

# Studies of Porcine Secretory IgA and its Component Chains in Relation to Intestinal Absorption of Colostral Immunoglobulins by the Neonatal Pig

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(Received 27th April 1972)

**Summary.** Porcine secretory component was identified after reductive dissociation of secretory IgA and isolated in the free form from sow milk.

Electrophoretically fast polypeptide chains were detected in the light chain fraction of reduced and alkylated porcine secretory IgA. These probably relate to J chain and were immunologically and electrophoretically distinct from secretory component. J chains were not present in the light chain fractions of reduced and alkylated porcine 7S IgG and 6·4S IgA.

Porcine colostrum IgA occurs in a wide range of molecular sizes and IgA lacking secretory component having  $S_{20,w}$  6·4 and 9·3 was isolated from colostrum and serum of suckling piglets.

A haemagglutination inhibition assay for 10·6S secretory IgA using specific rabbit anti porcine secretory component demonstrated that only a very small proportion of the IgA derived from colostrum is 10·6S IgA with secretory component.

## INTRODUCTION

The levels of specific immunoglobulins in porcine colostrum differ quantitatively from those demonstrated in the human (Chodirker and Tomasi, 1963) in that IgA is not the predominant immunoglobulin (Porter, 1969a). The differences found in the two species relate to the mode of transport of passive immunity from mother to offspring. Post-partum transport of antibodies by intestinal absorption from colostrum is of tremendous importance for survival in the pig. Unlike the human, there is no transmission of immunoglobulin *in utero*, and it is totally dependent for a time upon satisfactory intake and absorption of colostrum antibodies. High levels of colostrum immunoglobulins are thus fundamental to survival, and for the first few hours IgG predominates; however levels of immunoglobulin in porcine milk fall off rapidly as lactation progresses and IgA persists as the major secretory immunoglobulin after the first 2 or 3 days. (Porter and Noakes, 1969; Porter, Noakes and Allen, 1970; Vaerman, Arbuckle and Heremans, 1970; Curtis and Bourne, 1971).

Secretory IgA is an important antibody to *Escherichia coli* in porcine colostrum (Porter, 1969b). Our investigations have shown that the level of IgA in the serum of suckling piglets generally considerably exceeds adult levels. However, in contrast to secretory IgA of colostrum and milk, it possesses little or no *E. coli* antibody activity. (Porter, 1969b). This is consistent with findings in the human (Adinolfi, Glynn, Lindsay and Milne, 1966), but

the problem requires special consideration in the pig since piglet serum IgA relates directly to sow colostrum IgA.

Although secretory IgA has now been described in the pig by several other authors (reviewed by Porter and Allen, 1972) porcine secretory component has not been described to date. In fact Richardson and Kelleher (1970) failed to detect any evidence of it in reductive dissociation studies of secretory IgA.

In the present paper studies of porcine secretory component and colostrum IgA are described as a basis to the investigation of intestinal absorption of IgA by the neonatal piglet. More than one form of IgA appears in porcine colostrum and most of the IgA which appears in the serum of the suckling pig cannot be classed as secretory IgA either by physicochemical characteristics or immunological characteristics related to secretory component.

Reductive cleavage of porcine secretory IgA also demonstrates the presence of electrophoretically fast polypeptide chains, probably related to J chain, which are distinct from secretory component.

## MATERIALS AND METHODS

### *Micro-electrophoresis*

Protein samples and fractions were examined by immunoelectrophoresis using antisera raised in New Zealand white rabbits.

Disc electrophoresis was carried out in polyacrylamide gels using the methods of Orstein and Davis (1964).

### *Gel-filtration chromatography*

Chromatography was carried out using Sephadex G-200 columns (90 × 2.5 cm) and 0.1 M tris buffered saline pH 7.2.

### *Ion exchange chromatography*

Anion exchange chromatography was carried out on DEAE-cellulose using a column (30 × 2.0 cm). The stepwise elution entailed the use of four buffers, (1) 0.01 M NaH<sub>2</sub>PO<sub>4</sub> adjusted to pH 7.6 with 0.01 M NaOH, (2) 0.02 M NaH<sub>2</sub>PO<sub>4</sub> adjusted to pH 6.3 with 0.02 M NaOH, (3) 0.05 M NaH<sub>2</sub>PO<sub>4</sub>, (4) 0.3 M NaH<sub>2</sub>PO<sub>4</sub>. A fifth buffer 0.4 M NaH<sub>2</sub>PO<sub>4</sub> containing 2 M NaCl was used to wash firmly bound protein from the column after each run before regenerating with the starting buffer. The technique was described by Augustin and Hayward (1960).

Cation-exchange chromatography was carried out using CM-cellulose columns (20 × 2.0 cm). This technique was used to isolate IgG<sub>2</sub> immunoglobulin from fractions of pig serum obtained by anion exchange chromatography. For this purpose a fairly limited stepwise elution procedure using phosphate buffer pH 5.8 and molarities 0.005 M, 0.02 M, 0.04 M in sequence, were found to be adequate.

Samples were prepared for ion exchange chromatography by pressure dialysis against the starting buffer for 24 hours, or by passing through a Sephadex G-25 column (45 × 5 cm) equilibrated with starting buffer.

Flow rates up to 200 ml/hr were maintained with the ion exchange columns by use of a suitable hydrostatic head. Eluates were automatically scanned at 280 m $\mu$  and collected

using an LKB ultracollector and drop counter. Fractions were pooled from the eluates by reference to the elution pattern.

#### *Ultracentrifugation*

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

Density gradient ultracentrifugation was carried out by the isokinetic sucrose density technique of Noll (1967) using an MSE 50 superspeed centrifuge. Protein samples, 0.2 ml were layered on to the surface of 2.6 ml of a 10–39 per cent sucrose density gradient. The samples were centrifuged at 200,000 *g* for 19 hours.

