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Intestinal Secretion of Immunoglobulins and Antibodies to *Escherichia coli* in the Pig

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Summary. Immunoglobulins and antibodies against *Escherichia coli* 0141 have been studied in porcine intestinal secretions obtained from Thiry Vella loops prepared in the mid jejunum of 4 animals.

The molecular size of the secreted immunoglobulins were investigated by gel filtration and sucrose density gradient ultra-centrifugation. Intestinal IgM was found to have 7S characteristics and intestinal IgA mainly 11S characteristics similar to secretory IgA isolated from porcine milk.

Immune inhibition studies with rabbit anti-IgA-globulin serum produced complete elimination of *E. coli* 0141 antibodies detected by direct haemagglutination. In one animal incomplete antibody assayed by antiglobulin haemagglutination was identified in fractions associated with IgM and IgG.

Immunofluorescent studies were made to correlate immunoglobulins in the small intestinal tissue with weaning.

INTRODUCTION

Antibodies derived from the alimentary tract, the so called 'coproantibodies', have been the subject of considerable interest since Davies (1922) isolated specific antibody from faeces of patients with dysentery. Evidence for the intestinal origin of antibody to *Vibrio cholerae* was suggested by the work of Burrows, Elliot and Harvey (1947), Koshland and Burrows (1950), and Koshland (1953).

In recent years evidence has accumulated to suggest that IgA is the immunoglobulin involved in the immunological defence of all mucous surfaces (Tomasi 1967). The presence of IgA as the predominant immunoglobulin in human external secretions was first reported by Chodirker and Tomasi (1963) following the observation by Tomasi and Zigelbaum (1963) that IgA was the main immunoglobulin in parotid saliva.

In man the alimentary tract has been particularly well studied by the use of immunohistological techniques (Rubin, Fauci, Sleisenger and Jeffries, 1965; Crabbe, Carbonara and Heremans, 1965; Crabbe and Heremans, 1966; Gelzayd, Kraft and Fitch, 1967; Gelzayd, Kraft and Kirsner, 1968). This has shown the presence of large numbers of plasma cells containing immunoglobulins in which IgA predominates. There is evidence that a similar system exists in the pig. Secretory IgA was assayed in intestinal contents and found in the intestinal mucosa of the duodenum, jejunum and ileum (Porter and Allen, 1970).

The possible role of IgA in the immunological defence of the pig has been suggested by Porter (1969), Porter and Allen (1969), and Porter and Noakes (1969). The present

paper describes the secretion of immunoglobulins into the alimentary tract of young fistulated pigs. The importance of IgA as an intestinal antibody to *Escherichia coli* has been investigated and evidence for the secretion of IgM as a 7S subunit is presented.

MATERIALS AND METHODS

Isolation of specific immunoglobulins

The isolation of specific porcine immunoglobulins and the preparation of rabbit antisera specific for IgG, IgA and IgM has been described previously (Porter, 1969).

Chromatographic methods

Gel filtration chromatography was carried out on Sephadex G200 columns (45×2·5 cm) using 0·85 per cent NaCl in 0·1 M Tris-HCl buffer pH 7·2.

Anion exchange chromatography was carried out on diethyl-aminoethyl (DEAE) cellulose using the technique of Augustin and Hayward (1960).

Ultracentrifugation

Density gradient ultracentrifugation was carried out by the isokinetic sucrose gradient technique of Noll (1967) using an MSE50 superspeed centrifuge. Protein samples, 0·2 ml were layered on to the surface of 2·6 ml volumes of a 10–39 per cent sucrose density gradient. The samples were centrifuged at 200,000 ***g*** for 19 hours.

A Beckman model E centrifuge equipped with phase plate Schlieren diaphram was used in ultracentrifugal analysis of fractions. Sedimentation coefficients were determined at 20° at a speed of 59,780 rev/min.

Microelectrophoresis

Protein fractions were examined by immunoelectrophoresis using antisera raised in New Zealand White rabbits (Porter, 1964). Disc electrophoresis in polyacrylamide gels was done by the method of Orstein and Davis (1964).

Quantitative estimation of immunoglobulins

Immunoglobulin levels were estimated by the gradient immunodiffusion technique of Mancini, Carbonara and Heremans (1965).

Bacterial antibody tests

Haemagglutination and antiglobulin haemagglutination tests were done by the method of Buxton and Thomlinson (1961). Sheep red cells were modified with lipopolysaccharide from a haemolytic strain of *E. coli* (serotype 0141 : K85a, c(B) : H4) prepared by the method of Westphal, Ludovitz and Bister (1952).

