Immunology, 1974, **27**, 841.

Intestinal Antibody Secretion in the Young Pig in Response to Oral Immunization with *Escherichia coli*

P. PORTER, R. KENWORTHY, D. E. NOAKES AND W. D. ALLEN

Unilever Research, Colworth House, Sharnbrook. Bedford

(*Received* 22*nd February* 1973; *accepted for publication* 11*th April* 1974)

Summary. Intestinal immunoglobulins and antibodies in the local immune response to *E. coli* O somatic antigens have been studied in young fistulated pigs. Antibody levels in intestinal secretion were raised for approximately 2–3 weeks following a single local antigenic challenge with a heat-killed aqueous suspension of *E. coli*. A second challenge provoked a similar response suggesting a lack of immunological memory.

Antibody activity in the secretions was predominantly associated with IgA and immunofluorescent studies of biopsy specimens from young fistulated animals indicated that intestinal synthesis and secretion of IgA had commenced by the 10th day of life. Studies of piglets reared with the sow indicated that oral immunization with *E. coli* antigen after 10 days of age stimulated intestinal antibody secretion before weaning at 3 weeks.

The response of gnotobiotic pigs to oral immunization and infection was evaluated by immunofluorescent histology of the intestinal mucosa. Repeated oral administration of heat-killed *E. coli* O8 resulted in an immunocyte response in the lamina propria numerically comparable with that produced by infection with the live organism. The early response was dominated by cells of the IgM class whereas after 3 weeks IgA cells predominated. In the germ-free animal very few immunoglobulin-containing cells were detected.

In vitro studies of antibacterial activity indicated that the most probable mechanism of immunological control in the alimentary tract is bacteriostasis.

INTRODUCTION

In a recent report on 'oral enteric bacterial vaccines' a WHO study group (1972) concluded that there was not enough well substantiated basic information on immunization via the intestinal mucosa. Attention was drawn to the potential value of the young pig as an experimental model for *Escherichia coli*-associated enteritis and furthermore the analogous characteristics of its intestinal secretory antibody system (Porter, Noakes and Allen, 1970) make it a useful model for the human infant.

Pig enteric disorders which commonly occur during periods of stress are a considerable problem in normal farm management, particularly at weaning, when the young animal is challenged by a rapid proliferation of haemolytic *E. coli*. Pigs of 2–4 weeks of age have been regarded as having a critical antibody deficiency (Miller, Harmon, Ullrey, Schmidt,

Correspondence: Dr P. Porter, Unilever Research, Colworth House, Sharbrook, Bedford.

Luecke and Hoefer, 1962), since during this period passively acquired maternal antibody has declined to negligible levels and parenteral administration of bacterial antigens fails to induce a significant antibody response.

Immunoglobulin synthesis and secretion in the piglet intestine begins after the first few days of life and by the time of weaning at 3–4 weeks the lamina propria has become infiltrated with numerous lymphocytes and plasma cells synthesizing IgM and IgA (Allen and Porter, 1973). Hence there exists a potential for using oral immunization in the suckling animal to enhance its competence to cope with the challenge of bacterial infections. In this context we have attempted to control post-weaning enteric problems in the pig by oral immunization using a bacterial vaccine as a feed supplement (Porter, Kenworthy, Holme and Horsfield, 1973). The value of this approach was evident from field trials in which a significantly improved animal performance was obtained in the 2 weeks post-weaning.

The fundamental problem was to determine the age at which immunological responsiveness of the intestinal tissues to *E. coli* antigens develops. The present paper describes a series of investigations in fistulated and gnotobiotic pigs to examine the intestinal response to *E. coli* antigens in relation to the potential value of oral immunization as a preventive measure against enteric infection in the young pig.

MATERIALS AND METHODS

Microelectrophoresis

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

Chromatographic methods

Gel filtration chromatography was carried out on Sephadex G-200 columns (45 × 2·5 cm and 90 × 2·5 cm) using 0·85 per cent NaCl in 0·1 M Tris–HCl buffer, pH 7·2.

Isolation and quantitative estimation of immunoglobulins

The isolation of specific porcine immunoglobulins and the preparation of rabbit antisera has been described previously (Porter, 1969). The antisera were rendered specific for porcine γ-, μ- or α-chains by absorption with immunoglobulins coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia).

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

Bacterial antibody tests

Antibodies to *E. coli* were measured by the fluorescent bacterial antibody assay described by Cohen and Norins (1968) using fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit globulin and porcine immunoglobulin class-specific rabbit antiserum.

Antiglobulin haemagglutination tests were carried out with specific antisera for porcine α-, μ- and γ-chains in a manner similar to that described by Coombs, Jonas, Lachmann and Feinstein (1965).

Bacterial inhibition assays were carried out using four volumes of intestinal secretions. one volume of complement or saline and one volume of a bacterial suspension containing

approximately 10^5 viable *E. coli*/ml. A culture grown overnight in nutrient broth at 37° was suitably diluted to provide the bacterial suspension. Aliquots of each test were taken for examination immediately (0 hours) and after 3 and 5 hours of incubation at 37°, and the number of colony-forming units (CFU) estimated on sheep blood agar plates using the technique of Miles and Misra (1938).

