The results (Fig. 2) were similar to those obtained from the first sow and IgA persisted at reasonably consistent levels throughout 4 weeks of lactation. Thin layer gel filtration studies on Sephadex G-200 demonstrated that the protein pattern became consistent after the first 5 days (Fig. 3).

CHARACTERIZATION OF IMMUNOGLOBULINS AND *E. coli* ANTIBODIES BY GEL FILTRATION AND ANION EXCHANGE CHROMATOGRAPHY

A pooled sample of milk taken after the 1st week of lactation was fractionated by gel filtration on Sephadex G-200 and the immunoglobulins and antibodies were assayed in selected fractions. The gel filtration elution pattern for milk is compared with the pattern for colostrum in Fig. 4 and the considerable change in 7S immunoglobulin levels is immediately evident from the elution patterns.

E. coli somatic antibodies in colostrum were identified in a wider range of gel filtration fractions, than in milk. The antibodies in milk had very similar elution characteristics to IgA. The antibody activity in the colostrum and milk fractions was assayed after absorption with specific rabbit anti-IgA-globulin serum. This resulted in considerable inhibition of antibody activity in all fractions (Fig. 5). Residual activity in fractions at the exclusion peak was associated with IgM; this antibody is absorbed by the neonatal piglet (Porter, 1969). Incomplete antibody remaining in fractions in the second gel filtration peak was

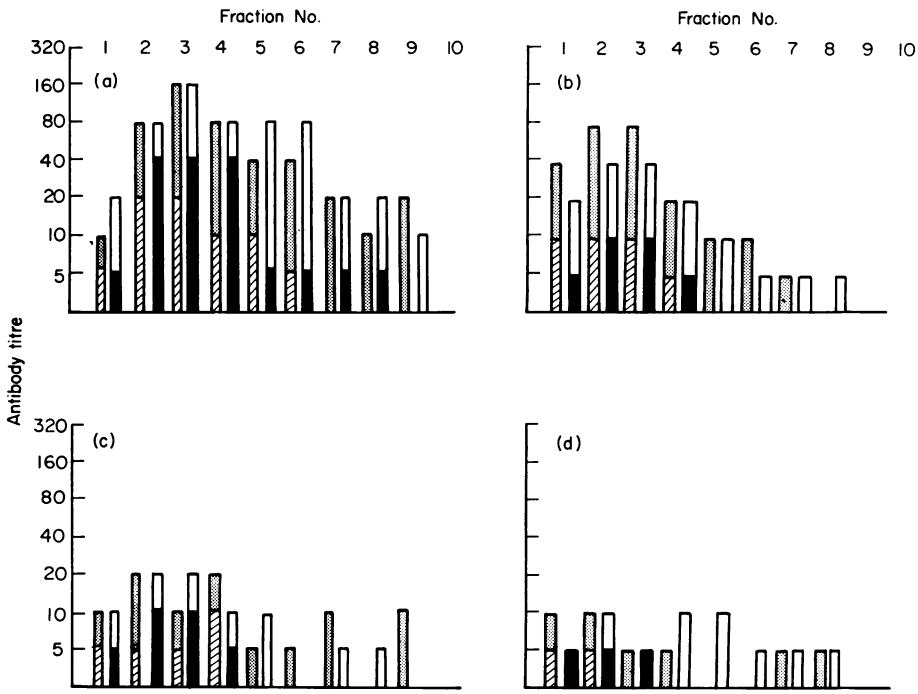

FIG. 5. (a) and (b) *E. coli* somatic antibodies in porcine colostrum (a) and milk whey (b) fractions obtained by gel filtration on Sephadex G-200 (Fig. 4). (c) and (d) *E. coli* somatic antibodies residual after absorption with specific rabbit anti-IgA-globulin serum. (c) Porcine colostrum and (d) milk whey. Hatched columns, *E. coli* 08 haemagglutination; stippled columns, *E. coli* 08 anti-globulin haemagglutination; solid columns, *E. coli* 0141 haemagglutination; open columns. *E. coli* 0141anti-globulin haemagglutination.

probably associated with IgG. Incomplete antibody to *E. coli* somatic antigens detectable by the anti-globulin haemagglutination test is associated with IgG in the serum of the chicken (Duffus and Allen, 1968) and the pig (Porter and Kenworthy, 1969). It is thus clear that a high proportion of the antibody detected in sow milk is associated with IgA.

