The functional role of secretory antibody systems in early development of the pig and the calf

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Nutrition and immunology

The relevance of immunology to nutrition has traditionally been limited to the phenomenon of transmission of maternal immunoglobulins to the foetus and neonate. Investigations of the intestinal absorption of colostral antibodies are usually cited in this context. This mechanism has a limited time span, only a matter of hours in the pig and calf, and there is no further augmentation of circulatory passive immunity. As no other analogy could be drawn between immunoglobulins and nutrients, and passive immunity is an essential require mentmainly of the neonate, investigations usually stop short of enquiring into any further role of antibody systems in nutrition.

Antibodies in secretions, particularly antibodies of the IgA class, are now known to play an important part in the protection of mucous epithelial surfaces. From the standpoint of the nutritionist the preservation of functional integrity of the intestinal epithelium is important; therefore, by its contribution in these terms IgA has an indirect role to play in nutrition. Also, the possibility of stimulation and control of this natural immunobiological system holds interesting prospects in modern agriculture. In the new intensive systems of management the young animal is exposed to increasing risk from gastro-intestinal disorders of bacterial origin. *Escherichia coli* are preponderant among those enteropathogens which may cause a decline in performance of the young animal, possibly leading to frank disease. The results of recent studies using germfree pigs monocontaminated with *E. coli* show the effects of bacteria on intestinal absorption (Kenworthy, 1973). In these animals there is a state of relative malabsorption closely associated with microbial proliferation. The germfree animal has virtually no immunoglobulins and antibodies (Porter & Kenworthy, 1970) but the intestinal secretory system develops rapidly within the first 3–4 d of challenge. Kenworthy (1973) reported that in this time period there is a considerable recovery from the disturbance in intestinal function although the infecting organism is present. These observations serve to emphasize the potential contribution of local intestinal antibody systems to health, performance and nutrition in the young animal.

From birth onwards the intestinal mucosa is bathed by immunoglobulins which are either maternal in origin or synthesized locally by immunocytes in the lamina propria. As a first step it is important to determine the interrelationship between the declining passive maternal antibodies and the developing mechanisms of active intestinal synthesis which provide continuous protection in early life.

Contrasting characteristics of immunoglobulins in bovine and porcine mammary secretions and absorption by the neonate

Studies with immunoglobulin class-specific antisera show that there is, in general, little or no transmission of immunoglobulins in utero. Therefore, intestinal absorption of ingested colostral immunoglobulins by the neonate supplies the passive immunity responsible for protection in early life. Bovine and porcine colostrum contains high levels of immunoglobulins and 75–80% is accounted for by IgG. The selective transfer of IgG_1 to the bovine mammary secretions is well described and is apparently a unique characteristic of the ruminant species. In the sow, the main colostral immunoglobulin is IgG_2.

The important protective role of maternal immunoglobulins is clearly evident in field studies with the calf where susceptibility to septicaemia caused by *E. coli* is generally related to a deficiency in absorption of maternal antibodies (Gay, Anderson, Fisher & McEwan, 1965). Logan & Penhale (1971) have assessed the importance of IgM and IgG classes of antibody in protecting the neonate, concluding that IgM makes a large contribution.

All investigations on this subject, to date, have neglected to consider the role of colostral immunoglobulins in local defence of the intestinal epithelium of the neonatal calf. It is in this context that attention has been drawn to the peculiar role of maternal secretory 11 *s* IgA in the calf (Porter, 1972). Comparative studies of the porcine and bovine species serve to emphasize the probable significance of these observations.

The neonates of both species absorb maternal immunoglobulins for the first few hours of life. In the pig there is selectivity against the absorption of colostral secretory 11 *s* IgA, and the antibody function of this molecule is mainly confined to the lumen of the intestine (Porter, 1969). In contrast there is no selectivity against absorption of secretory 11 *s* IgA in the calf and the immunoglobulin profile of its serum rapidly becomes similar to that of the colostrum ingested (Porter, 1971). Therefore, in the young ruminant there is a temporary condition in which high levels of secretory 11 *s* IgA are found in the blood circulation. This is a unique situation not recorded in other mammals under normal conditions, and it is interesting that there is a rapid decline in the serum-IgA level during the first week of life. The main reason for this decline is an apparent loss of IgA into external secretions. The secretions of the gastro-intestinal tract are important in this system and therefore the passively acquired antibody may have a role in local defence.

In relation to these observations on the neonatal calf, it is interesting that in the mammary secretions of the cow there is a rapid decrease in total immunoglobulin content, so that, after the first few days of lactation, all immunoglobulin and antibody activities have declined to very low levels. This again contrasts with the pig, in which IgA is an important antibody throughout lactation, and also contributes to local intestinal defence. Clearly milk immunoglobulins must contribute little to local intestinal protection in the calf, and this peculiar characteristic of the young ruminant of absorbing maternal secretory 11 *s* IgA and losing it again by some transudative

process into the alimentary tract must compensate to some extent for the deficiency in the milk.