Rabbit serum was used as a source of complement. Animals were selected for low serum antibody titres and the sera were absorbed with the organism to be tested and also bentonite to remove lysozyme.

Lysozyme was assayed against *Micrococcus lysodeikticus* using the method of Osserman and Lawler (1966) and expressed in terms of the equivalent weight in μg/ml of three times crystallized egg white lysozyme.

Immunofluorescent histochemistry

Cell counts were performed on paraffin sections of small intestinal tissue prepared by the method of Saint Marie (1962) and stained with FITC-conjugated rabbit anti-globulins. All fluorescent cells present in twenty fields, selected at random from the lamina propria, were counted using a × 40 objective, 0·65 numerical aperture. Localization of immunoglobulins was studied in tissues taken from three levels of small intestine, (duodenum, jejunum and ileum), Peyer's patches and spleen.

Germ-free animals

The animals were derived by hysterotomy and reared in isolators constructed from polyvinyl chloride as described by Kenworthy (1970). In the immunological investigation described in the text some of the animals were infected by introducing 10 ml of pure broth culture of *E. coli* O8:K87(B):K88a,b(L) into a small volume of liquid feed. In the group of animals maintained germ-free, oral immunization was carried out twice daily with 5 ml of an aqueous suspension of the same organism (approximately 2×10^9 organisms/ml), heat-killed by autoclaving (121°, 15 psi, 30 minutes). All dosing solutions were introduced into the animal isolators in sealed containers which were sterilized by 2 per cent peracetic acid. Two animals were maintained as controls; one of these was germ-free, the other (pig 541, Table 2) was accidentally contaminated with a Gram-positive aerobic spore bearing *Bacillus* at least 8 days before post-mortem.

Fistulated animals

Thiry–Vella loops, 30–40 cm in length, were prepared in the mid-portion of the jejunum in pigs 4–9 days of age as 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. Anaesthesia was induced by intravenous injection of 12 mg of methohexitone sodium ('Brietal' Elanco Ltd, London) per kilogram of body weight and maintained with cyclopropane/oxygen.

Surgery was normally carried out on animals aged 4–9 days and studies of secretions were conducted over a period of approximately 5 weeks.

The secretion from Thiry–Vella loops was collected over a period of several hours in small polythene bags attached to the end of each cannula.

Tissues for sequential immunofluorescent analyses were obtained from the loops using a paediatric peroral biopsy capsule (Sebus, Fernandes and Bult, 1968). Care was taken to

minimize interference from inflammation arising from the procedure by ensuring that the biopsies were removed from a different region of the loop on each occasion.

RESULTS

IMMUNOGLOBULINS AND PROTEINS IN INTESTINAL SECRETIONS OF FISTULATED ANIMALS

The Thiry–Vella loop provides access to intestinal secretion without contamination from other sources such as saliva, bile and gastric secretions. However, the surgical interference can result in occasional abnormal protein profiles in the secretions attributable to inflammatory lesions and serous exudation. In order to establish the suitability of surgically prepared animals the intestinal secretions were examined by electrophoresis. Immunoelectrophoresis consistently showed the main serum fractions present in the secretions to be albumin, two or more α-globulins and the immunoglobulins. With regard to the latter there was clearly a deficiency in the strength of the IgG as compared with the serum counterpart (Fig. 1).

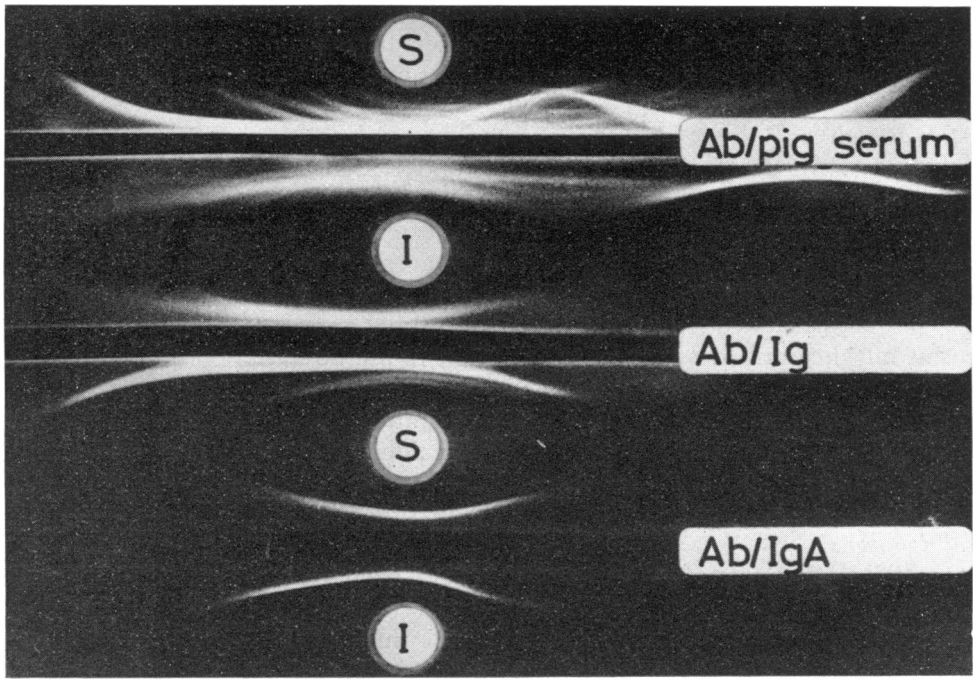

FIG. 1. Immunoelectrophoresis of pig serum (S) and intestinal secretions (I) demonstrating the presence of serum-derived antigens in the secretions. The electrophoretograms are precipitated with rabbit antisera prepared against serum proteins, immunoglobulins and secretory IgA.

