Clin. exp. Immunol. (1975) **21**, 407–418.

LOCALIZATION OF IMMUNOGLOBULINS IN INTESTINAL MUCOSA AND THE PRODUCTION OF SECRETORY ANTIBODIES IN RESPONSE TO INTRALUMINAL ADMINISTRATION OF BACTERIAL ANTIGENS IN THE PRERUMINANT CALF

W. D. ALLEN AND P. PORTER

Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford

(*Received* 19 *December* 1974)

SUMMARY

Immunofluorescent studies of intestinal tissues from young preruminant calves demonstrate the presence of two main populations of immunocytes synthesizing IgA and IgM. These cells had infiltrated the lamina propria of the intestine as early as 4 days of age. There was little evidence of any significant involvement of IgG1 in intestinal immune synthesis of calves at this age although activity was demonstrable in the ileum and colon of one calf. In general there were more IgG2-synthesizing cells than IgG1, but these were few compared with the main populations of IgA and IgM cells.

Local antigenic stimulus to the intestinal mucosa of young fistulated calves using extracts of heat-killed Gram-negative bacteria produced antibody in the secretions over a period of approximately 3 weeks. A second administration of a similar antigenic dose produced a similar response indicating the requirement for continuous stimuli to maintain a measurable level of antibody secretion. Gel filtration and antiglobulin assays indicated that the antibacterial activity was predominantly associated with IgA and that IgM also played a significant role.

Oral administration of bacterial antigens to colostrum-fed calves from 5 to 8 days of age produced a faecal antibody response, indicating that intestinal secretion could be successfully interrelated with the declining passive antibody to maintain an almost continuous level of intestinal antibody in early life.

INTRODUCTION

Although the concept of oral vaccination against microbial enteritis is not new, progress has been made only recently in exploiting its practical potential. Early work was hindered by inadequate appreciation of the essentially local nature of the immune mechanism involved and oral vaccines were rejected because they failed to induce a detectable serum response. It is now realized that circulating antibody level is a poor criterion by which to

Correspondence: Dr W. D. Allen, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford.

judge an oral immunizing agent (World Health Organization Technical Report, 1972) and one which is probably totally irrelevant.

The young animal in modern intensive systems of farm management is particularly susceptible to enteric infection with Gram-negative bacilli with consequent deterioration in nutritional performance and increased mortality. Studies in the young pig have shown that orally administered antigens of pathogenic *Escherichia coli* stimulate the gut-associated lymphoid system and render the animal better able to withstand post-weaning enteritis. A vaccine has been trialled as a feed supplement and the benefits of this approach are reflected in improved animal performance during the critical postweaning period (Porter *et al.*, 1973). Demonstration of the existence of a local intestinal immune system (reviewed by Porter & Allen, 1972) and determination of the earliest age at which active immunoglobulin synthesis occurred in the gut mucosa (Allen & Porter, 1973) were crucial to the development of this oral immunization procedure.

Early weaned calves feeding on milk substitute diets are susceptible to digestive disorders and are particularly vulnerable to *E. coli* infection during their first 3 weeks of life (Roy *et al.*, 1955). Oral immunization again appears to be a practical approach to improving health and nutrition.

Alimentary tract physiology in the preruminant calf is similar in many respects to that of monogastric species and recent evidence suggests the existence of an analogous secretory immune mechanism (Porter, 1973). However, there is some confusion in the literature as to the nature of the principal immunoglobulin involved, with IgG1 (Curtain, Clark & Dufty, 1971) or IgA (Butler *et al.*, 1972) both having been implicated. Furthermore, no evidence has been presented which would indicate the earliest age at which intestinal synthesis of immunoglobulin begins.

These problems are investigated in the text of this paper which describes immunofluorescent studies of the functional development of intestinal immune mechanisms in the young calf and examines the response to local administration of bacterial antigens.

MATERIALS AND METHODS

Isolation of specific immunoglobulins and conjugation of specific antisera with fluorescein isothiocyanate

The techniques for the isolation of bovine immunoglobulins and the preparation of $\gamma 1$, $\gamma 2$, α and μ chain-specific rabbit antisera and conjugation with fluorescein isothiocyanate (FITC) have been described previously (Porter & Noakes, 1970; Allen & Porter, 1970).

Preparation of tissues

Tissues were obtained at post-mortem (immediately after death) from healthy animals varying in age from 4 days to 24 months. They included four levels of the alimentary tract, duodenum, jejunum, ileum and colon.