#### *Quantitative estimation of immunoglobulins*

Immunoglobulins were assayed by the radial immunodiffusion technique of Mancini, Carbonara and Heremans (1965). The isolation of specific porcine immunoglobulins and the preparation of rabbit antisera have been described (Porter, 1969b).

#### *Preparation of rabbit anti-porcine secretory component*

The normal procedure for preparation of an antiserum for secretory component has been to absorb an antiserum for secretory IgA with homologous serum or serum IgA. From fifteen different rabbit antisera this technique yielded only weak activity for free and bound secretory component and these were unsatisfactory for the purpose of measurement or immunofluorescent studies. It seemed that a more satisfactory reagent might be obtained if secretory component could be released from secretory IgA. For these purposes a preparation of secretory IgA was subjected to reductive dissociation with 2-mercaptoethanol (2-ME) and alkylation.

Porcine secretory IgA was treated with 0.1 M 2-ME in 0.1 M tris buffer pH 0.8 for 4 hours. An equal volume of 0.2 M iodoacetamide and alkylation was allowed to proceed for 2 hours followed by dialysis against 0.1 M tris buffer pH 8.0 for 24 hours. The products of reduction and alkylation of secretory IgA were separated by gel filtration on Sephadex G-200 (45 × 2.5 cm). Using the  $\alpha$ -chain absorbed antisera for secretory IgA, secretory component could be detected in lower molecular weight fractions which were pooled, concentrated and rechromatographed on Sephadex G-200 using reverse flow. Fractions containing secretory component were selected for injection with Freund's adjuvant into rabbits. These antisera proved to be improved reagents for secretory component,  $\alpha$ -chain and light chain activity was removed by absorption with serum IgA.

#### *Isolation of free secretory component*

Sow milk whey was concentrated five-fold by dialysis against 30 per cent polyethylene glycol and 100 ml was fractionated by gel filtration on Sephadex G-150 (80 × 6 cm). Eluates containing secretory component unassociated with  $\alpha$ -chain were detected by immunological double diffusion and were precipitated by treatment with 55 per cent saturated  $(\text{NH}_4)_2\text{SO}_4$ . The precipitate was dissolved in 0.01 M phosphate buffer pH 7.6 and further fractionated on DEAE cellulose. Fractions rich in secretory component were eluted with 0.01, 0.05 and 0.1 M buffers. These fractions contained other proteins which migrated faster towards the anode on analytical electrophoresis at pH 8.4 in 5 per cent polyacrylamide gels. The migration position of secretory component was detected by sec-

tioning the unstained gel and testing by immunodiffusion in agar against the antiserum. Similarly secretory component was isolated by elution in saline of the appropriate sections from numerous unstained gel electrophoretograms.

#### *Assay of secretory IgA in the serum of piglets receiving colostrum*

The levels of secretory IgA in post colostrum piglet serum were below the sensitivity of assay by precipitin techniques such as radial immunodiffusion using rabbit anti secretory component. An assay technique involving haemagglutination inhibition was developed using tanned sheep erythrocytes sensitized with porcine secretory IgA agglutinated with rabbit anti secretory component. Serum IgA or  $\alpha$ -chain did not inhibit in this system.

Washed sheep erythrocytes were treated with tannic acid at a concentration of 1:40,000 in phosphate buffered saline pH 7.2 at 37° for 10 minutes. The tanned cells were washed and suspended in 200 volumes of phosphate buffered saline pH 6.4 and sensitized with secretory IgA by adding 1 volume a solution of secretory IgA approximately 1 g/100 ml to 4 volumes of tanned cell suspension and maintaining the suspension at 37° for 30 minutes. The sensitized cells were centrifuged at 1500 rev/min, washed with a solution of rabbit serum (1:100) in saline and resuspended in 100 volumes of the rabbit serum solution.

The haemagglutination end point of secretory IgA sensitized tanned cells was assessed in serial dilutions of the rabbit anti-secretory component antiserum. The end point reciprocal dilution was 64 and a 20-volume dilution of the antiserum was selected for use in the inhibition test. The test comprised 3 volumes anti-secretory component (1/20 dilution) incubated with 2 volumes of test sample prepared in serial dilutions and 1 volume of secretory IgA sensitized tanned cells. The inhibition test was calibrated with a standard solution of secretory IgA and had a limit of sensitivity of 8  $\mu$ g/100 ml. Piglet serum was absorbed with washed packed sheep erythrocytes before being assayed in the test.

#### *Animals*

Colostrum was collected from pigs at parturition and casein and free fat were removed by centrifugation at 40,000 rev/min at 20° for 30 minutes (Bohren and Wenner, 1961).

Blood was taken from piglets prior to, and during the first 48 hours following receipt of colostrum.

## RESULTS

### PORCINE SECRETORY COMPONENT AND J CHAIN

The elution profile on Sephadex G-200 of porcine secretory IgA dissociated with 0.1 M  $\beta$ -ME is shown in Fig. 1 and analysis of three selected fractions in 5 per cent polyacrylamide gels at pH 8.4 containing 8 M urea are shown in Fig. 2. Fast migrating components distinct from light chains were evident in fraction 3. Rabbit anti-secretory IgA absorbed with serum IgA was used to detect secretory component activity in the gel filtration profile. Although secretory component appeared in fraction 3 there was no evidence of any relationship to the electrophoretically fast components which failed to react with antiserum after sectioning of unstained gels. Also free secretory component migrated only slowly in the gels under the same conditions of electrophoresis.