The dissimilarity from the serum protein profile was further established by electrophoresis in polyacrylamide gels (Fig. 2) to provide substantial evidence that the proteins in the secretions did not derive from serous exudation due to inflammation or surgical trauma. On the basis of these studies ten pigs were selected for experimentation. The protein content of the secretions varied in a range approximately 250–600 mg per 100 ml

Semiquantitative evidence from immunoelectrophoretic profiles with specific antiglobulin antisera clearly indicated that IgA was the predominant immunoglobulin. Quantitative assays by radial immunodiffusion (Mancini *et al.*, 1965) indicated that IgA represented approximately 90 per cent of the total immunoglobulin in the secretions of pigs 3–8 weeks of age, a finding consistent with previous evidence (Porter *et al.*, 1970).

The gel filtration profiles of intestinal secretions on Sephadex G-200 were remarkably similar in all animals. A typical example is given in Fig. 3, showing the elution characteristics of intestinal IgA, IgM and IgG as well as lysozyme which was readily detectable.

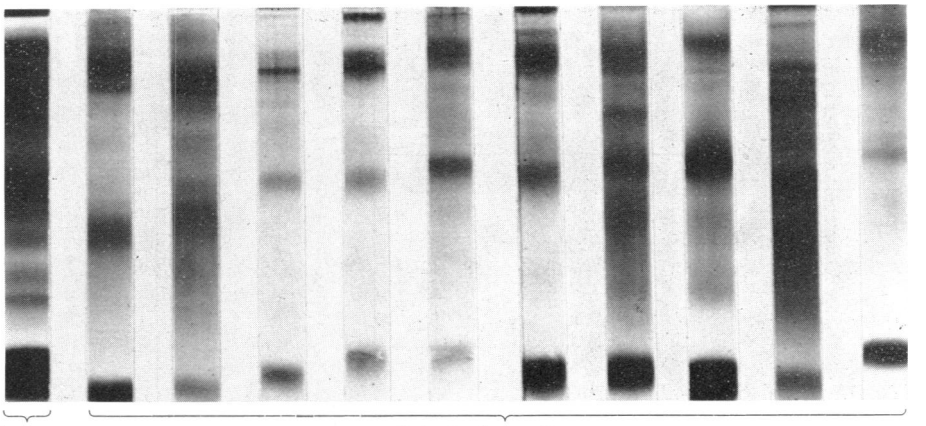

FIG. 2. Polyacrylamide disc electrophoresis of proteins in intestinal secretions obtained from fistulated pigs compared with a normal serum pattern.

LOCAL ANTIBODY RESPONSE TO *E. coli* ANTIGENS IN FISTULATED ANIMALS

Studies were carried out in two weaned animals from 5 weeks of age to determine whether local application of bacterial antigens in intestinal loops produced a measurable secretion of antibodies. A 5-ml aqueous extract from a boiled suspension of *E. coli* (2×10^9 organisms per ml) was administered into the loop of each pig. Two pathogenic strains were used (O141 and O139), one for each animal, the patterns of change in antibody response being shown in Figs 4a and 4b respectively. A peak of antibody secretion was registered in both animals within 7–10 days of application of antigen, but the activity declined to pre-immunization levels within 3–4 weeks. A second administration of antigen into the loops produced a very similar response to that obtained in the first application. There was no evidence of increased immunoglobulin or antibody secretion, nor increase in duration of the response following the second dose of antigen, thus suggesting a lack of memory in the secretory immune system.

In the animal stimulated with O139 the fluorescent antiglobulin bacterial antibody assay was carried out to assess the importance of the different immunoglobulin classes in the secretions. During the peak of activity in the secretion following each local challenge the main response was attributable to IgA but IgG and IgM antibodies were also detectable (Fig. 4c). In the animal stimulated with heat-killed *E. coli* O141, gel filtration of the secretions and haemagglutination antiglobulin assays on the fractions confirmed this finding (Fig. 3).

Immunofluorescent studies on biopsy specimens of small intestinal tissues obtained from fistulated animals demonstrated that by the 10th day of life IgA was being synthesized in the immunocytes of the lamina and transported across the epithelial cells to the lumen. We therefore examined the possibility of stimulating an intestinal immune response to bacterial antigen in fistulated animals at this early age. A comparison was made between four animals which were maintained on the sow and four animals weaned at 4 days of age, reared in cages and fed on cows' milk; one animal in each group was used as a control.