Small blocks of tissue, about 1 cubic cm were fixed immediately in chilled ethanol, or quenched in isopentane cooled with liquid nitrogen. The quenched blocks were stored in liquid nitrogen until required. Paraffin blocks were prepared from the ethanol fixed tissues by the method of Saint Marie (1962).

Fluorescent staining procedure

Following removal of wax by chilled xylol and rehydration in cold ethanol, sections were

rinsed in phosphate-buffered saline (PBS) pH 7·1, before staining. Both direct and indirect staining techniques were used with incubation conditions ranging from 30 min at room temperature to overnight at 4°C. For the indirect method a sheep anti-rabbit FITC antiserum (Wellcome) was used. The stained sections were mounted in buffered glycerine at pH 8·5 just before microscopic examination.

The specificity of the reactions were controlled by tests similar to those described earlier (Allen & Porter, 1970), except that tissues from newborn, colostrum-deprived calves were not examined.

Microscopy

Fluorescent stained sections were examined by dark ground microscopy using a Reichert Zetopan microscope with an HBO 200 light source. A U.G.1 exciter filter was used with an absorption GG9 filter. This filter was particularly useful for counteracting the intense blue autofluorescence which frequently occurs in sections prepared by the Saint Marie (1962) technique.

Colour transparencies were taken on Kodak Ektachrome high speed daylight film (160 A.S.A.). Black and White photographs were either taken on Ilford FP4 or copied from colour transparencies.

Counts were made of all cells showing fluorescent staining of cytoplasm in twenty fields selected at random, using a ×40 objective, 0·65 N.A. and a ×8 eyepiece. As fluorescent cells seldom occurred in the villous cores observations were confined to lamina propria lying between the crypt villous junction and the muscularis mucosa.

Estimates of variation between specimens in the ratio of lamina propria area to crypt epithelium area were made on replicate sections stained by haematoxylin and eosin. These were projected onto the viewing screen of a Projectina microscope using a ×40 objective 0·75 N.A.; and the relative amounts of lamina propria and epithelial tissues measured using a 1 cm squared grid.

Antibody studies in fistulated animals

Thirty–Vella loops were prepared in the mid position of the jejunum of three Ayrshire calves aged 2–4 days. Anaesthesia was induced by the intravenous injection of methohexitone sodium (Brietal Elanco Ltd, London) and after endotracheal intubation, was maintained by cyclopropane and oxygen. Thirty–Vella loops 30–40 cm in length were prepared by the method of Markowitz (1954) using Perspex gutter cannulae (1·2 cm, i.d.; 1·5 cm, o.d.). The continuity of the small intestine was restored by side to side anastamosis and the cannulae were exteriorized through stab incisions in the right flank of the calf.

Secretions from the loops were collected in small polythene bags attached to the end of each cannula over a period of several hours.

Antibody studies in orally immunized calves

A first experiment was carried out with *Salmonella dublin* using 24-hour shake flask cultures (1×10^{10} organisms per millilitre) sterilized by autoclaving (121°C, 15 psi, 30 min). The O antigen level assayed by haemagglutination inhibition as described by Porter *et al.* (1973) was 40 HI units/ml.

Ten calves were paired and the sterilized culture was added twice daily to their liquid milk replacer in volumes 0, 0·02, 0·2, 2 and 20 ml over a period of 3 weeks. In addition one pair of calves was given an oral drench of 10 ml of sterile culture twice daily over the same period. Dosing commenced at an age of 5–8 days.

In a second experiment investigations were carried out with mixed sterile cultures of *Salmonella typhimurium* and *E. coli* O137 prepared as above. The O antigen level assayed by haemagglutination inhibition was 160 HI u/ml for *S. typhimurium* and 40 HI u/ml for *E. coli* O137. Eight calves were paired and the sterilized cultures added daily to their liquid milk replacer in volumes 0·02, 0·2, 2 and 10 ml.

Faecal samples were taken daily and blood samples were taken twice weekly. Antibody assays were carried out by antiglobulin haemagglutination on serial dilutions of blood serum and saline extracts of faeces.

RESULTS

Fluorescent localization of immunocytes in the intestinal tissues

Localization of immunoglobulins in sections of intestinal tissue stained with fluorescein-conjugated antisera to either IgA or IgM showed both immunoglobulins in the apical cytoplasm of crypt epithelial cells at all intestinal levels examined. Fluorescent staining was concentrated in the region of the terminal web extending into the supranuclear cytoplasm (Fig. 1). A less intense staining was observed in the intercellular spaces of the crypt epithelia. Cells in the deeper regions fluoresced more strongly than those nearer the crypt villous junction.