The electrophoretically fast components from reduced and alkylated secretory IgA were not detectable in the light chain fraction of 7S IgG or 6.4S IgA following cleavage with 2-ME. These components probably relate to the J chain recently described in rabbit and

human secretory IgA (Halpern and Koshland, 1970). This electrophoretically fast polypeptide chain has been suggested to be responsible for linkage of 7S subunits in structural configurations of 11S IgA and 18S IgM.

It was not possible with any antisera prepared against secretory IgA—fifteen rabbits, one sheep—to show the spur on precipitation lines formed between secretory IgA and

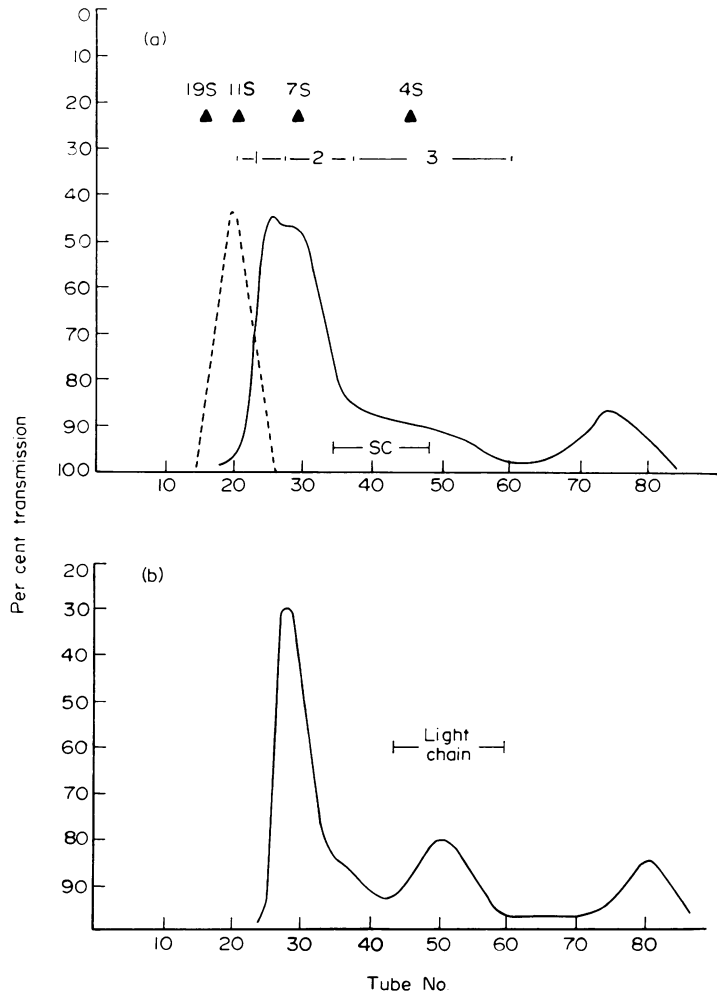


FIG. 1. Chromatography on Sephadex G-200 equilibrated with 8 M urea of secretory IgA (a) and serum IgG (b) after treatment with 0.1 M 2-ME and alkylation of dissociation products. Elution of untreated secretory IgA (broken line) and 19S 11S 7S and 4S markers are shown.

serum 6.4S IgA. This would have been the simplest indication of an additional antigen on the secretory immunoglobulin. However rabbit antisera for secretory IgA reacted with free secretory component producing a reaction of partial identity with secretory IgA. Rabbit anti-secretory component agglutinated tanned sheep erythrocytes sensitized with secretory IgA. This was the basis of the haemagglutination inhibition assay for secretory IgA used later in this text.

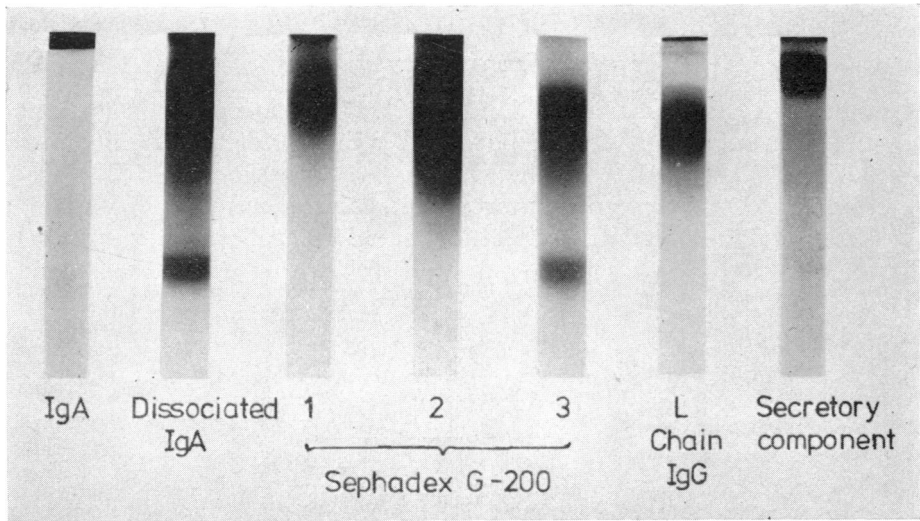


FIG. 2. Electrophoresis in 5 per cent polyacrylamide gel pH 8.4 8 M urea of secretory IgA secretory component, reduced and alkylated secretory IgA and gel filtration fractions of Fig. 1 showing the presence of electrophoretically fast polypeptide chains which are absent from the light chain fraction of IgG.