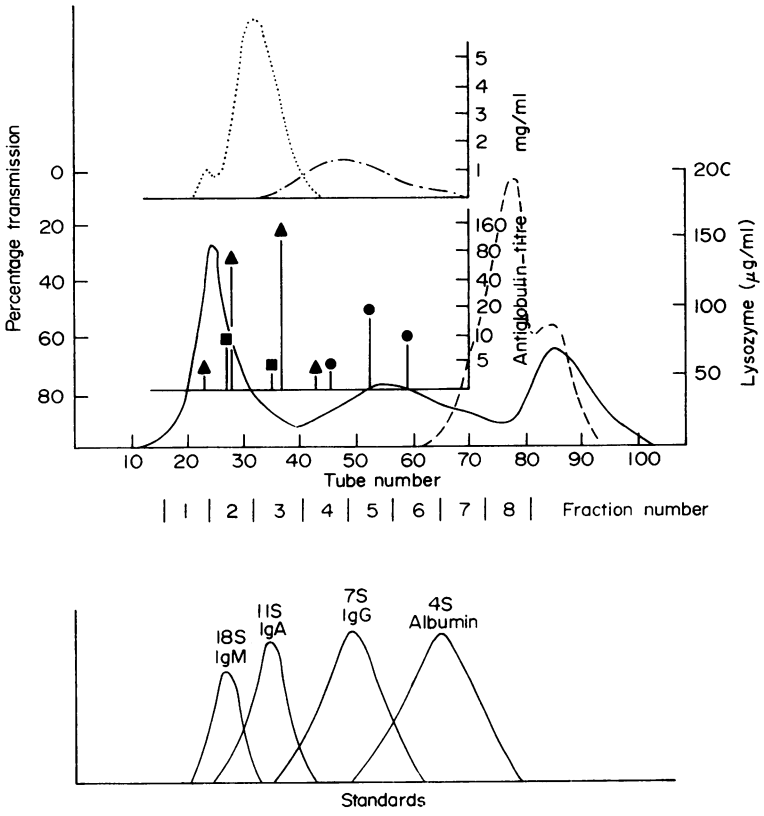

FIG. 3. Gel filtration of intestinal secretions on Sephadex G-200 showing the elution characteristics of immunoglobulins and lysozyme. (· · · ·) IgA. (· – · –) IgG. (– – –) Lysozyme.
The intestinal secretions were obtained from a fistulated animal after local immunization with heat-killed *E. coli* O141. Antiglobulin antibodies in selected fractions were detected by haemagglutination with specific rabbit anti-immunoglobulin heavy chains. (●) IgG. (▲) IgA. (■) IgM.

The antibody response in intestinal secretions following local challenge of each animal with 5 ml of heat-killed *E. coli* O141 (2×10^9 bacteria/ml) is shown in Fig. 5. The nature of the response was similar to that obtained previously in the older animals (Fig. 4), thereby demonstrating that stimulation of local intestinal immune response in the young pig prior to normal weaning was practicable.

ANTIBACTERIAL TESTS

A typical study of antibacterial activity of intestinal secretions is shown in Table 1; the

secretions were obtained from a fistulated pig 10 days after local immunization of the intestinal loop with heat-killed O149. Heating the secretions at 56° before the test failed to produce any change in the reaction, the mean number of colony-forming units CFU/ml after incubation with *E. coli* O149 for 3 and 5 hours was practically the same, irrespective of whether the secretions had been heated or not. By comparison of bacterial counts for these periods of incubation with intestinal secretions prior to immunization or a saline