No staining of villous epithelium was detected with either antiserum; however, mucin coating the brush border frequently showed a reaction with anti-IgA serum and very occasionally with anti-IgM.

In the lamina propria numerous lymphoid cells with clearly defined cytoplasmic staining mainly in the intercryptal region between the muscularis mucosa and the crypt villous junction (Fig. 1) and frequently close to the crypt epithelial cell basement membrane. Occasionally, stained cells were found in the lamina propria of villi, more especially those staining for IgM. There was no marked variation in the location of reactive cells within the lamina between different levels of intestine.

In contrast to the finding for IgM and IgA, little evidence of secretion of IgG1 across the intestinal epithelium was found, and only very occasionally was IgG2 detected in the supranuclear cytoplasm of crypt epithelial cells. This latter reaction was much weaker than that obtained for either IgA or IgM. No staining of intraluminal mucin was found with either anti-IgG1 or anti-IgG2.

The distribution of cells showing cytoplasmic staining IgG1 or IgG2 was similar to that described for IgA or IgM-containing cells, being mainly confined to those located in the intercryptal lamina propria; only an occasional cell was seen in the lamina of the villous cores. Cells in the intercryptal region did not appear to be so closely associated with the basement membrane as did those containing IgM and IgA.

Estimations of the relative numbers of cells containing the various immunoglobulins were made on sections of intestinal tissues from six 5-week-old calves (Table 1). The cells counted were those which showed a clearly defined cytoplasmic staining. In some preparations, particularly those stained with antisera to IgG1 and IgG2, in which extracellular

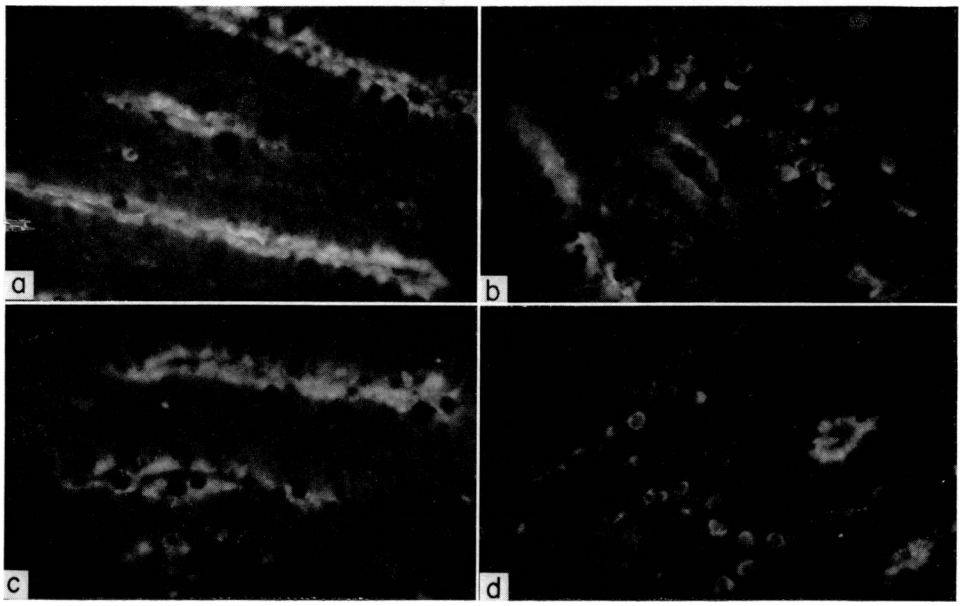

FIG. 1. Bovine jejunum stained with rabbit anti-bovine immunoglobulin conjugated with FITC. (a) and (b) show the localization of immunoglobulin IgA in the apical cytoplasm of crypt epithelial cells (a) and immunocytes in the intercryptal lamina propria (b). (Magnification × 576.) (c) and (d) show a similar localization of immunoglobulin IgM. (Magnification × 576.)