TABLE 1

HAEMAGGLUTINATING ANTIBODY LEVELS TO *E. coli* IN PORCINE COLOSTRUM AND MILK AND THE EFFECT OF ABSORPTION WITH RABBIT ANTI-IgA-GLOBULIN SERUM

Time after parturition	0141		0141 after absorbing IgA		08		08 after absorbing IgA	
	Indirect	Anti-globulin	Indirect	Anti-globulin	Indirect	Anti-globulin	Indirect	Anti-globulin
0 hours	20	80	20	80	80	160	40	80
3 hours	40	160	40	80	80	160	40	80
10 hours	320	1280	20	80	320	640	40	80
1 day	120	320	20	80	160	320	10	40
2 days	20	80	5	20	40	160	20	40
3 days	20	80	10	40	10	160	10	40
4 days	10	40	0	10	10	40	10	20
5 days	5	20	0	5	40	80	20	20
7 days	0	20	0	5	20	80	20	40

The change in proportion of *E. coli* antibodies in association with IgA during the 1st week of lactation is indicated by inhibition studies recorded in Table 1. In the first 24 hours, during the period of intestinal absorption by the neonatal piglet, IgM plays a prominent role as antibody. This was particularly evident from the assays for antibody to 0141 remaining after absorption with specific rabbit anti-IgA globulin serum. In the following period the antibody became predominantly associated with IgA.

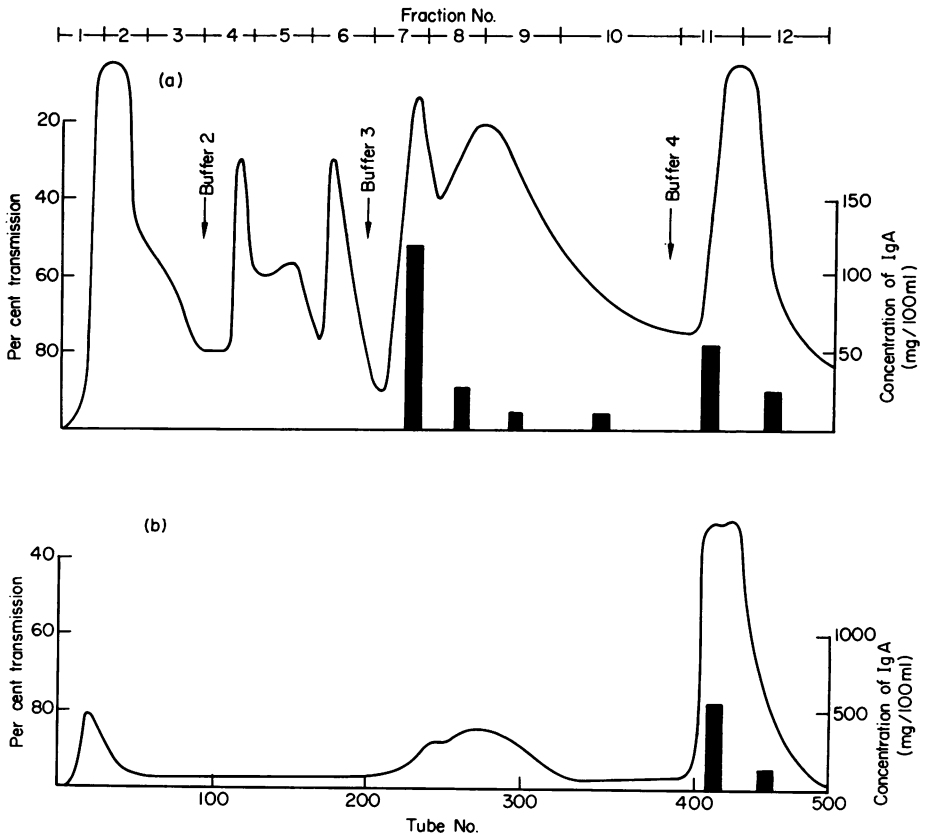

FIG. 6. Comparative chromatographic studies of porcine serum (a) and milk whey (b) on DEAE-cellulose using a stepwise elution schedule. The elution characteristics of IgA are shown in various fractions for which the pooling data are given.

The elution characteristics of IgA in milk were studied on DEAE-cellulose. The milk chromatogram is compared with that of serum in Fig. 6 and two features are immediately evident. Firstly there was very little material eluted with buffers 1, 2 and 3 in the milk chromatogram whereas in the serum chromatogram the greater proportion of the serum proteins were eluted with these buffers. Clearly very little serum protein passed across the mammary acinar epithelium into the milk. The second feature of this study was that milk IgA appeared late in the chromatogram, being eluted mainly with buffer 4 whereas serum IgA appeared mainly in early eluates. Gel filtration studies of the milk eluate from

DEAE-cellulose with buffer 4 provided an elution pattern not dissimilar to that of whole milk whey in Fig. 4; the IgA appeared in fractions which included components with ultracentrifugal characteristics S_{20} 11 to 18S, and was identified with acid mucopolysaccharides which stained with alcian blue. Possibly an association with such acidic components resulted in the late appearance of milk IgA in the anion exchange chromatogram.