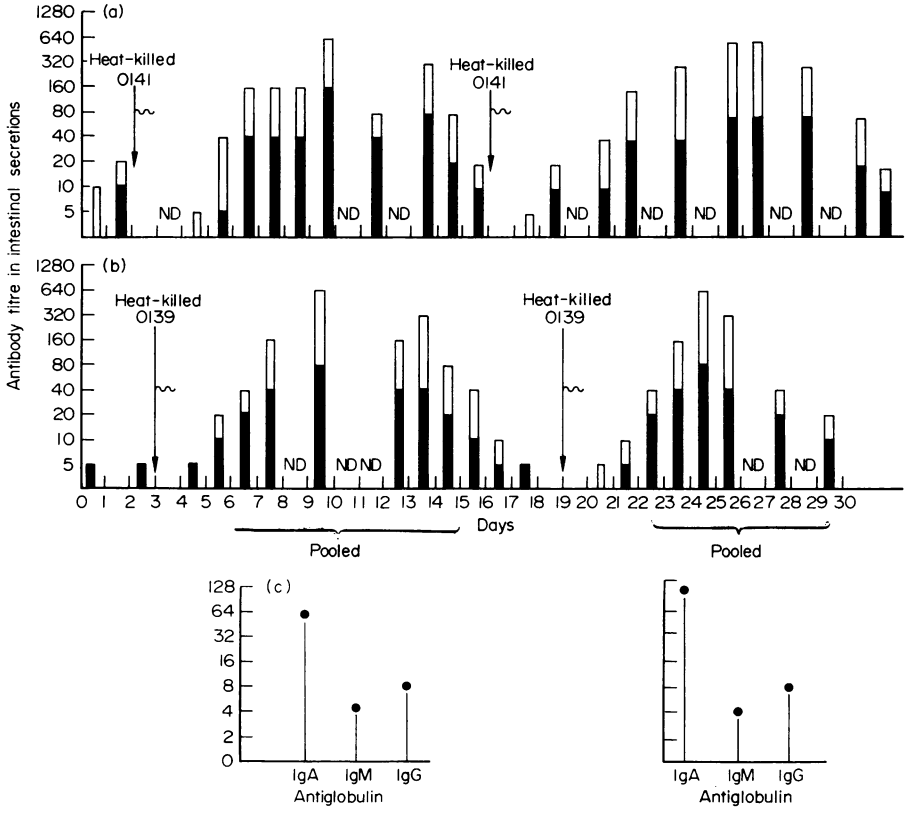

FIG. 4. Variation of intestinal antibody secretion in a Thiry–Vella loop of jejunum in young pigs following administration of heat-killed *E. coli*.
(a) Local antibody secretion in response to *E. coli* O141 in pig 230.
(b) Local antibody secretion in response to *E. coli* O139 in pig 309.
(c) Fluorescent bacterial antibody assays of activity in specific immunoglobulin classes in pooled secretions after local immunization with *E. coli* O139.
ND = not determined.

control, there was a considerable reduction in CFU/ml, clear evidence of bacteriostasis in both the heated and unheated immune secretions. In absence of added complement there was no evidence of bacteriolysis, further substantiating the absence of complement components in the intestinal secretions.

Antiglobulin studies indicated that the major antibody activity in intestinal secretions was attributable to IgA (see Fig. 3). Lysozyme has previously been shown to be important for bacteriolysis with IgA and complement. The level of lysozyme in the secretions

exceeded 100 μg/ml, which would be more than adequate to participate in bacteriolysis since *in vitro* test have previously been conducted with 30 μg/ml (Hill and Porter, 1974).

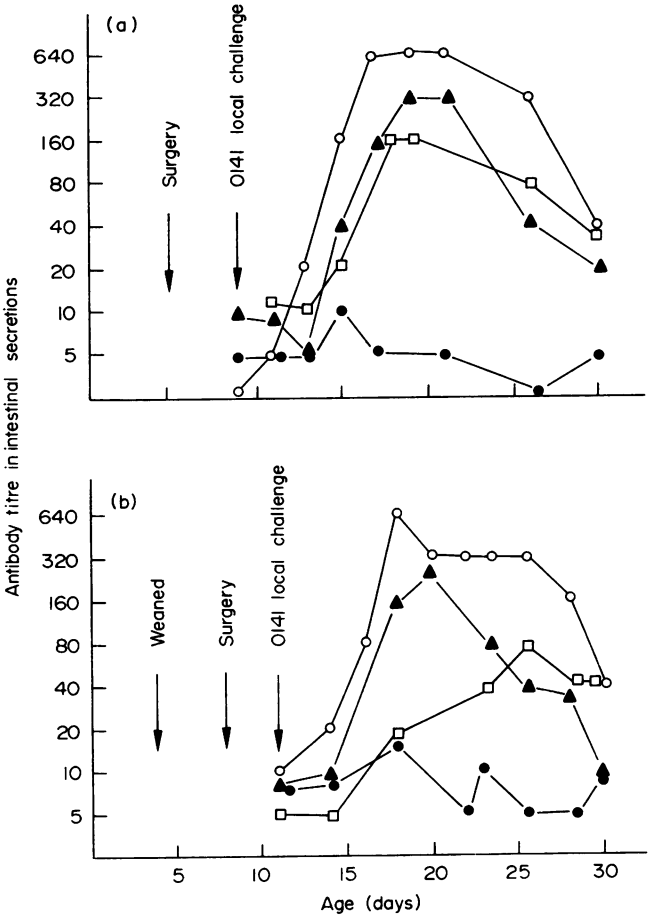

FIG. 5. Local intestinal antibody secretion after administration of heat-killed *E. coli* O141 to fistulated baby pigs.
(a) Animals maintained on the sow.
(b) Animals weaned at 4 days of age and raised on reconstituted spray dried cows' milk.
(●) Control animals. (○), (▲) and (■) animals locally challenged with heat-killed *E. coli* O141.

INTESTINAL IMMUNOGLOBULINS IN ORALLY IMMUNIZED AND MONOCONTAMINATED GERM-FREE PIGS

The studies carried out using germ-free animals facilitated an examination of the alimentary tract response in relation to a single infecting organism or to repeated oral administration of a heat-killed suspension of the same organism. This response was observed in terms of the number of immunocytes infiltrating the lamina propria of the small intestine and other lymphoid tissues assessed by immunofluorescence with specific α- and μ-chain antisera (Table 2). Little or no activity was attributable to IgG in the

alimentary tract response and therefore studies with γ-chain antisera are not recorded with the observations of numbers of cells staining for IgA and IgM.

Very few cells were detected in the alimentary tract of the germ-free animal even at 40 days of age, thus providing an excellent zero base line against which to assess the effect of any treatment. Clearly the diet, an autoclaved cows' milk reconstituted from spray-dried whole milk powder, provided little or no antigenic stimulus as indicated by the virtual absence of immunoglobulin-containing cells in the lamina propria.