TABLE 1. Number of cells synthesizing IgA, IgM, IgG1 and IgG2 in the alimentary tract of young calves

Organ	Immuno-globulin	Calf number					
		585	588	589	590	591	592
Duodenum	IgA	564	742	1045	461	591	588
	IgM	251	251	118	349	274	302
	IgG1	1	0	6	0	7	19
	IgG2	69	38	24	54	20	48
Jejunum	IgA	441	229	345	584	664	755
	IgM	90	184	87	189	156	271
	IgG1	0	0	1	5	5	1
	IgG2	52	9	1	3	9	1
Ileum	IgA	300	215	376	296	403	219
	IgM	45	81	65	133	143	110
	IgG1	0	0	0	221	0	0
	IgG2	1	9	0	7	1	0
Colon	IgA	—	297	266	199	114	79
	IgM	—	15	39	104	54	38
	IgG1	—	0	1	107	1	0
	IgG2	—	2	4	14	1	0

Counts are expressed as the number of cells per twenty fields (× 40 objective).

immunoglobulin was considerable, there were many cells showing stained cell membranes only. These were specifically excluded from the counts.

The pattern of distribution of immunoglobulin-containing cells at the different levels of intestine was similar for all six animals. By far the greater number of reactive cells stained either for IgA or IgM; moreover, the number of cells containing IgA was generally between three and four times higher than that of cells containing IgM. Whilst a small number of IgG2-containing cells were found, very few cells showed cytoplasmic staining for IgG1. It is interesting that a relatively high number of cells containing both immunoglobulins were present in the intestinal tissues of the 4-day-old calf, the cells staining for IgA and IgM

TABLE 2. Distribution of IgA- and IgM-containing cells in jejunum of calves of different ages

	Age (months)								
	4 days	3	3	6	6	12	18	24	24
IgM	40	294	241	54	125	182	178	197	110
IgA	53	296	602	176	249	334	112	459	640
Ratio M:A	1:1·3	1·0	2·5	3·3	2·0	1·8	0·6	2·3	5·8

Counts are expressed as the number of cells per twenty fields (×40 objective).

TABLE 3. Proportion of lamina propria occurring in a ×40 microscopic field

	Animal number						Mean of
Tissue	585	588	589	590	591	592	animals
Duodenum	50·9 (2·7)	44·9 (2·4)	45·5 (3·3)	54·7 (2·5)	45·4 (3·1)	40·0 (3·7)	46·9
Jejunum	46·9 (2·9)	50·7 (3·9)	56·5 (3·2)	58·0 (2·8)	49·4 (4·0)	46·8 (4·3)	51·4
Ileum	60·4 (2·3)	53·5 (3·9)	67·3 (3·6)	52·9 (4·0)	53·7 (3·6)	53·9 (3·2)	57·0
Colon	—	42·1 (2·4)	43·2 (4·1)	45·8 (2·8)	35·5 (3·1)	47·0 (3·9)	42·7

The results are expressed as the area of lamina propria as a percentage of the total field. A mean of twenty fields (s.d.) is shown.

being more or less equal (Table 2). This finding forms a basis for the subsequent studies on intestinal synthesis and secretion of antibodies.

Comparison of the relative area of lamina propria and crypt epithelium surveyed by examining twenty random fields showed a close similarity of crypt : lamina ratio between animals (Table 3). Rather more laminal tissue was surveyed in jejunum and ileum than in duodenum and colon. The ratio of lamina tissue in the duodenum and jejunum was 1 : 0·9.

Immunoglobulins and antibodies in intestinal secretions of fistulated animals

The intestinal secretions were examined by electrophoretic techniques in order to establish the suitability of the surgically prepared animals for studies of local intestinal immune function. Inflammatory lesions and serous exudation produces an abnormal

protein profile often showing the majority of serum proteins. Immunoelectrophoretic profiles typical of intestinal secretions in the young calf are shown in Fig. 2 and compared with that of serum. Precipitin arcs indicative of immunoglobulins IgG2, IgA and IgM were readily demonstrated. IgG1 was either absent or present in negligible amount bearing out the immunofluorescent evidence on cellular synthesis in the lamina propria. In immunoelectrophoresis using rabbit anti-bovine secretory IgA two precipitin arcs were evident indicating the presence of free secretory component. The antiserum produced only a single arc in the immunoelectrophoretogram of calf serum.