The effect of treating intestinal secretions or chromatographic fractions with anti-IgA-globulin serum was investigated by adding 1 vol. of the specific antiserum to 3 vols of the fraction.

Immunofluorescent histochemistry

The technique of preparing fluorescein isothyocyanate (FITC) conjugated rabbit antisera and immunofluorescent detection of porcine immunoglobulins in cryostat sections of intestinal tissue has been described (Allen and Porter, 1970).

TABLE 1

IMMUNOGLOBULINS AND *E. coli* ANTIBODIES IN PORCINE INTESTINAL SECRETIONS AND THE EFFECT OF ABSORPTION WITH RABBIT ANTI-IgA-GLOBULIN SERUM ON ANTIBODY

Sample	Total protein (mg/100 ml)	Immunoglobulins (mg/100 ml)			*E. coli* 0141 haemagglutination titre			
		IgA	IgG	IgM	normal		after absorbing IgA	
					direct	antiglobulin	direct	antiglobulin
25/3	250	102	D	12	40	20	0	20
26/3	470	118	D	26	20	80	0	20
27/3	470	130	D	35	40	80	0	40
28/3	–	140	D	–	40	80	0	40
31/3	500	80	D	10·5	20	80	10	40
1/4	270	102	D	10·5	20	40	10	40

D—detected but too little to be assayed.

Experimental animals

Thiry Vella loops were prepared in the mid portion of the jejunum of 3 Large White pigs (2, 4 and 5) aged 4–9 days and 1 Large White pig (230) aged 42 days. Anaesthesia was induced by intravenous injection of 12 mg of methohexitone sodium ('Biretal' Elanco Ltd., London) per kg body weight. After endotracheal or nasal intubation anaesthesia was maintained with cyclopropane/oxygen.

The Thiry Vella loops, 30–40 cm in length, were prepared by the method described by Markowitz (1954) using perspex gutter type cannulae (1 cm i.d.). The continuity of the small intestine was restored by side to side anastomosis and the cannulae were exteriorized through stab incisions in the right flank of the pig.

The precise position of each loop was determined at post mortem examination. In pig 230, killed at 15 weeks of age, the loop was situated 810 cm from the pylorus and 690 cm from the ileo caecal junction. In pigs (2, 4, 5) which were killed at 5 weeks of age the loops were prepared in the mid jejunum 480 to 540 cm from the pylorus and 450 to 510 cm from the ileo-caecal junction.

RESULTS

SERUM PROTEINS AND IMMUNOGLOBULINS IN PORCINE INTESTINAL SECRETIONS

Secretions obtained from the intestinal loops were examined by immunoelectrophoresis to see whether the protein profile was relatively consistent. This technique consistently