TABLE 1

ANTIBACTERIAL ACTIVITY OF INTESTINAL SECRETIONS AFTER LOCAL IMMUNIZATION

Incubation conditions	Mean number of colony-forming units after incubation for:		
	0 hours	3 hours	5 hours
E. coli O149 +saline	$9{\cdot}0 \times 10^4$	$3{\cdot}0 \times 10^7$	$> 10^9$
E. coli O149 +intestinal secretion	$4{\cdot}0 \times 10^4$*	$3{\cdot}0 \times 10^6$	$> 10^9$
	$3{\cdot}0 \times 10^4$†	$1{\cdot}0 \times 10^4$	$3{\cdot}0 \times 10^4$
E. coli O149 +intestinal secretion heated at 56°	$5{\cdot}0 \times 10^4$	$4{\cdot}0 \times 10^5$	$> 10^9$
	$8{\cdot}0 \times 10^4$†	$2{\cdot}0 \times 10^5$	$4{\cdot}0 \times 10^4$
E. coli O149 +intestinal secretion +complement	$6{\cdot}0 \times 10^4$*	$2{\cdot}6 \times 10^6$	$> 10^9$
	$3{\cdot}0 \times 10^4$	$1{\cdot}0 \times 10^4$	$5{\cdot}0 \times 10^3$

* Intestinal secretions from Thiry–Vella loop before local immunization with heat-killed *E. coli* O149.

† Intestinal secretions taken 10 days after local immunization with heat-killed *E. coli* 0149.

Repeated oral immunization with the heat-killed *E. coli* O8 resulted in a response, in terms of cell numbers, similar to that produced by infection with the live organism in a comparable period. The response in the lamina propria during the first few days was dominated by cells of the IgM class and after 3 weeks cells of the IgA class predominated. These cells were located almost without exception in the tissues surrounding the crypts, and both IgA and IgM immunoglobulins were demonstrated in the apical cytoplasm of crypt epithelial cells. Only on very infrequent occasions were cells found in the core of the villous. Examination of small intestinal contents and tissue extracts using fluorescent bacterial antibody assay with specific anti-μ-, α- and γ-chain antisera established that *E. coli* O8 antibodies were present in the IgM and IgA classes. Little or no activity was attributable to IgG.

Studies of immunoglobulin-containing cells in Peyer's patches were carried out in six animals, and here again the indications were that IgM cells preceded IgA cells in their appearance in these tissues. In the animals which had been subjected to long-term challenge either by oral immunization or infection, IgM cells were present in greater numbers than IgA cells, although the latter predominated in the small intestinal lamina.

IgA cells could not be detected in the spleen of any of the animals. Furthermore, in serum antibody studies using the antiglobulin haemagglutination test (Coombs *et al.*, 1965) no activity was attributable to IgA.

TABLE 2

COMPARATIVE DISTRIBUTION OF CELLS SYNTHESIZING IgA+IgM IN THE INTESTINE AND SPLEEN OF GNOTOBIOTIC PIGS AFTER ORAL CHALLENGE

Pig number	Age (days)	Treatment	IgA cells					IgM cells				
			Duodenum	Jejunum	Ileum	Peyer's patch	Spleen	Duodenum	Jejunum	Ileum	Peyer's patch	Spleen
542	40	Germ-free	1	0	0	0	0	4	0	1	2	28
530	20	Oral *E. coli* antigen for 2 days	5	1	1	—	0	28	46	4	—	19
538	22	Oral *E. coli* antigen for 4 days	0	2	0	—	0	8	12	3	—	21
532	20	Infected with live *E. coli* for 1 day	0	0	0	—	0	0	0	0	—	58
531	20	Infected with live *E. coli* for 2 days	1	1	0	0	0	4	11	1	9	32
537	22	Infected with live *E. coli* for 4 days	0	0	0	—	0	15	3	0	—	23
543	40	Oral *E. coli* antigen for 22 days	177	247	81	53	0	176	64	25	103	26
544	42	Oral *E. coli* antigen for 24 days	219	98	27	2	0	86	37	11	19	17
545	42	Infected with live *E. coli* for 24 days	352	193	18	45	0	244	63	12	110	57
541	40	Accidental contamination Gram-positive *Bacillus*	144	55	2	0	0	54	37	2	2	20

DISCUSSION

The participation of secretory antibodies in determining the normal flora of the alimentary tract has not been evaluated. McClelland, Samson, Parkin and Shearman (1972) demonstrated that IgA in gastrointestinal secretions agglutinated a wide range of enteric organisms and postulated that agglutinates even in the absence of lysozyme and complement may reduce the ability of an organism to colonize the gut, possibly enhancing its clearance by intestinal peristalsis. Secretory IgA in the presence of complement and lysozyme lyses *E. coli* (Adinolfi, Glynn, Lindsay and Milne, 1966; Hill and Porter, 1974) and also promotes phagocytosis (Girard and Kalbermatten, 1970; Wernet, Brau, Knop and Rowley, 1971).

In the present investigations of intestinal secretions obtained from Thiry Vella loops of pigs locally immunized with heat-killed *E. coli*, there were adequate levels of lysozyme to facilitate bacteriolysis in the presence of added complement. However, since it was not possible at this stage to demonstrate conclusively the presence of complement components it appears that bacteriostasis is the most likely antibacterial mechanism attributable to IgA in the lumen of the gut. Evidence from recent studies of protection in young pigs by oral administration of anti-*E. coli* antibodies substantiates this thesis (Wilson and Svendson, 1971).