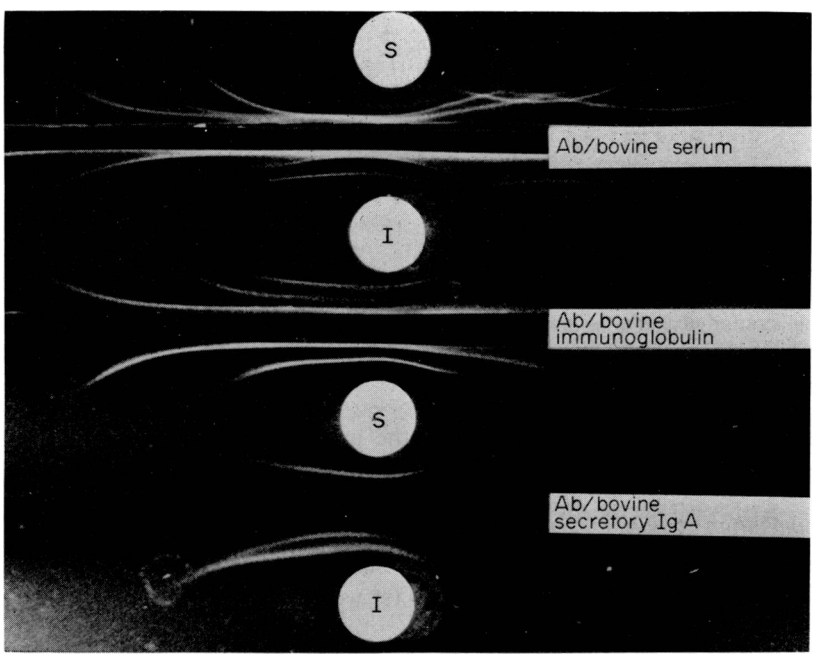

FIG. 2. Immunoelectrophoretograms of calf serum (S) and intestinal secretion (I) developed with antisera of various specificities. Precipitin arcs indicative of immunoglobulins IgG2, IgA and IgM are present in the intestinal secretion reactions. Two arcs were obtained in the reaction between intestinal secretion and rabbit anti-secretory IgA indicating the presence of IgA and free secretory component.

Local application of bacterial antigens (5 ml of aqueous extract from a boiled suspension of 2×10^9 organisms per millilitre) in the intestinal loop produced a measurable secretion of antibodies. The patterns of change in antibody secretion in response to *E. coli* and *Salmonella* antigens are shown in Fig. 3. A peak of antibody secretion was registered within 7 to 10 days of application of antigen but the activity declined within 3 weeks. A second administration of the antigen produced a similar response in terms of time, level and duration of antibody secretion; a finding consistent with that previously reported for the pig (Porter *et al.*, 1974) and indicative once again of apparent lack of immunological memory in the intestinal secretory immune system. Examination of the secretions by gel filtration and

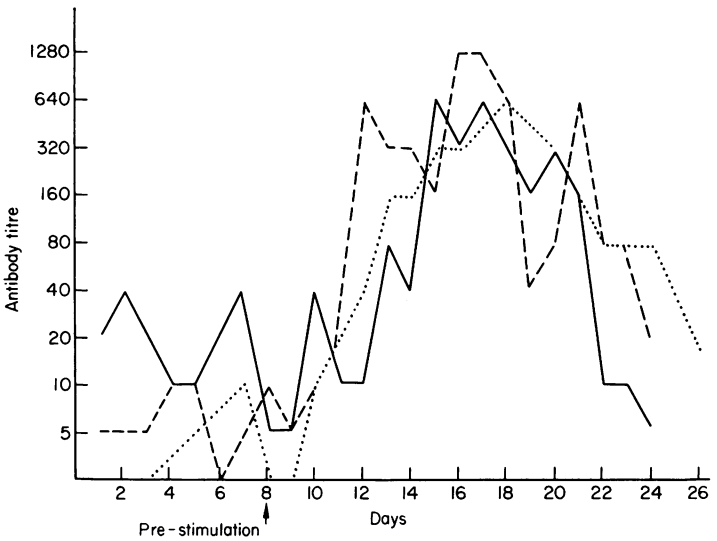

FIG. 3. Local intestinal antibody secretion in intestinal loops following administration of heat-killed bacteria. The level of antibody secretion reached a peak within 7–10 days of administration declining to low levels during the following 2 weeks. (——) Calf number 17, heat-killed *E. coli*. Calf number 18 (– – –), heat-killed *E. coli*. Calf number 19 (· · · ·), heat-killed *S. dublin*.

antiglobulin techniques demonstrated that the antibacterial activity was predominantly associated with IgA (Fig. 4).