Recent in vitro studies of immunoglobulin synthesis in bovine tissues using organ culture substantiate the evidence from quantitative immunoglobulin assays in milk, confirming a state of IgA deficiency in the mammary gland (Butler, Maxwell, Pierce, Hylton, Asofsky & Kiddy, 1972). Whether this lactation anomaly is a consistent feature of the ruminant species is not known but similar findings in our examination of the milk from sheep and goats suggests that this is a distinct possibility. It is of interest that in other herbivores, e.g. the rabbit and the horse, which rely on bacterial activity in the caecum for cellulose degradation, the milk contains comparatively high levels of IgA (Vaerman, 1971). This of course suggests that the IgA deficiency in the ruminant mammary gland relates directly to the need for development of rumen function in the young calf and the potent anti-bacterial activity of IgA might inhibit the microflora which promotes rumen development.

The developing secretory immune system

The foetus is sheltered from antigenic challenge and in general emerges into life ill-equipped in terms of lymphoid development and immunologic information with which to counter the challenge of its new environment. The gut mucosa receives a continuous stimulus from a variety of antigens, in particular the gut flora. In the ruminant, the complex fore-stomach or rumen, from which the species derives its name, is undeveloped in the neonate. Therefore for the first few weeks after birth when the animal is given milk or other liquid feeds the young ruminant is virtually a monogastric animal, and, as might be expected, there is a development of immunologic function in the alimentary tract which resembles that of the pig (Porter, Noakes & Allen, 1972).

In both species a characteristic IgA system develops as part of the secretory immune mechanism and secretion of the immunoglobulin can be detected during the second week of life. The antibody has all the physico-chemical characteristics of secretory 11 *s* IgA described in man and immunochemical examination of mucous secretions provides evidence of free- and bound-secretory component in the gut contents of the pig (Bourne, Pickup & Honour, 1971) and calf (Mach & Pahud, 1971; Porter *et al.* 1972).

Immunofluorescent studies on intestinal tissues demonstrate that secretory component is synthesized independently from IgA and is associated entirely with crypt epithelium and intestinal mucins (Porter *et al.* 1972, Allen & Porter, 1973*a*, *b*). Possibly the most important biological function which could be attributed to secretory component would be that of retaining the immunoglobulin at high concentration in the mucin lining the epithelium. Therefore, the complexed secretory component may behave as an adhesive factor assisting in the establishment of the antibody barrier; also, there is evidence that it assists in the maintainance of antibody function in the gut by reducing susceptibility to enzyme degradation.

Until recently it has been generally accepted that IgA is the major immunoglobulin synthesized in the gut and that it is present in approximately 80% of plasma

cells in the lamina propria (Crabbe, Carbonera & Heremans, 1965). However reported observations relate almost entirely to the alimentary tract response in the adult (see Doe, 1972). With the possible exception of examination of immune response to oral polio-virus vaccines in the human infant (Keller, Dwyer, Oh & D'Amodio, 1969), little attention has been given to the development of the intestinal immune response of the young.

Observations on the young pig (Allen & Porter, 1973*b*) and calf (Porter *et al.*, 1972) indicate that most of the immunoglobulin-synthesizing cells which first infiltrate the gastric mucosa are involved in the production of IgM. The important role of IgM in the early stages of development of secretory immunity was further confirmed by immunofluorescent studies of intestinal tissues stained sequentially with fluorescein-conjugated rabbit anti-IgM followed by rhodamine-conjugated rabbit anti-IgA. This demonstrated the presence of two populations of cells in the lamina; also certain cells appeared to be dual stained, suggesting that these were synthesizing both classes of immunoglobulin. In the adult animal most of the intestinal cells synthesize IgA and cells synthesizing IgM are not very important. Recent studies in the germfree mouse show that intestinal-IgA synthesis can be suppressed by frequent injections of goat anti-mouse IgM (Lawton, Asofsky, Hylton & Cooper, 1972). Therefore the evidence suggests that the primary immune response in the immunocytes of the alimentary tract is attributable to IgM and that there is a switch in antibody synthesis to IgA which becomes the predominant local antibody as the system matures.

It is interesting that in the secretory immune system in the early period of its development IgM predominates in the lamina propria and IgA predominates in the intestinal secretions. Therefore, a selective transport mechanism must exist for the transfer of the immunoglobulin to the external surface. Recently, electron microscopy studies have been done on the mechanism of secretion (Allen, Smith & Porter, 1973). These results indicate that IgA is transferred from the plasma cell to crypt epithelial cells in the form of discrete vesicles which migrate to the supra-nuclear cytoplasm before being secreted into the lumen of the gut. This mechanism will also explain the difference in levels of IgA in the serum and secretions.