The maintenance of integrity and function of the small intestinal epithelium is likely to be very important in control of *E. coli*-associated post-weaning diarrhoea in the young pig. In considering the possible application of oral immunization, the main point to be established was that a sufficiently early onset of local immune mechanisms occurred. In this context the successful studies with polio virus immunization carried out in children orally dosed at birth provided an interesting precedent (Sabin, Michaels, Ziring, Krugman and Warren, 1963), more especially since polio virus neutralizing coproantibodies of the IgA class appeared within 2–3 weeks of immunization (Keller, Dwyer, Oh and D'Amodio, 1969). The present studies of the pig demonstrate that *E. coli* intestinal antibodies of the IgA class can be stimulated in the first 3 weeks of life. Furthermore studies of intestinal secretions from young animals surgically prepared with isolated intestinal loops provide satisfactory evidence that agglutinating antibody, mainly associated with IgA, appears in the lumen within a few days of the administration of antigens into the small intestine. This response is also obtained in surgically prepared animals that have been maintained on the sow.

In our experiments intestinal antibody secretion did not persist at an elevated level for more than approximately 3 weeks after antigenic challenge with heat-killed *E. coli*. Freter and Gangarosa (1963) recorded essentially similar observations in relation to oral immunization with heat-killed *V. cholerae* and decided that repeated doses were required to maintain a detectable coproantibody level. Ogra and Karzon (1969), in studies of the immune response to local intranasal administration of inactivated polio vaccine, suggested that the secretory immune system appeared to have no immunological memory. This is surprising, since one would expect any immunization that induces the proliferation of antigen-reactive cells to leave some kind of trace. Nevertheless, support for this observation is provided in the present studies with fistulated animals. Furthermore, a second dose of the same antigen induced an almost identical short-lived response quite unlike the secondary response normally associated with systemic immunity.

Although IgA was the predominant immunoglobulin and *E. coli* antibody in intestinal

secretions of young pigs it was evident from the immunofluorescent studies in orally immunized gnotobiotic pigs that IgM synthesis contributed significantly to the total response in the cells of the intestinal lamina. The early appearance of IgM-containing plasma cells in the small gut following oral administration of live or dead *E. coli* substantiates the previous evidence that IgM appears early in antibody responses of the external secretory immune system (Allen and Porter, 1973). In this context it is significant that Cebra (1969) noted in rabbits injected with *Trichinella* that there was a relative increase in intestinal IgM cells between 7 and 13 days after injection. Furthermore, in germ-free mice repeated injections of goat anti-mouse IgM may cause a decrease in numbers of IgM cells in the spleen, and also a virtual absence of IgA cells in the gut, a result consistent with IgM-producing cells being the precursors of IgA (Lawton, Asofsky, Hylton and Cooper, 1972).

IgM-producing cells predominated in the Peyer's patches of gnotobiotic pigs for at least the first 6 weeks, and also accounted for a considerable proportion of the cell population in the lamina propria of the small gut. These findings differ from those reported in germ-free mice (Crabbe, Nash, Bazin, Eyssen and Heremans, 1969; Bazin, Levi and Doria, 1970) in which oral immunization induced antibodies exclusively of the IgA type. A further point of difference was the absence of IgA-containing cells in the spleen of the young pig and the lack of circulating IgA antibody. Nevertheless, our findings are consistent with those of other authors (Kriebel, Kraft and Rothberg, 1969; Lejeure, Uecker, Blumel and Raetigg, 1970; Girard and Kalbermatten, 1970), and thus the weight of evidence suggests that oral immunization gives rise to IgA-producing cells restricted mainly to the lamina propria of the gastro-intestinal tract.

An understanding of the cellular response in the intestinal mucosa and local antibody mechanisms in the gut lumen is fundamental to the successful application of oral immunization. Nevertheless, for a satisfactory evaluation of the principle, the burden of demonstrating efficacy rests on the outcome of clinical studies. Several investigations of oral administration of immunoglobulins leaves no doubt as to the protective value of local antibody in the gut of the pig (Kohler and Bohl, 1966; Miniats, Mitchell and Barnum, 1970; Wilson and Svendsen, 1971); but these investigations relate to the neonate in which there is a complete dependence on passive immunity. The potential for control of post-weaning, *E. coli*-associated enteritis by active induction of local antibody has only recently been examined and in extensive field trials there is further substantiation for our earlier observation that oral immunization prior to weaning results in a significantly improved growth and nutritional performance in the immediate post-weaning period (Porter *et al.*, 1973). Furthermore, orally immunized animals show an increased ability to resist experimental enteric infection with pathogenic *E. coli*. In these terms, therefore, oral immunization can make a significant contribution to animal health and performance.

REFERENCES

Adinolfi, M., Glynn, A. A., Lindsay, M. and Milne, C. M., (1966). 'Serological properties of γA antibodies to *Escherichia coli* present in human colostrum.' *Immunology*, **10,** 517.

Allen, W. D. and Porter, P. (1973). 'The relative distribution of IgM and IgA cells in intestinal mucosa and lymphoid tissue of the young pig and their significance in ontogenesis of secretory immunity.' *Immunology*, **24,** 493.

Bazin, H., Levi, G. and Doria, G. (1970). 'Predominant contribution of IgA antibody-forming cells to an immune response detected in extra-intestinal lymphoid tissues of germ-free mice exposed to antigen by the oral route.' *J. Immunol.*, **105,** 1049.