Oral immunization studies

Passively derived maternal antibodies to *Salmonella* and *E. coli* antigens could be detected in the serum and faeces during the 1st week of life. The activity declined to low

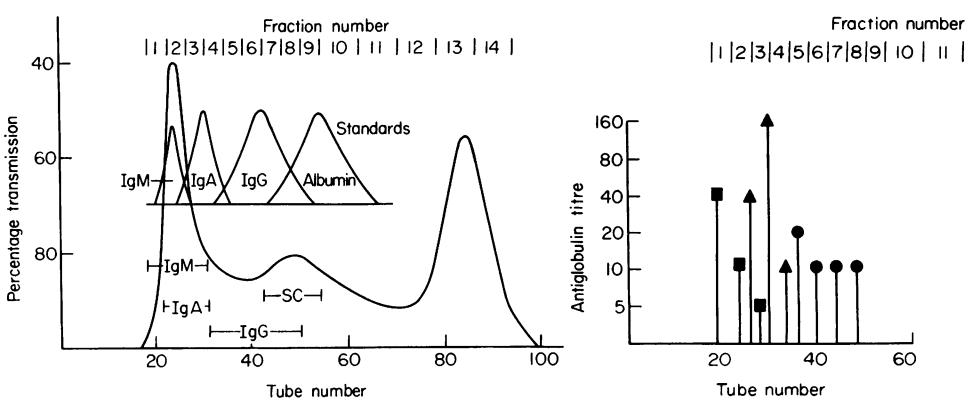

FIG. 4. Gel filtration studies of intestinal secretions on Sephadex G-200 showing the elution characteristics of immunoglobulins and anti-*Salmonella dublin* antibodies detected by anti-globulin haemagglutination with specific rabbit anti-immunoglobulin heavy chains to IgG (●), IgA (▲) and IgM (■). Antibody activity is predominantly associated with IgA.

levels in both the serum and the faeces during this period. Oral immunization produced no detectable antibody response in serum even at the highest levels of antigen administration. Antibodies were detectable in saline extracts of faeces (copro antibodies) 3–4 days after initial dosing and the minimum effective dose required to produce an antibody response of this type was 0·2 ml (8 HI units) *Salmonella dublin*; 0·02 ml (3·2 HI units) *Salmonella typhimurium*; and 0·2 ml (8 HI units) *E. coli* O137.

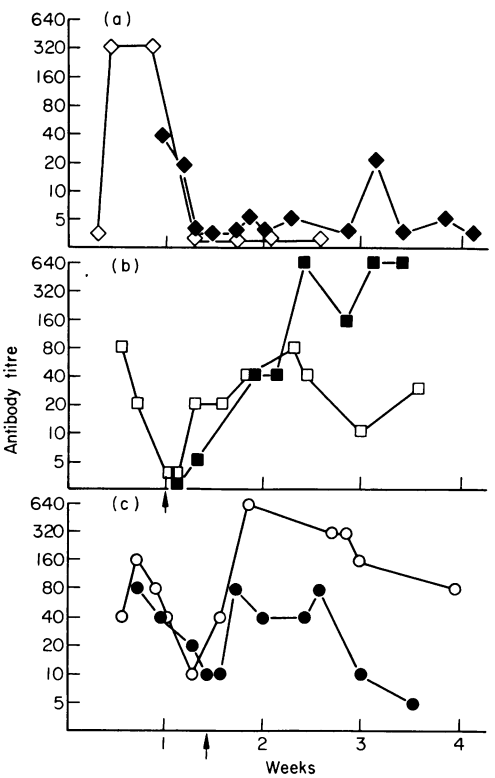

FIG. 5. Antibody levels in faecal extracts from calves after oral immunization with heat-killed *Salmonella dublin*. The initial peak reflects maternally derived colostral and milk antibodies. The subsequent response relates to oral immunization with different dose levels of antigen. (a) Control. (b) 2 ml included in feed. (c) 10 ml included in feed.

Typical results showing the change in pattern of faecal antibody response are given in Fig. 5; the early peak of antibody relates to maternal immunoglobulins and the subsequent response relates to oral immunization with different levels of antigen. Levels of activity were maintained only as long as antigen administration continued. The method of administration via the feed was as effective as direct oral dosage.

Oral administration of antigens at the dose levels under investigation, produced no detectable antibody response in the serum up to 5 weeks. However, there was a priming effect on the circulatory antibody response demonstrable by studies of serum antibodies produced by intramuscular injection of 1·5 ml of a saline suspension of heat killed organisms (2×10^9 organisms per millilitre). This was manifest in a rapid production of antibody

with a very much higher titre than in control animals. This priming effect was only evident in animals which registered a positive antibody response in the faecal assay, following oral immunization.