IMMUNOFLUORESCENT LOCALIZATION OF IgA IN THE MAMMARY GLAND OF THE SOW

Mammary gland tissue from four sows was examined. Three animals had been lactating for 5–6 weeks and one was approximately 1 week *pre-partum*. IgA was demonstrated in the granular tissue of all the animals. It was generally dispersed throughout the lobules of the lactating sows, although there was some variation in activity in different parts of the mammary gland from any one individual. The glandular tissue of the *pre-partum* pig consisted mainly of thin sheets of cells surrounding large globules of colostrum; both the cells and the colostrum contained IgA.

Apart from the luminal contents of the ducts, which stained strongly, no IgA was detected in the interlobular septa of the lactating animals. A few IgA containing cells were seen in the septa of the *pre-partum* pig.

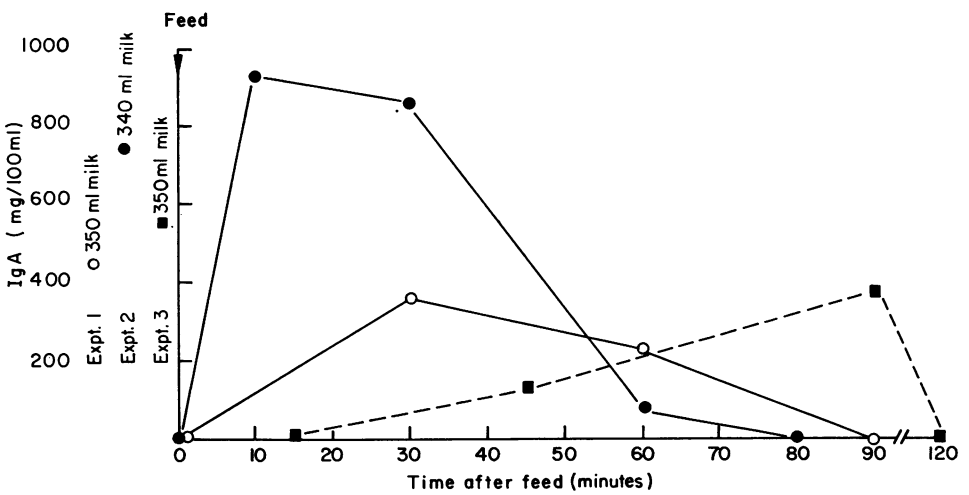

FIG. 7. Studies of the passage of milk IgA through the intestines of young pigs prepared with re-entrant fistulae. ○, Experiment 1, duodenal re-entrant (35 ml milk); ●, Experiment 2, duodenal re-entrant (340 ml milk); ■, Experiment 3, jejunal re-entrant (350 ml milk).

In sections stained with fluorescent anti-IgG and examined as part of the controls to these studies, the distribution of IgG in the tissue of the mammary gland was essentially the same for both *pre-* and *post-partum* animals; being generally present throughout the glandular tissue and interlobular connective tissue. However, it was interesting that the intensity of fluorescence in the *pre-partum* animals was considerably lower than that observed for *post-partum* animals although the conditions for fixation and staining were identical. No marked difference in intensity of fluorescence was observed in the detection of IgA.

STUDIES OF MILK IgA IN THE ALIMENTARY TRACT OF THE YOUNG PIG

The results of three experiments using animals with re-entrant fistulae in the duodenum and jejunum are shown in Fig. 7. Each animal was fed a known amount of sows milk for which the IgA level had been assayed, indicated to the left of the figure. The level of IgA in the digesta passing through the re-entrant fistulae was assayed in samples taken at intervals after administration of the milk.

In experiments 1 and 2 IgA could be detected in duodenal contents for periods exceeding 1 hour after the ingestion of the sows milk, whilst in experiment 3 IgA persisted in jejunal contents for a much longer period. An interesting feature was that high levels of IgA were identified in digesta collected from the duodenum within 10 minutes of feeding. The experiments were repeated in two more animals which were fed smaller volumes of sows milk and sampled at more frequent intervals. The assays of IgA in the digesta at known intervals after feeding are recorded in Table 2. IgA was present in digesta passing into the duodenum within 5 minutes of feeding and in digesta in the jejunum within 10 minutes of feeding.