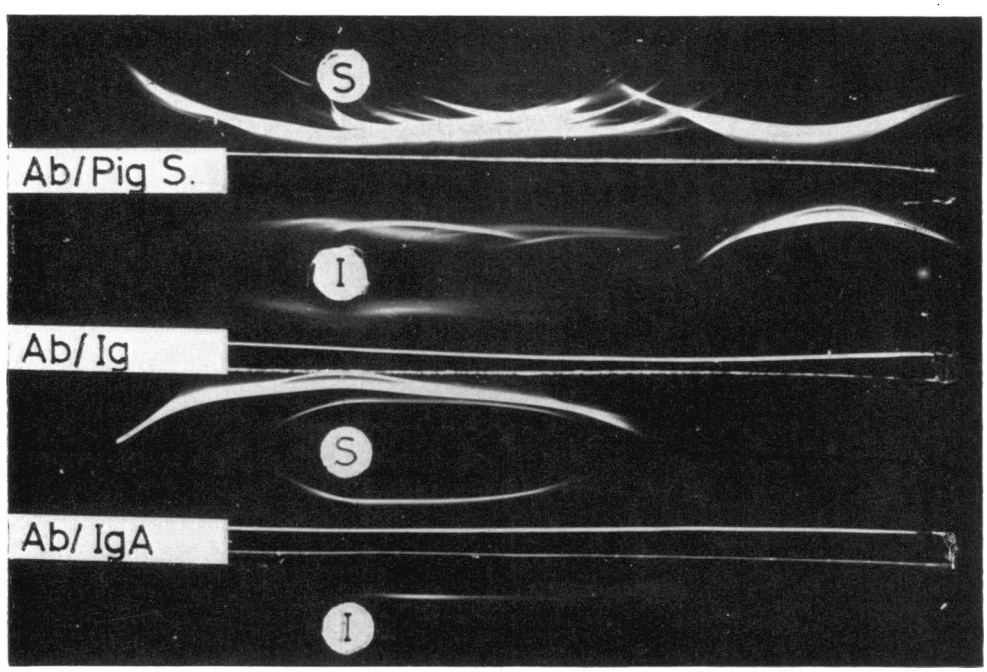

FIG. 1. Immunoelectrophoresis of pig serum (S) and intestinal secretions (I) demonstrating the presence of serum derived antigens in the secretions. The electrophoretograms are precipitated with rabbit antisera prepared against serum proteins, immunoglobulins and colostral IgA.

showed that the main fractions derived from serum were albumin, two or more alpha globulins, and IgA (Fig. 1).

In one animal (pig 230) it was possible to collect intestinal secretions into a bag attached to the lower cannula. Normally a 12-hour collection yielded approximately 2 ml of secretions which was sufficient for protein and immunoglobulin assays. In the other 3 pigs samples could be obtained only by irrigation of the loops with saline and concentration of the macromolecular components by dialysis against 30 per cent polyethylene glycol.

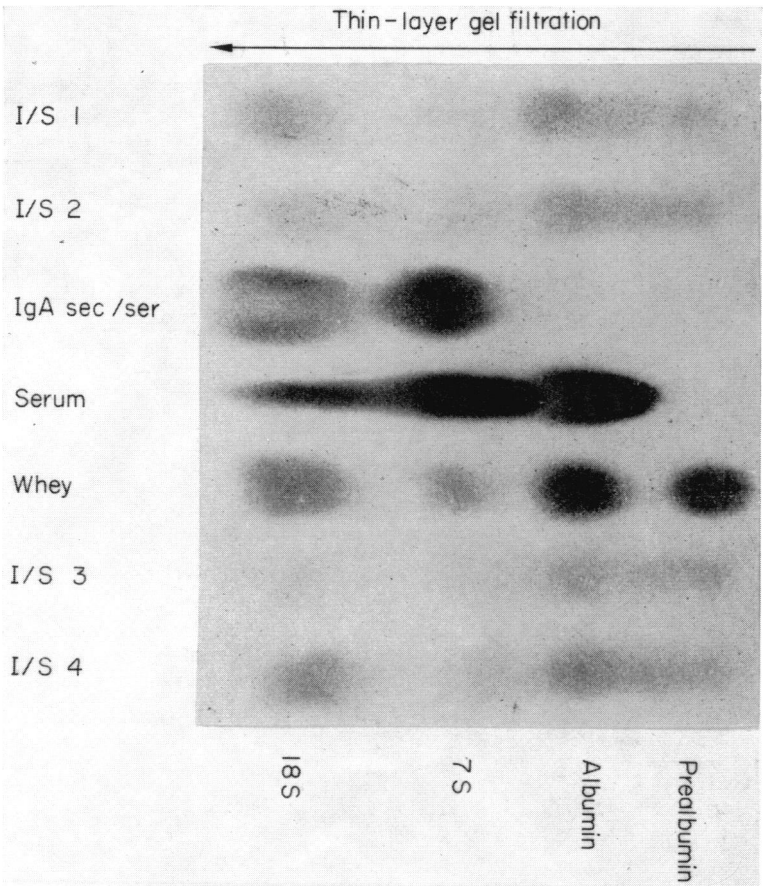

FIG. 2. Thin layer gel filtration on Sephadex G200 of proteins in intestinal secretions I/S 1–4 compared with serum, sow milk whey, secretory IgA isolated from milk and serum IgA.

Protein and immunoglobulin levels in secretions from pig 230 are shown in Table 1 together with assays of antibodies to *E. coli* 0141 antigen. IgA was the predominant immunoglobulin assayed by radial immunodiffusion. Absorption of the secretions with specific rabbit anti-IgA-globulin serum produced a complete inhibition of antibody activity measured by direct haemagglutination. However, incomplete antibody activity measured by the antiglobulin haemagglutination test remained, suggesting that IgG or IgM may also have a role as antibody in intestinal secretions.