DISCUSSION

The initial association of immunoglobulins with intestinal function in the young calf is by way of absorption of colostral antibody. The effective absorption of maternal immunoglobulins is considered to be of prime importance for survival, however, impaired absorption of maternal antibodies, unrelated to colostral levels, is a feature of many calves (Staley, 1971). Furthermore, whilst the young of most other mammalian species are also sustained by antibodies in the maternal milk throughout lactation there is no similar provision in the bovine (Porter, 1972).

Bovine mammary tissues are deficient in immunoglobulin synthesis, in particular IgA, which is predominant in most mammalian exocrine systems (Butler *et al.*, 1972) and in consequence bovine milk after the first few days of lactation is unlikely to significantly contribute to local intestinal defence in the calf. In such circumstances, it will be appreciated that the earliest onset of active antibody synthesis and secretion in the intestinal mucosa of the young calf may be crucial to the animal's survival.

Active synthesis and secretion of antibodies in the intestinal tissues can have little value in the context of neonatal defence but immunocytes infiltrate the lamina propria as early as 4 days of age. Calves orally dosed with bacterial antigens at 5 days of age responded quickly with the secretion of antibodies indicating that intestinal synthesis of antibodies can suitably interrelate with declining passive maternal antibodies to provide a continuum of local antibody function in the alimentary tract of the calf.

Our previous studies of the ontogenesis of immunoglobulin synthesis in the intestinal mucosa of the young pig have shown that IgA secretion commenced on approximately the 10th day. Immunohistological studies of the localization of secretory component and α chain show close similarity in the nature of secretion of IgA in the two species. A further important point of comparison between the two species was large numbers of IgM cells in the lamina propria and evidence of its secretion across the epithelial lining, indicating once again the complementary role that this immunoglobulin must play with IgA in local defence (Allen & Porter, 1973). Immunization via the intestinal mucosa gives rise to secretory antibodies residing predominantly in these two immunoglobulin classes with bacteriostatic or bacteriolytic function. Their localization in and on the intestinal mucosa provides the basis of an antibody interface between host and environment.

The role of locally stimulated immunoglobulin synthesis in the control of the alimentary tract flora has been under examination for many years (Bezredka, 1927), because immunization via the oral route presents a most practical approach to control of most enteric syndromes. Recent evidence on the antibacterial properties of intestinal antibodies (Girard and Kalbermatten, 1970; McClelland *et al.*, 1972; Wernet *et al.*, 1971) is a favourable basis from which to examine this type of immunoprophylaxis. The current studies of fistulated calves show that the nature and duration of local antibody secretion in response to a single antigenic challenge is similar to that previously recorded in the pig (Porter *et al.*, 1974); lasting no more than 3–4 weeks. Thus, quite unlike the response associated with systemic immunity, no memory is implied and continuous antigenic stimulation is required to

maintain a measurable level of antibody secretion; a finding consistent with that of other authors (Freter & Gangarosa, 1963; Ogra and Karzon, 1969).

Studies with *E. coli* in the mouse have shown that oral immunization induces protection against the lethal effects of live bacteria administered by the intraperitoneal route (Mochmann *et al.*, 1971). However, this is a totally unnatural route of infection quite divorced from potential control by intestinal immunoglobulins. In previous studies in the pig (Porter *et al.*, 1973; Porter, Kenworthy and Allen, 1974) we have elected to record the beneficial effects in terms of such parameters as weight gain, nutritional performance, clinical symptoms and requirement for medication. The post-weaned young pig provides a natural model for evaluating the benefits of oral immunization. Similarly Roy and his colleagues (1955) have identified a valuable test model in the calf reared on milk replacer diets in an environment created by continuous occupancy. In such animals the natural build up of infection was shown to produce a significant deterioration in health and performance during the first 3 weeks of life and this is the model system we have selected to evaluate oral immunoprophylaxis (Porter, Kenworthy & Thomson, 1975). With it we have demonstrated significant benefits in health and nutritional status of developing young calves which indicate in practical field terms the value of oral immunization.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Mr M. E. Prior, Mrs L. Pugh and Miss P. M. Smith for their skilful technical assistance.

REFERENCES

ALLEN, W.D. & PORTER, P. (1970) The demonstration of immunoglobulins in porcine intestinal tissue by immunofluorescence with observations on the effects of fixation. *Immunology*, **18**, 799.