TABLE 2

STUDIES OF THE PASSAGE OF MILK IgA THROUGH THE INTESTINES OF PIGLETS WITH RE-ENTRANT FISTULAE (IgA ASSAYED IN DIGEST AT GIVEN INTERVALS AFTER FEEDING)

Time after feeding (minutes)	Piglet A duodenum IgA(mg/100 ml)		Time after feeding (minutes)	Piglet B jejunum IgA(mg/100 ml)
	Experiment 1	Experiment 2		
0	0	0	0	–
5	96	240	10	35
15	435	575	15	160
25	480	730	20	200
35	400	600	30	260
45	330	660	40	320
55	–	104	50	350
			60	220
			70	200
			90	96
			120	–
Volume of sow milk fed (ml)	110	100		200

DISCUSSION

Studies of the immunoglobulin composition of human colostrum have demonstrated that IgA is the major component (De Muralt *et al.*, 1960; Chodirker and Tomasi, 1963) and similar observations have been recorded for rabbit colostrum (Cebra and Robbins, 1966). The present studies in the pig demonstrate that IgG is the main colostral immunoglobulin although the level of this component falls rapidly in the first 2–3 days of lactation. Clearly the high levels of IgG in early lactation support the physiological requirement of the pig for intestinal absorption of immunoglobulin. Unlike the human infant and the rabbit this is essential to the pig owing to the absence of transfer of immunoglobulins *prepartum*. The period of lactation during which colostral IgG and IgM persist at optimum levels is very similar to the natural intestinal absorptive phase of the neonate which has been established to be 24–36 hours (Lecce and Morgan, 1962).

The dramatic change in immunoglobulin composition of sow milk in the first 2 days of lactation in which IgA becomes the predominant immunoglobulin probably reflects the different roles played by the immunoglobulins in the protection of the neonatal animal. Previous studies of immunoglobulins and *E. coli* antibodies have demonstrated that the main serum antibody is IgM whereas the main colostral antibody is IgA. The IgA antibody is not acquired as part of the circulating passive immunity of the neonatal piglet (Porter, 1969). The fact that it persists as the major immunoglobulin and the source of *E. coli* antibody in the gut throughout lactation surely suggests that its main function is to provide protection in the alimentary tract.

Secretory IgA is highly resistant to the action of proteolytic enzymes (Tomasi, 1967) a factor which must assist in the retention of its antibody function in the gastro-intestinal tract. An additional factor facilitating the action of milk IgA as an antibody in the intestine of pig is the rate at which it passes from the stomach after ingestion. After a single feed IgA continues to pass through the small intestine for a longer period than the intervals in the normal feeding pattern of the suckling pig. Thus it would be reasonable to infer that in the normal animal, milk IgA is constantly present as an immunological defence in the small intestine. This would represent the first line of protection until the normal secretory immune system is established in the intestinal mucosa.

Studies in man (Adinolphi *et al.*, 1966), pig (Porter, 1969) and rabbit (Genco and Taubman, 1969) provide evidence that colostral IgA antibodies are not acquired from the serum but synthesized locally in the mammary gland. Recent investigations in the sheep indicate that the inoculation of bacterial antigens into the non-lactating mammary gland can give rise to a local immune response (Outteridge, Mackenzie and Lascelles, 1968) and the specific antibody detected in the lacteal secretions has been provisionally identified as IgA.

Previous studies using isotopic components in the rabbit and goat (Askonas, Campbell, Humphrey and Work, 1954) and in cattle (Larson and Gillespie, 1957) have demonstrated that colostral immunoglobulins are derived from the serum without degradation or resynthesis. Homologous γ-globulin given to pregnant cattle is transferred to the mammary secretions in the week immediately prior to parturition. Electrophoretic studies of bovine colostral immunoglobulins have provided some evidence that there may be selective transfer of immunoglobulins across the mammary acinar epithelium (Larson, 1952; Murphy, Aalund, Osebold and Carrol, 1964; Peirce and Feinstein, 1965). This evidence is based on semi-quantitative observations from electrophoretic profiles and neglects to characterize the immunoglobulins other than by electrophoretic mobility; it also fails to take into account the possibility of synthesis in the mammary gland. Some support for the local synthesis of IgA is provided by the immunofluorescent studies of mammary tissue taken from the sow approximately 1 week *pre-partum* when IgA was clearly evident and IgG, which is the predominant colostral immunoglobulin was barely detectable.

Secretory IgA differs from serum IgA in possessing a complexed non-globulin component called the 'secretory piece' (Tomasi, 1967) and, therefore, it appears in a higher molecular size range than serum IgG. This is demonstrated in the Sephadex G-200 gel filtration studies of porcine milk and serum (Fig. 4). However, gel filtration studies of colostum taken at parturition demonstrate that IgA appears in a wide range of molecular sizes, including molecules equivalent in size to serum IgA as well as milk secretory IgA. Thus it appears that IgA in early lactation may be derived partly from the serum and partly from the mammary gland.

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