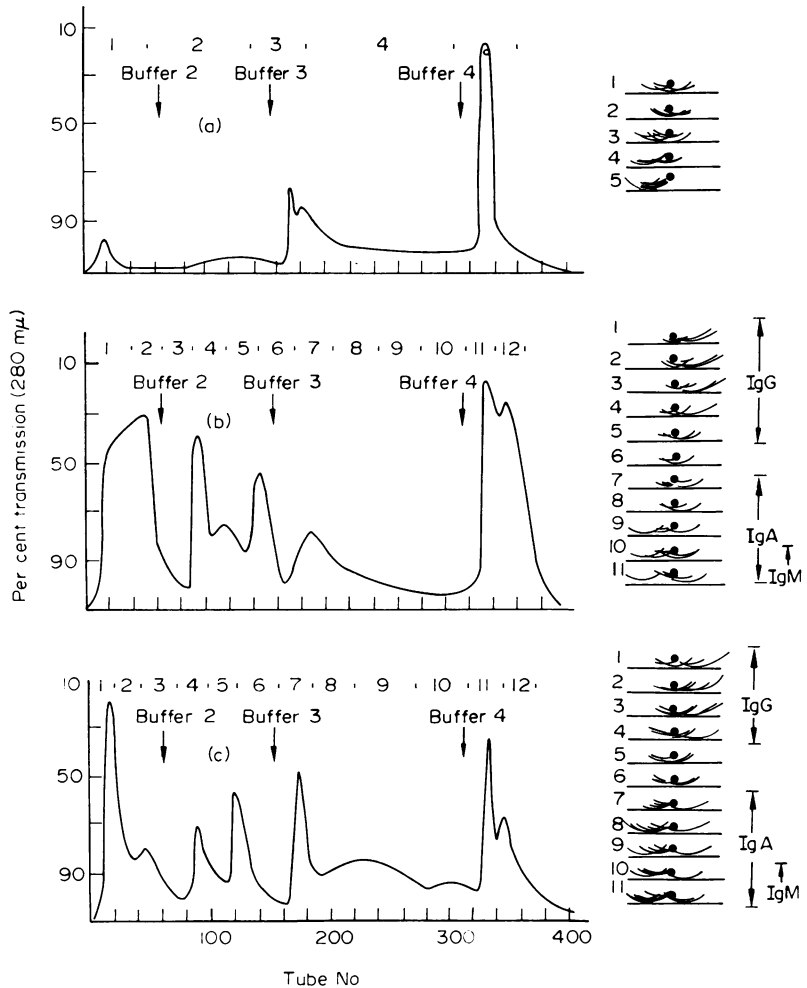


FIG. 3. DEAE cellulose chromatography of pre-colostrum piglet serum colostrum and post-colostrum piglet serum (a, b and c figures respectively). Immunoelectrophoretic analyses of fractions and identification of immunoglobulins in selected fractions indicated on the right.

## CHROMATOGRAPHIC STUDIES OF PIGLET SERA AND COLOSTRUM WITH CHARACTERIZATION OF IgA

Previous studies of secretory IgA in porcine milk by chromatography on DEAE cellulose, using the stepwise elution technique of Augustin and Hayward (1961), have shown that a partial separation of serum IgA and secretory IgA could be obtained (Porter, Noakes and Allen, 1970). Eluates with buffer 3 contain predominantly 6·4S IgA whereas secretory IgA appears mainly in eluates with buffer 4. This promised to be a fruitful starting point for studies of IgA in colostrum and post-colostrum piglet serum. Elution patterns for DEAE-cellulose chromatograms of precolostral and post-colostrum piglet sera and colostrum are shown in Fig. 3 giving the regions of elution of IgA, IgG and IgM.

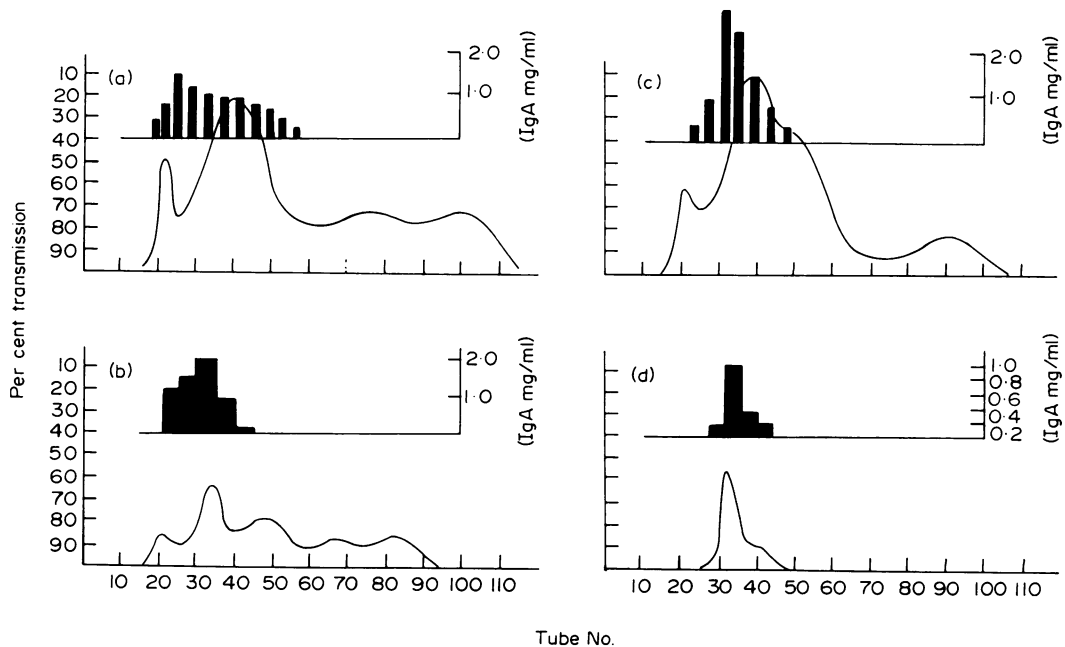


FIG. 4. Gel filtration of DEAE cellulose buffer 3 eluates (see Fig. 3) of colostrum (b) and post-colostrum serum (d) compared with whole colostrum (a) and piglet serum (c). Assays of IgA in selected fractions.