Cebra, J. J. (1969). 'Immunoglobulins and immunocytes.' *Bact. Rev.*, **33,** 109.

Cohen, I. R. and Norins, L. C. (1968). 'Antibodies of the IgM, IgG and IgA classes in newborn and adult sera reactive with Gram-negative bacteria.' *J. clin. Invest.*, **47,** 1053.

COOMBS, R. R. A. JONAS, W. E., LACHMANN, P. J. and FEINSTEIN, A. (1965). 'Detection of IgA antibodies by the red cell linked antigen–antiglobulin reaction: antibodies in the sera of infants to milk proteins.' *Int. Arch. Allergy*, **27,** 321.

CRABBE, P. A., NASH, D. R., BAZIN, H., EYSSEN, H. and HEREMANS, J. F. (1969). 'Antibodies of the IgA type in intestinal plasma cells of germ free mice after oral or parental immunization with ferritin.' *J. exp. Med.*, **130,** 723.

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

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

HILL, I. R. and PORTER, P. (1974). 'Studies of bactericidal activity of *Escherichia coli* of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA.' *Immunology*, **26,** 1239.

KELLER, R., DWYER, J. E., OH, W. and D'AMODIO, M. (1969). 'Intestinal IgA neutralizing antibodies in new born infants following polio virus immunization.' *Pediatrics*, **43,** 330.

KENWORTHY, R. (1970). 'Effect of *Escherichia coli* on germ-free and gnotobiotic pigs. I. Light and electron microscopy of the small intestine.' *J. comp. Path.*, **80,** 53.

KOHLER, E. M. and BOHL, E. H. (1966). 'Studies of *Escherichia coli* in gnotobiotic pigs. III. Evaluation of orally administered specific antisera.' *Canad. J. comp. med. vet. Sci.*, **30,** 233.

KRIEBEL, G. W., KRAFT, S. C. and ROTHBERG, A. M. (1969). 'Locally produced antibody in human gastrointestinal secretion.' *J. Immunol.*, **103,** 1268.

LAWTON, A. R., ASOFSKY, R., HYLTON, M. B. and COOPER, D. M. (1972). 'Suppression of immunoglobulin class synthesis in mice.' *J. exp. Med.*, **135,** 277.

LEFEVRE, H., UECKER, W., BLUMEL, P. and RAETTIGG, H. (1970). 'Untersuchungen Zur Oralen immunisierung.' *Zbl. Bakt. I. Abt. Orig.*, **213,** 548.

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

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

MANCINI, G. CARBONARA, O. and HEREMANS, J. F. (1965). 'Immunochemical quantitation of antigens by single radial immunodiffusion.' *Immunochemistry*, **5,** 235.

MILES, A. A. and MISRA, S. (1938). 'The estimation of the bactericidal power of the blood.' *J. Hyg. (Camb.)*, **38,** 732.

MILLER, E. R., HARMAN, B. J., ULLREY, D. E., SCHMIDT, LUECKE, R. W. and HOEFER, J. A. (1962). 'Antibody absorption, retention and production by the baby pig.' *J. Amer. Sci.*, **21,** 309.

MINIATS, O. P., MITCHELL, L. and BARNUM, D. A. (1970). 'Response of gnotobiotic pigs to *Escherichia coli*.' *Canad. J. comp. Med.*, **34,** 269.

OGRA, P. L. and 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.

OSSERMAN, E. F. and LAWLOR, D. P. (1966). 'Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia.' *J. exp. Med.*, **124,** 921.

ORSTEIN, L. and DAVIS, B. J. (1964). 'Disc electrophoresis—method and application to human serum proteins.' *Ann. N.Y. Acad. Sci.*, **121,** 321.

PORTER, P. (1969). Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet.' *Biochim. biophys. Acta (Amst.)*, **181,** 381.

PORTER, P., KENWORTHY, R., HOLME, D. and HORESFIELD, S. (1973) *Escherichia coli* antigens as dietary additives for oral immunization trials with pig creep feeds. *Vet. Rec.*, **92,** 630.

PORTER, P., NOAKES, D. E. and ALLEN, W. D. (1970). 'Secretory IgA and antibodies to *Escherichia coli* in porcine colostrum and milk and their significance in the alimentary tract of the young pig.' *Immunology*, **18,** 245.

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

SABIN, A. A., MICHAELS, R. H., ZIRING, P., KRUGMAN, S. and WARREN, J. (1963). 'Effect of oral polio virus vaccine in new born children. II. Intestinal resistance and antibody response at 6 months in children fed type 1 vaccine at birth.' *Pediatrics*, **31,** 641.

SEBUS, J., FERNANDES, J. and BULT, J. A. VAN DER (1968). 'A new twin hole capsule for peroral intestinal biopsy in children.' *Digestion*, **1,** 193.

WERNET, P., BREU, H., KNOP, J. and 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.

WILSON, M. R., SVENDSON, J. (1971). 'Immunity to *Escherichia coli* in pigs. The role of milk in protective immunity to *E. coli* enteritis.' *Canad. J. comp. Med.*, **35,** 239.

World Health Organisation Technical Report (1972). 'Oral enteric bacterial vaccines.' Serial number 500.