Comparative studies of intestinal secretions from the 4 animals, using thin layer Sephadex G200 gel filtration showed a fairly consistent protein profile in which components with a molecular size similar to secretory IgA, serum albumin, and milk prealbumin were present (Fig. 2). The level of components migrating in the 7S region of the chromatogram was consistently low. Preparations of serum IgA and milk secretory IgA were used to indicate the regions of migration of 7S and 11S components.

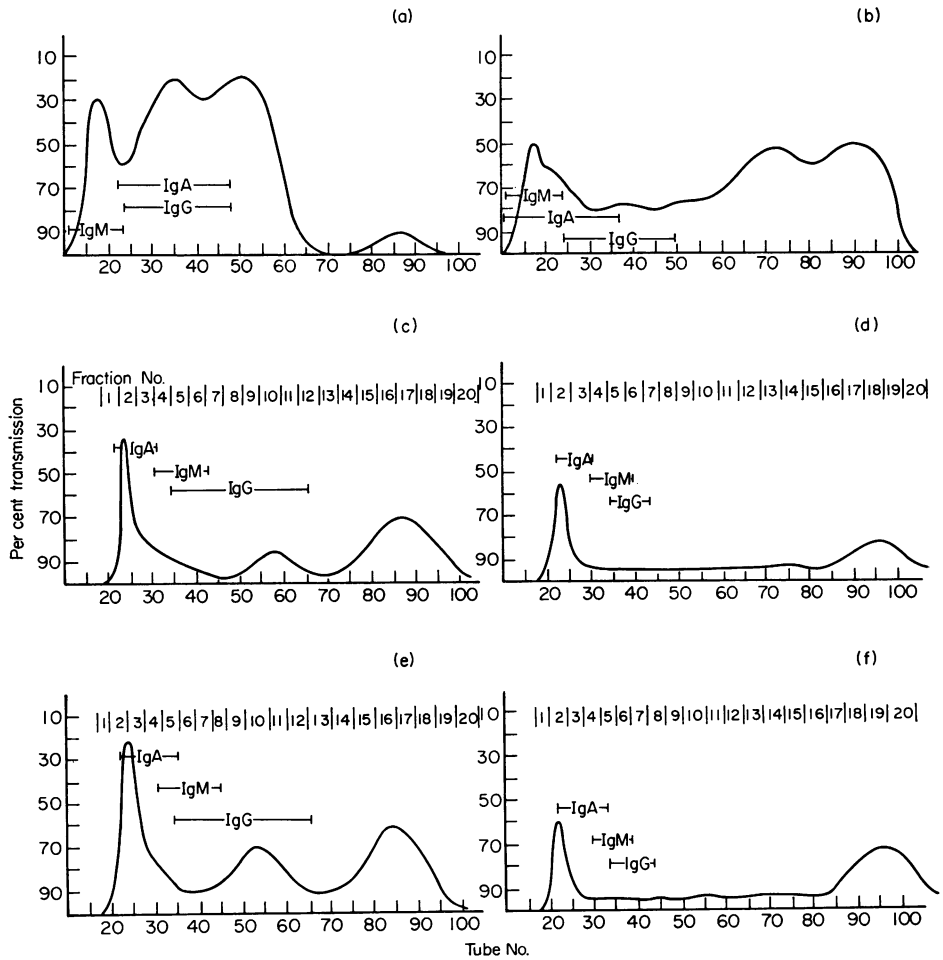

FIG. 3. Gel filtration of porcine serum (a) milk whey (b) and intestinal secretions (cdef) on Sephadex G200 giving pooling data and indicating elution of immunoglobulins.

STUDIES OF IMMUNOGLOBULIN AND *E. coli* ANTIBODIES IN PORCINE INTESTINAL SECRETIONS BY GEL FILRATION AND ULTRACENTRIFUGATION

The molecular size of the immunoglobulins in intestinal secretions from 3 animals was examined by gel filtration on Sephadex G200. Comparative studies with serum and milk in which immunoglobulins were detected by immunodiffusion using specific rabbit antisera to IgG, IgA and IgM are shown in Fig. 3. The elution characteristics of intestinal

secretory IgA were comparable to secretory IgA which is the major immunoglobulin in sow milk. In 2 of the animals 7S IgG was detectable and in all 3 animals IgM was identified in the 7S region of the chromatogram. In the fractions of lower molecular weight a component was identified which gave a reaction of identity with 7S IgG. The elution characteristics of these components are shown in the chromatograms in Fig. 3.