Colostrum IgA was identified in fractions eluted with buffer 3 as well as buffer 4. Immunodiffusion studies of fractions 7–10 with rabbit anti-secretory component failed to produce a precipitin, whereas positive reactions were obtained for fractions 11 and 12 eluted with buffer 4. This provided preliminary evidence that a non-secretory form of IgA was present in colostrum and available for intestinal absorption by the neonatal piglet.

The only immunoglobulin identifiable in pre-colostrum piglet serum is a subunit of IgG (Stertzl, Kostka, Riha and Mandel, 1960). A chromatogram of pre-colostrum piglet serum, pooled from six pigs of different litters, provided no evidence of any other immunoglobulin. The comparative chromatogram of post-colostrum piglet serum pooled from seven pigs of different litters demonstrated considerable absorption of colostrum immunoglobulins. The main changes in protein profile occur in eluates with buffer 1 and 2, indicative of consider-

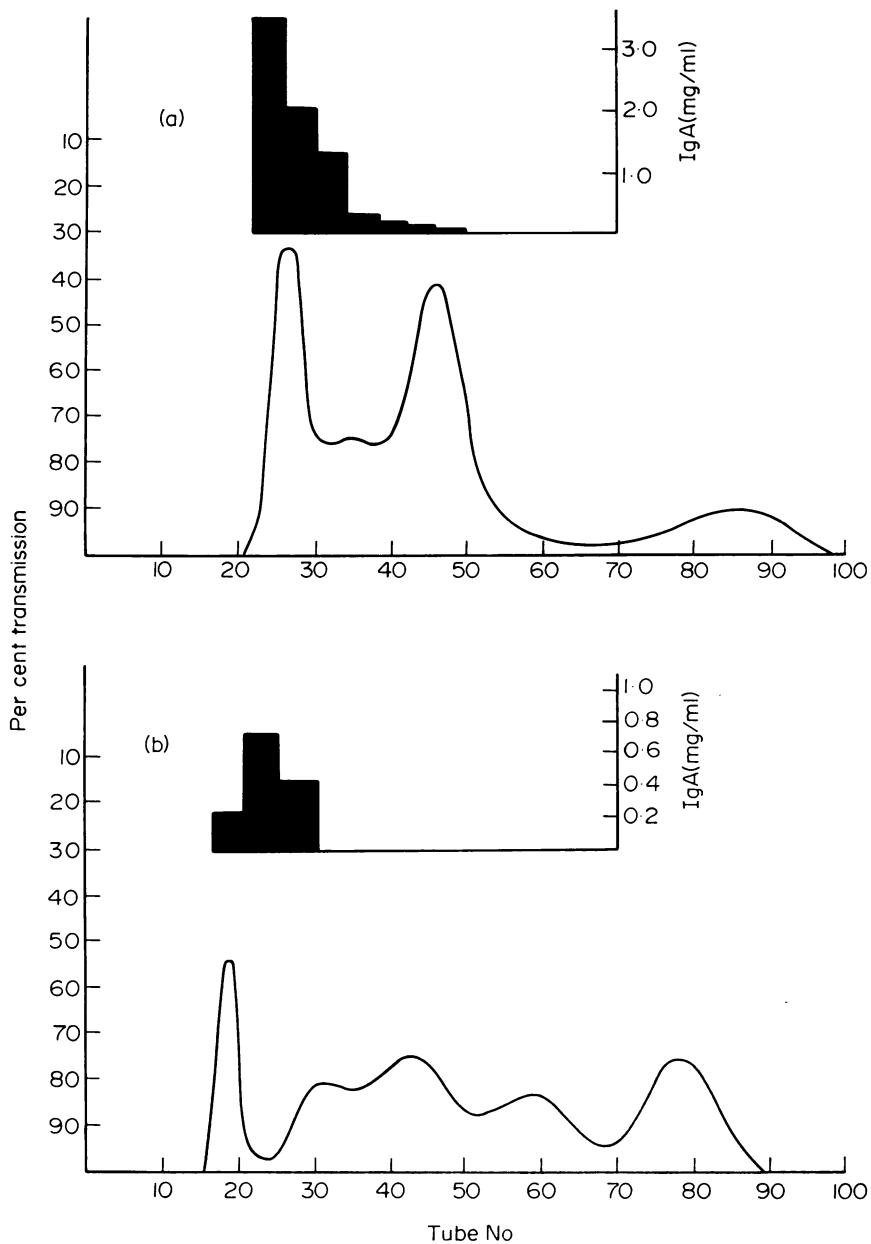


FIG. 5. Gel filtration of DEAE cellulose buffer 4 eluates (see Fig. 3) of post-colostrum serum (a) and colostrum (b) with assay of IgA in selected fractions.



able absorption of IgG. The difference in quantity of protein eluted with buffer 3, supported by the immunoelectrophoretic analyses of these fractions, provided preliminary semiquantitative evidence of a considerable proportion of IgA in post-colostral piglet serum which was present as the non-secretory form, i.e. lacking secretory component. Confirmation of this was produced by immunodiffusion studies with rabbit anti-secretory component which failed to produce a precipitin with post colostrum serum fractions 7-10. In studies of fractions 11 and 12 of post-colostral piglet serum, weak activity with rabbit anti-secretory component was identifiable.