It is important in the context of nutrition that in the local immune system the mechanisms of active synthesis and secretion of antibody in the intestinal mucosa start functioning in the first week of life, although the high levels of passively-acquired maternal antibodies produce a potentially suppressive effect. This suggests that there are opportunities for oral immunization while the young animal is suckled, with the attractive prospect of increasing the protective characteristics of the local epithelial defence mechanisms before weaning.

The effects of oral immunization on animal performance

It is almost fifty years since a treatise on local immunization was first published (Bezredka, 1927). However, the concept has not been successfully exploited because of persistent attempts to correlate protection produced by vaccination with the

level of antibody in the blood circulation. The concept of intestinal antibody (copro-antibody) was advanced by Burrows, Elliott & Havens (1947) and the recent definition of local immune mechanisms (Heremans, Crabbe & Masson, 1966; Tomasi, 1967) confirmed that the antibody defences of the body can be divided into virtually separate systems (local and circulating), therefore strengthening the prospect of producing effective vaccines for oral administration.

Previous observations have particular relevance to alimentary tract infections in the young animal. Enteric disorders associated with *E. coli* are found during periods of stress, often associated with dietary change or change of environment. The piglet presents an interesting model for this syndrome because at weaning the young animal is frequently challenged by a rapid proliferation of haemolytic *E. coli* (Buxton & Thomlinson, 1961; Kenworthy & Crabbe, 1963). In common with *E. coli*-associated diseases in general, post-weaning enteritis is attributable to only a limited number of serotypes. Therefore, an oral vaccine might be produced and its effect on animal performance studied in the post-weaning period.

Studies on intestinal secretions from young fistulated animals provided evidence for an IgA antibody response which rapidly followed local challenge with aqueous suspensions of heat-killed *E. coli*. This response was also obtained in orally-dosed animals before weaning so that there was no indication of interference by maternal antibodies. However, one immediate drawback was that antibody secretion persisted only for 2–3 weeks following a single antigenic challenge. Also, a second challenge provided a similar response of the same duration; therefore, in contrast to the circulatory antibody system, the local intestinal mechanism showed no sign of memory and required repeated stimulus. Apparently a creep diet might be a useful carrier for the bacterial antigens as each animal would receive an antigenic stimulus every time it ate. In this context it was encouraging that intestinal antibody secretion in animals given creep diets supplemented with *E. coli* antigens compared well with animals which were dosed daily. These diets are made available to the young animals for periods of 2–4 weeks before weaning and the voluntary intake gradually increases and the piglets are removed from the sow.

The value of this approach is reflected in improved performance in the immediate post-weaning period. Using standard feeding-trial organisation sixty-two litters of young pigs were fed on a creep diet supplemented with *E. coli* antigens and their growth rates were compared with sixty-two litters of pigs given unsupplemented diets Antigen-fed pigs showed significantly greater weight gains than control pigs in the 4-week post-weaning period (Porter, Kenworthy, Holme & Horsfield, 1973).

In clinical trials in which animals were deliberately infected with pathogenic *E. coli*, the orally-immunized animals showed a more rapid clearance of the infecting organism, better faeces scores, quicker recovery and fewer deaths. Also the results of these studies of serum antibodies following infection in these animals suggested a further factor. As stated earlier the action of local immunity is separate from that of the circulatory antibody system. We have not been able to demonstrate a blood-serum antibody response to oral immunization; the activity appears to be directed almost exclusively towards local antibody production. However, we observed that

the orally-immunized animals responded quickly with the production of serum antibodies following infection, but there was a time period of nearly 7 d before a serum-antibody response was registered in control animals. Clearly oral immunization primes systemic immunity as well as stimulating local immunity, and this is an added bonus to the processes of defence.

The effects of oral immunization cannot be considered in normal nutrient-value terms. The significant weight gains are the indirect result of an improved competence to combat a bacterial challenge in the post-weaning period. It was interesting that the effect was greatest in smaller pigs so that poorer animals achieved a better level of performance. In these terms oral immunization is clearly capable of making inroads into the ever present problem of subclinical infection and its deleterious effects on nutrition. There are long-term benefits which might result from consistent use on a farm, possibly improving the health pattern over a period of years and, therefore, solving an important problem in intensive-management systems. The fact that we are working with natural biological systems is worth emphasizing. Instead of viewing the intestine simply as an absorptive organ for nutrients we also see its potential in providing a protective system for its important absorptive surface. In simple terms we are endeavouring to programme the developing intestinal antibody system of the young animal to cope with the anticipated challenge of its environment, thereby improving health and performance.

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Printed in Great Britain