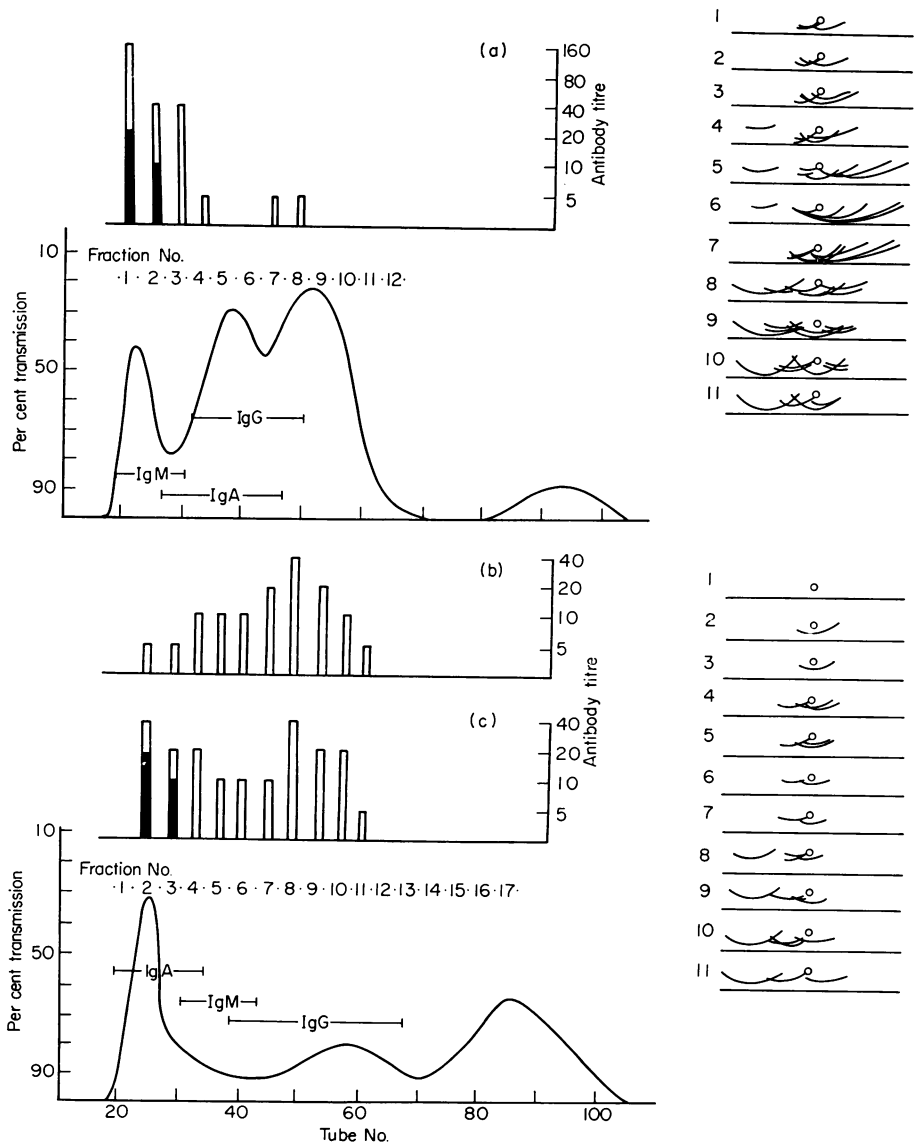

FIG. 4. Gel filtration of porcine serum and pooled intestinal secretions on Sephadex G200 giving pooling data, immunoelectrophoretic analyses of fractions using rabbit anti pig serum and also identification of antibodies to *E. coli* 0141. ■ Direct haemagglutination; □ Antiglobulin haemagglutination. (a) Antibodies in serum fractions; (b) Antibodies in intestinal fractions after inhibition with rabbit anti-IgA-globulin; (c) Antibodies in intestinal fractions.

In intestinal secretions from pigs 2 and 4, *E. coli* antibodies were found only in association with IgA. In the secretions from pig 230 a wider range of elution of antibody was obtained (Fig. 4). Absorption of the fractions with specific rabbit anti-IgA-globulin serum completely eliminated antibody activity detectable by direct haemagglutination, but incomplete antibody could still be assayed by the antiglobulin haemagglutination tests in fractions associated with IgG and IgM. This confirmed the findings in Table 1 and suggested that IgG and IgM may also play a protective role in intestinal secretions.