Further to these studies, the fractions of colostrum and serum containing IgA were examined by gel filtration on Sephadex G-200 with a view to obtaining molecular size characteristics of the immunoglobulin. Studies of the DEAE cellulose buffer 3 eluates of post-colostral serum and colostrum are shown in Fig. 4 and compared with elution patterns for whole serum and colostrum. The elution of IgA was assayed in the chromatograms using the radial immunodiffusion technique (Mancini *et al.*, 1965). Ultracentrifugal analysis of the fractions containing the peak of IgA provided ( $S_{20,w}$ ) values of 6.42 and 6.33 for the colostrum IgA and serum IgA fractions respectively.

A complex mixture of components in a wide range of molecular sizes was eluted from DEAE cellulose with buffer 4. Gel filtration studies of these fractions of colostrum and serum are shown in Fig. 5. As was expected colostrum IgA appeared predominantly in fractions close to the exclusion volume, and secretory component was also demonstrated. The IgA in this fraction of post-colostral serum had similar gel filtration elution characteristics to that in colostrum. Secretory component was identified in fractions 1 and 2 but not in 3, 4 and 5 where the main proportion of the IgA appeared.

#### ULTRACENTRIFUGAL STUDIES OF IgA IN COLOSTRUM AND PIGLET SERUM

The fraction of post-colostral piglet serum eluted from DEAE-cellulose with buffer 4 was further examined by sucrose density gradient ultracentrifugation. The protein profile is shown in Fig. 6 and compared with profiles for IgM, secretory IgA, 7S IgG and albumin. IgA was assayed throughout the fractions and most of the IgA had a molecular size intermediate to 7S IgG and 11S secretory IgA. Only a small proportion of the IgA appeared in molecular size ranges characteristic of secretory IgA.

Ultracentrifugal analysis was carried out on high molecular weight gel filtration fractions containing IgA from post-colostral piglet serum and colostrum obtained as described in the previous section. Ultracentrifuge patterns of milk secretory IgA, colostrum IgA and piglet serum IgA are shown in Fig. 7. The high molecular weight fraction of piglet serum IgA occurred predominantly as a 9S peak with a slight shoulder at approximately 10.6S. Two other small peaks migrating at 13S and 18S were also present in this fraction. As reported previously (Porter, 1969a) the main peak in the high molecular weight fraction of colostrum was approximately 9S preceded by a shoulder at 10.6S and two other peaks of 13 and 18S. A purified fraction of milk secretory IgA had a sedimentation coefficient  $S_{20,w}$  10.6S preceded by a small faster sedimenting peak of approximately 16S.

In an attempt to improve the quality of the piglet serum IgA fractions, a pooled sample of serum was treated with 12 per cent  $\text{Na}_2\text{SO}_4$  and the globulin precipitate was prepared for chromatography on DEAE cellulose (Fig. 8). The main component in fractions eluted with buffers 3 and 4 was IgA and these were concentrated and further fractionated on Sephadex G-200. The two different molecular species of IgA identified in these gel filtra-

tion patterns were subject to ultracentrifugal analysis. The isolate of IgA obtained from buffer 3 eluates provided a single migrating peak with a sedimentation coefficient ( $S_{20,w}$ ) 6.41 and the isolate from buffer 4 eluates had a sedimentation coefficient ( $S_{20,w}$ ) 9.33.

#### QUANTITATIVE STUDIES OF SECRETORY IgA IN PIGLET SERA

The levels of secretory IgA in whole piglet serum were too low to assay satisfactorily by

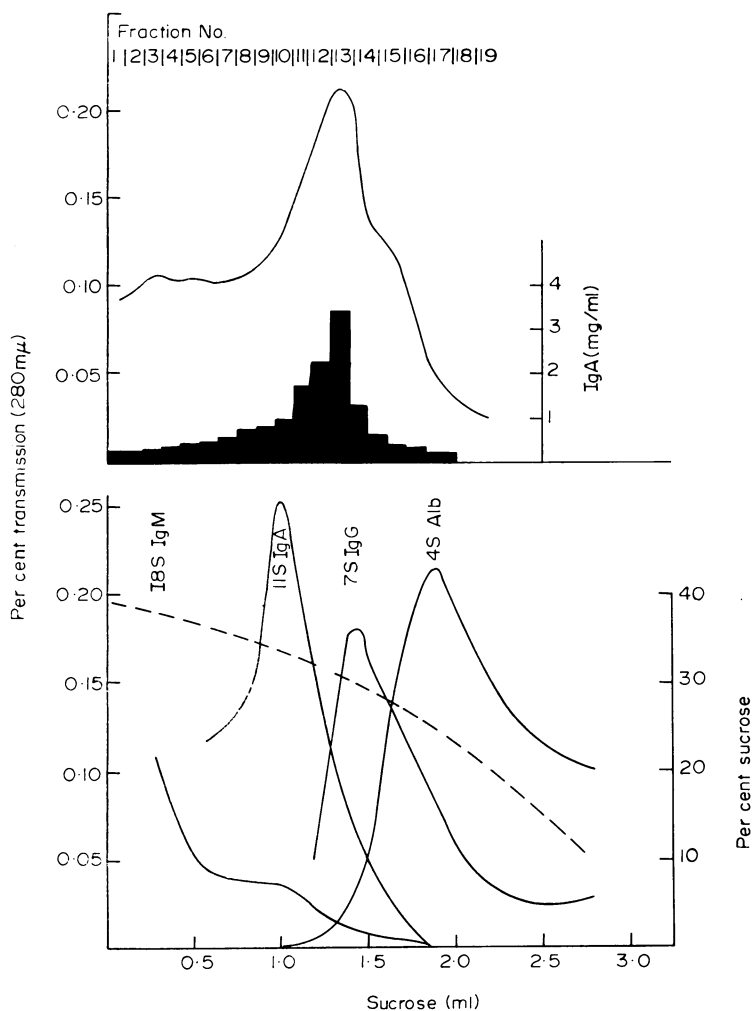


FIG. 6. Sucrose density gradient centrifugation studies of post-colostral serum IgA from Fig. 5 (a).