ALLEN, W.D. & PORTER, P. (1973) The relative distribution of IgM and IgA cells in intestinal mucosa and lymphoid tissues of the young unweaned pig and their significance in ontogeny of secretory immunity. *Immunology*, **24**, 493.

BEZREDKA, A. (1927) *Local Immunization*. Williams & Wilkins, Baltimore, Maryland.

BUTLER, J.E., MAXWELL, C.F., PIERCE, C.S., HYLTON, M.B., ASOFSKY, R. & KIDDY, C.A. (1972) Studies on the relative synthesis and distribution of IgA and IgG, in various tissues and body fluids of the cow. *J. Immunol.* **109**, 38.

CURTAIN, C.C., CLARK, B.L. & DUFTY, J.H. (1971) The origins of the immunoglobulins in the mucous secretions of cattle. *Clin. exp. Immunol.* **8**, 335.

FRETER, R. & GANGAROSA, E.J. (1963) Oral immunization and production of coproantibody in human volunteers. *J. Immunol.* **91**, 724.

GIRARD, J.R. & KALBERMATTEN, A. (1970) Antibody activity in human duodenal fluid. *Europ. J. clin. Invest.* **1**, 188.

MARKOWITZ, J. (1954) *Experimental Surgery*. Balliere, Tindall & Cox, London.

MCCLELLAND, D.B.L., SAMSON, R.R., PARKIN, D.M. & SHEARMAN, D.J.C. (1972) Bacterial agglutination studies with secretory IgA prepared from human gastrointestinal secretions and colostrum. *Gut*, **13**, 450.

MOCHMANN, H., OCKLITZ, H.W., HERING, L., SCHMIDT, E.F. & RICHTER, H. (1971) Influence of dosage and boostering upon effectiveness of oral immunisation against dyspepsia coli infections in mouse protection test. *Zbl. Bkt I*, **216**, 24.

OGRA, P.L. & KARZON, D.T. (1969) Distribution of polio virus antibody in serum, nasopharynx and alimentary tract following segmental immunization of lower alimentary tract with polio vaccine. *J. Immunol.* **102**, 1423.

PORTER, P. (1972) Immunoglobulins in bovine mammary secretions. Quantitative changes in early lactation and absorption by the neonatal calf. *Immunology*, **23**, 225.

PORTER, P. (1973) Functional heterogeneity of the bovine immune system. *J. Amer. vet. med. Ass.* **163,** 789.

PORTER, P. & ALLEN, W.D. (1972) Classes of immunoglobulins related to immunity in the pig. *J. Amer. vet. med. Ass,* **160,** 511.

PORTER, P., KENWORTHY, R. & ALLEN, W.D. (1974) Effect of oral immunization with *Escherichia coli* antigens on post weaning enteric infection in the young pig. *Vet. Rec.* **95,** 99.

PORTER, P., KENWORTHY, R., HOLME, D.W. & HORSEFIELD, S. (1973) *Escherichia coli* antigens as dietary additives for oral immunization of pigs. *Vet. Rec.* **92,** 630.

PORTER, P., KENWORTHY, R., NOAKES, D.E. & ALLEN, W.D. (1974) Intestinal antibody secretion in the young pig in response to oral immunization with *Escherichia coli. Immunology,* **27,** 841.

PORTER, P., KENWORTHY, R. & THOMPSON, I. (1975) Oral immunization and its significance in the prophylactic control of enteritis in the preruminant calf. *Vet. Rec.* (In press.)

PORTER, P. & NOAKES, D.E. (1970) Immunoglobulin IgA in bovine serum and external secretions. *Biochem. biophys. Acta* (Amst.), **214,** 107.

ROY, J.H., PALMER, J., SHILLAM, K.W.G., INGRAM, P.L. & WOOD, P.C. (1955) The nutritive value of colostrum for the calf. *Brit. J. Nutr.* **9,** 11.

SAINT MARIE, G. (1962) A paraffin embedding technique for studies employing immunofluorescence. *J. histochem. cytochem.* **10,** 250.

STALEY, T.E., JONES, E.W. & BUSH, L.J. (1971) Maternal transport of immunoglobulins to calf. *J. Dairy Sci.* **54,** 1323.

WERNET, P., PREU, H., KNAP, J. & ROWLEY, D. (1971) Antibacterial action of specific IgA and transport of IgM, IgA and IgG from serum into the small intestine. *J. infect. Dis.* **124,** 223.

WORLD HEALTH ORGANIZATION TECHNICAL REPORT (1972) Oral enteric bacterial vaccines. Serial number 500.