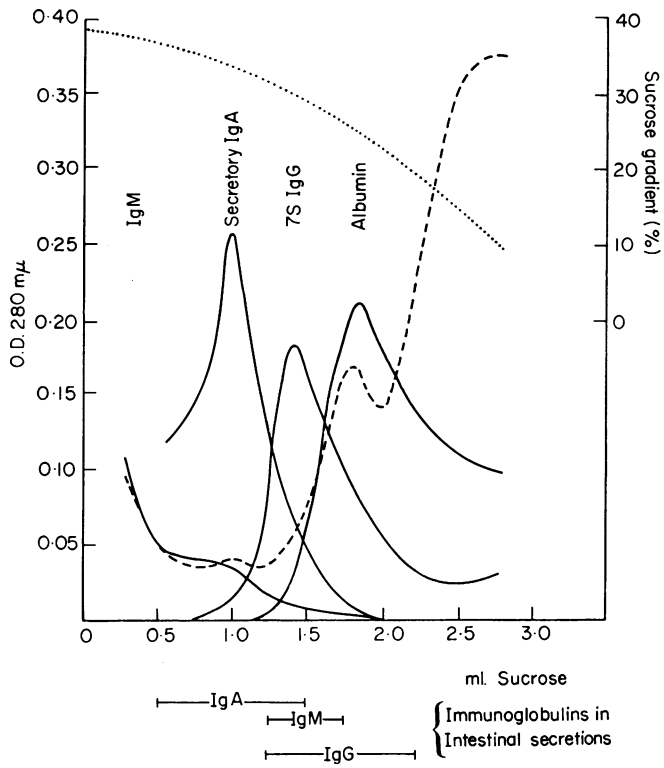

FIG. 5. Sucrose density gradient centrifugation studies of intestinal secretions and selected components to confirm the molecular size characteristics of intestinal immunoglobulins.

The molecular size of the immunoglobulins in intestinal secretions was also studied by ultracentrifugation (Noll 1967). The density gradient centrifugation patterns of intestinal secretions (pig 230) and serum are compared in Fig. 5. The calculated form of the sucrose gradient (10–39 per cent sucrose) and the position of purified preparations of albumin, 7S IgG, 11S secretory IgA and 18S IgM are shown. Intestinal secretory IgA was identified mainly in the 11S region and IgM was confirmed to have 7S characteristics by density gradient centrifugation.

STUDIES OF IMMUNOGLOBULINS IN THE INTESTINE BEFORE AND AFTER WEANING

The passage of milk antibodies through the alimentary tract has been previously studied (Porter and Noakes, 1969) and the evidence suggests that in the normal animal reared on

the sow, milk IgA is constantly present as an immunological defence in the small intestine. It might be expected therefore that the intestinal secretory immune mechanisms might not come into effect until the animal was weaned, and investigations were undertaken to see if this were so. Individual animals from a litter of 10 piglets were killed at 2 day intervals from the 15th day. Weaning took place on the 21st day.

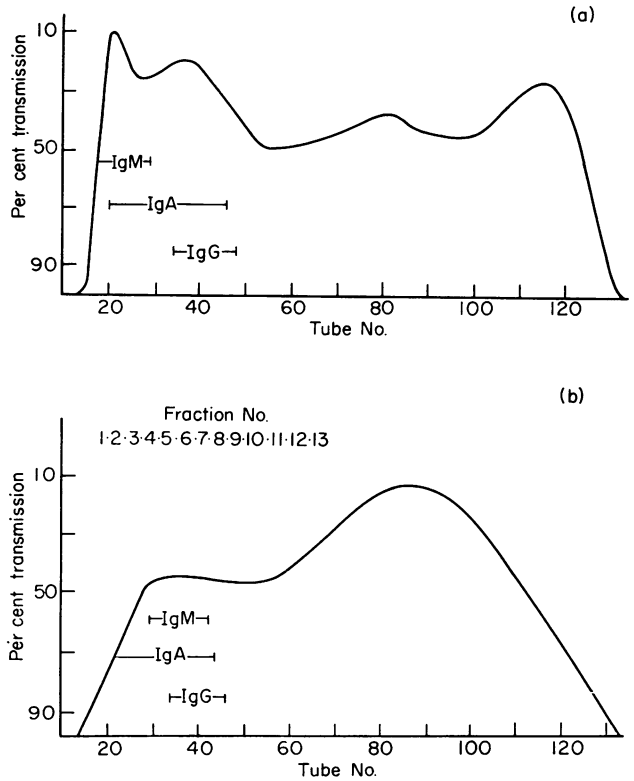

FIG. 6. Sephadex G200 gel filtration of extracts of intestinal contents of animals before weaning (15-day suckling), (a) and after weaning, (b) with elution characteristics of immunoglobulins.