precipitin techniques using rabbit anti secretory component antiserum. The haemagglutination inhibition technique was adapted for assay and under the conditions described, levels of secretory IgA as low as  $8 \mu\text{g}/100 \text{ ml}$  could be determined. Immunoglobulin IgA, was assayed by radial immunodiffusion in samples of sera of suckling piglets of five different litters. The serum level of IgA ranged from 350 to 620  $\text{mg}/100 \text{ ml}$  ( $578 \pm 108 \text{ mg}/100 \text{ ml}$ ). The serum level of secretory IgA assayed by haemagglutination inhibition ranged from 2.7 to 10.1  $\text{mg}/100 \text{ ml}$  ( $5.2 \pm 1.1 \text{ mg}/100 \text{ ml}$ ).

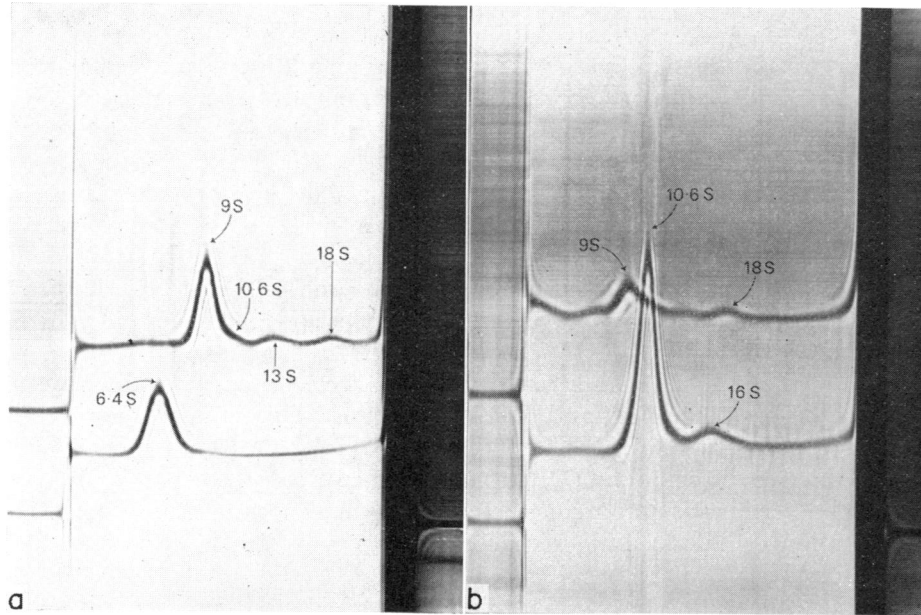


FIG. 7. Ultracentrifugal analysis of IgA fractions prepared from post-colostrum piglet serum colostrum and milk. (a) Fractions of post-colostrum piglet serum IgA prepared by chromatography on DEAE cellulose and Sephadex G-200 (Figs 4 and 5). (b) High molecular weight fraction of colostrum IgA (upper). Secretory IgA from milk (lower).

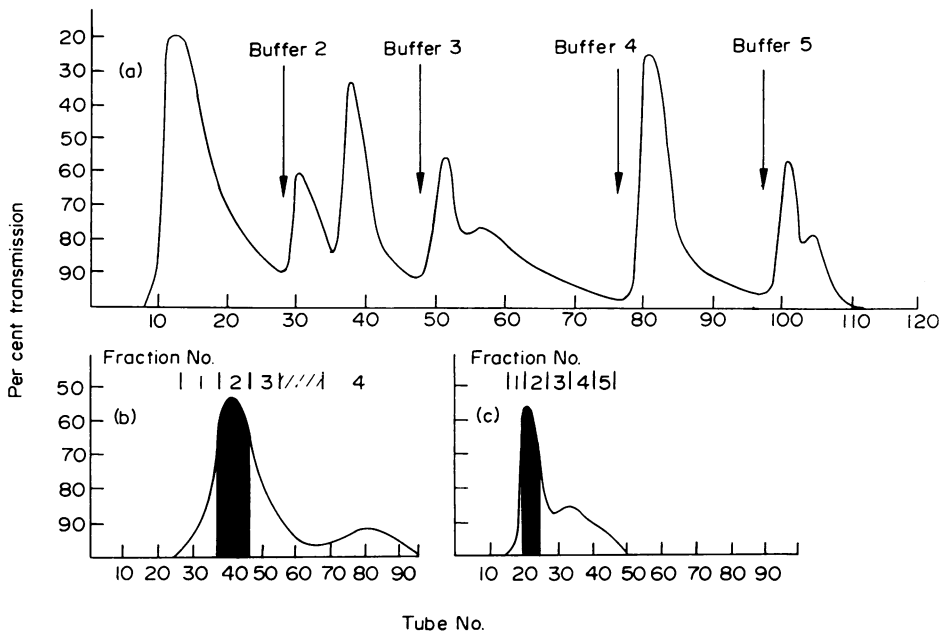


FIG. 8. DEAE cellulose chromatography (a) of globulin from post-colostrum serum precipitated with 12 per cent  $\text{Na}_2\text{SO}_4$ , followed by gel filtration of eluates from buffer 3 (b) and buffer 4 (c). Indicates fractions of IgA used in ultracentrifugal analysis.