The Sephadex G200 profile of extracts of small intestinal contents from animals before weaning was not unlike that of sow milk whey. IgA, IgG and IgM with elution characteristics similar to those in milk were identified. In animals examined after weaning the gel filtration profile was ill defined (Fig. 6). Normal elution characteristics were demonstrated for IgG and IgA but again intestinal secretory IgM appeared with 7S characteristics. Secretory IgA was readily detected by immunodiffusion in extracts of intestinal contents in animals before weaning and also in animals in the first 5 days after weaning. The latter observation suggested that the secretory immune mechanism might be rapidly effective after the removal of the protection provided by sow milk or that it was already effective before weaning.

To examine these possibilities immunofluorescent studies were made on the tissues of the small intestine. IgA was located in the tissues of suckling animals of 15 days and older. There was little variation in the location of IgA in all the tissues examined (duodenum, jejunum, ileum), and similar observations were recorded for weaned and unweaned

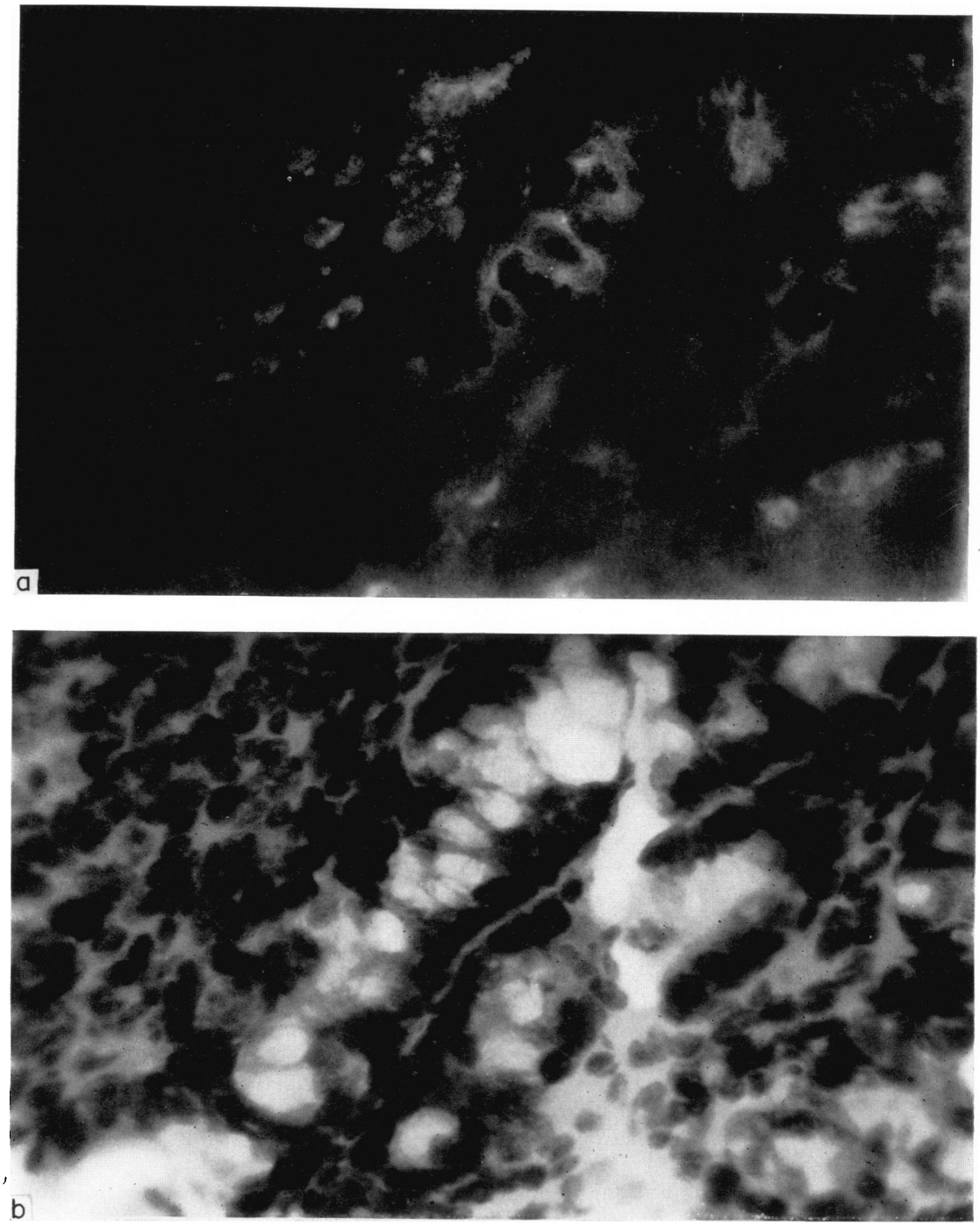

FIG. 7. (a) Small intestinal tissue from 15-day suckling pig stained with fluorescein conjugated antiserum to IgA. Fluorescence can be seen in the apical cytoplasm of the epithelial cells of the crypt and also in a plaque of cells in the intercrypt lamina propria. × 800. (b) Same field stained by haematoxin and eosin. × 800.

animals. IgA was confined to the apical cytoplasm of epithelial cells occupying the lower part of the crypt (Fig. 7) and the lamina propria contained a number of brightly fluorescing plasma cells.

DISCUSSION

A variety of antiviral and antibacterial antibodies are associated with secretory IgA in human colostrum, parotid saliva and nasal secretions (Tomasi and Bienenstock, 1968), but the immunoglobulins in the alimentary tract have not received much attention. Much of the work reported on coproantibodies probably relates mainly to the IgA system, but investigations on patients with ataxia telangiectasia tend to suggest that where there is a deficiency of IgA its role in external secretions may be taken over by IgM (Stobo and Tomasi, 1967; Edelmann and Davis, 1965). Furthermore it has been suggested that a common secretory mechanism may operate for IgA and IgM (Brandtzaag, 1968).

Recent studies, however, of intestinal fluid drawn from the ligament of Treitz area in normal men have shown that IgA is the predominant immunoglobulin present (Plaut and Keonil, 1969) and studies of the small intestinal contents of the pig provide similar results (Porter and Allen, 1969a). Thus the immunoglobulin found in intestinal contents could be derived from such sources as saliva, bile, and pancreatic, or gastric secretions; our experiments clearly suggest that the immunoglobulins demonstrated in the *lamina propria* may be transported across the intestinal epithelium and may perhaps contribute to an immunological defence at the site of production.

E. coli antibodies in intestinal secretions detected by direct haemagglutination were entirely associated with IgA. However IgM and IgG were also present in significant amounts. IgM is of particular interest since it is the main serum antibody. Its presence in intestinal secretions and localization by immunofluorescence in the intestinal crypt epithelium provide support for the view of Brandtzaag (1968) that a common mechanism of secretion may be operative for IgA and IgM. However an anomaly exists in that the secreted IgM does not appear as an 18S immunoglobulin but has characteristics of a 7S immunoglobulin.

Secretory IgA is not as readily degraded by proteolytic enzymes as IgM and it has been suggested that 'secretory piece' blocks sites on IgA which are subject to enzymic action (Tomasi, 1967). Cleavage of IgM might possibly occur *in vivo*, perhaps by intracellular proteolysis, but it would then be expected that both molecular sizes would be found. This was not the case in 4 animals.

It was not possible to demonstrate 7S IgM in porcine serum but this component has been found in horse serum (Sandor, Korach and Mattern, 1964). In man also there is evidence for 7S IgM in the serum of patients with disseminated lupus erythematosus (Rothfield, Frangione and Franklin, 1965; Stobo and Tomasi, 1967).

The extent to which secreted antibodies are responsible for maintaining the integrity and function of the small intestine still remains speculative. The possibility that dysfunction of this system may be an aetiological factor in alimentary tract disease in man has been suggested (Eidelman, 1968). From this viewpoint the development of intestinal antibodies in the young pig may play an important role in immunological defence in the first weeks of life when serum antibody acquired from the colostrum falls to very low levels. In this context it is interesting that immunofluorescent studies provide evidence of IgA secretion in piglets before weaning. Secretory IgA is the major antibody in sow milk and it might be

expected that an almost continuous presence of this component in the intestine of the suckling pig might suppress the stimulus of the immunological mechanisms. However this is apparently not the case and secretory intestinal antibody capacity may well contribute to the defence of the animal in this critical period of life when the level of serum antibodies is known to be low (Brown, Speer, Quinn, Hays and Catron, 1961).

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