## DISCUSSION

To date secretory component has been described as an additional antigenic determinant in secretory IgA of the human (Tomasi, Tan, Solomon and Prendergast, 1965), rabbit (Cebra and Small, 1967) bovine (Mach, Pahud and Isliker, 1969) goat and sheep (Pahud and Mach, 1970) dog (Ricks, Roberts and Patterson, 1970). The classic spur in immunodiffusion of secretory 11S IgA and serum IgA showing that the secretory immunoglobulin possesses an antigenic fragment lacking in serum IgA, was claimed to have been demonstrated in the pig. (Bourne, 1969.) However, Richardson and Kelleher (1970) failed to demonstrate this with antisera raised in three rabbits and in the present studies antisera raised against porcine secretory IgA in fifteen rabbits and one sheep all failed to give evidence of this.

Absorption of the antisera with serum IgA did however provide evidence of an additional antigen on secretory IgA and it was clear that if porcine secretory component existed it was a poor antigen in the intact 11S IgA molecule. It was not until fractions taken after reductive dissociation of 11S IgA were used that satisfactory antisera defining this additional determinant on porcine secretory IgA were obtained. This possibly relates to the observation of hidden antigenic determinants on secretory component bound in human secretory IgA (Brandtzaeg, 1968).

Evidence that the additional antigen from porcine secretory IgA was distinct from  $\alpha$ -chain and light chains was provided by absorption of the antiserum with serum 6·4S IgA.

Other components probably relating to J chain (Halpern and Koshland, 1970) were demonstrated in the products of reductive dissociation of porcine secretory IgA. These peptide chains were present in the light chain fraction obtained by gel filtration and were demonstrated to be distinct from the additional antigenic determinant by polyacrylamide gel electrophoresis combined with immunodiffusion.

Final conclusive evidence that the additional antigenic determinant was analogous to secretory component in other species was provided by isolation of free component from sow milk whey and demonstration of immunological cross reactivity with secretory IgA. Furthermore, immunofluorescent localization of secretory component in porcine intestinal epithelium is similar to that described in man (Allen and Porter, 1972). Thus having defined porcine secretory component and prepared a satisfactory antiserum for its detection, the study of intestinal absorption of secretory IgA by the neonate was facilitated.

It is generally accepted that in the young of a number of species, cattle, sheep, goats, horses, pigs, maternal antibodies secreted in the colostrum and ingested in early life are absorbed across the intestinal epithelium to provide circulating passive immune defence. Previous studies in the pig (Porter, 1969b) suggested that this was only partially true at least in relation to IgA, and the present studies indicate that secretory IgA accounts for only a very small proportion of the IgA present in the circulation of the suckling neonatal pig.

The fact that IgA appears in porcine colostrum unassociated with secretory component suggests that this is the most probable source of the absorbed IgA. It seems unlikely that colostrum secretory IgA is changed in the alimentary tract by processes of digestion or by some unknown mechanism in the intestinal epithelium since secretory IgA was demonstrated to pass the epithelium in low levels both by the immunofluorescent studies and by immunochemical studies of serum IgA in the suckling pig. It is more reasonable to look for a cause of the lack of absorption of secretory IgA.

To explain selective transmission of immunoglobulins in a number of species, it has been suggested that attachment to specific receptors on or in the cells might occur. Evidence supporting this thesis has been reviewed by Brambell (1969). The portion of an immunoglobulin responsible for transmission is in the Fc fragment (Brambell, Hemmings, Oakley and Porter, 1960; Morris, 1963); therefore if the lack of absorption of secretory IgA is attributable to the structural configuration of the immunoglobulin, the possible implication is that secretory component is covering receptors on the Fc fragment.

Tomasi and Bienenstock (1968) have considered the same thesis in attempting to account for the relative predominance of IgA in external secretions. Thus the preferential secretory mechanism might relate to a specific recognition site present on the IgA molecule. The biological role of secretory component remains unaccounted for and as yet there is no evidence to suggest that it plays any part in the transport of IgA. If the suggestions from the present findings on absorption have any relevance to secretion, such a thesis would require that secretory component became attached to IgA close to the external surface, otherwise hindrance in transport might be expected.

The high levels of the non secretory form of IgA which occur in porcine colostrum probably originate in the transudate of serum immunoglobulin which crosses the mammary acinar epithelium in the initial stages of lactation. This therefore would not represent true secretory IgA synthesized in the mammary gland and which persists as the major immunoglobulin and *E. coli* antibody in later lactation (Porter, Noakes and Allen, 1970). Secretory IgA in milk occurs predominantly as a 10·6S molecule whereas gel filtration and ultracentrifugal analysis shows that 6·4S and 9·3S forms predominate over 10·6S secretory IgA in colostrum. Evidence has also been produced for the presence of 7S IgA in human colostrum (Tomasi, Tan, Solomon and Prendergast, 1965) and nasal secretions (Rossen, Alford, Butler and Vannier, 1966; Butler, Rossen and Waldmann, 1967). If the lower molecular weight forms occur in porcine colostrum as a transudate of immunoglobulin, then the level of IgA in milk may represent in some measure the ability of the mammary gland to synthesize and secrete it.

The findings described here for the pig are quite different from those in the bovine. In bovine colostrum, IgA occurs predominantly in an 11·2S secretory form and the neonatal calf shows no selection in the intestinal absorption of immunoglobulins (Porter, 1971). The immunoglobulin profile in post-colostral calf serum closely resembles that in the colostrum and high levels of secretory IgA are found in the blood circulation of the calf during the first week of life. It will be interesting to see how far secretory IgA contributes to systemic immunity in other species in which the neonate derives maternal antibodies from the colostrum.

#### ACKNOWLEDGMENTS

This work was carried out with the technical assistance of Mrs L. Pugh, Mr M. E. Prior and Mr D. Leadbeater.

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