Immunologically mediated nutritional disturbances associated with soya-protein antigens

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The immunologist in nutrition or the nutritionist in immunology is generally aware of three main areas where the intestine interacts with immune function. In neonatal life passive protection of the intestinal mucosa by antibodies ingested with the mother's colostrum and milk is important. Furthermore there is the generation of antibody synthesized and secreted locally in the intestinal mucosa forming a barrier towards the penetration of macromolecules and bacterial products thereby retaining intestinal integrity and function (Porter, 1976).

Immunologic sensitivity and intestinal disfunction is a topic of immense interest drawing together nutritional, medical and immunological sciences. The characteristics of the immune response in the intestinal tract are complex and only recently has a more satisfactory understanding been obtained. In the past, extensive studies have been carried out on the subject of dietary sensitivity at a clinical level but there have seldom been opportunities to examine the problem on a large scale together with its nutritional implications. We propose to present a topic which embraces a wide area of gut immunology and has a substantial basis in nutrition.

The levels of serum antibody generated in calves feeding on soya proteins are quite remarkable. This phenomenon presents an apparently unique model for investigations of intestinal immune response to antigen absorbed from the lumen.

Soya protein and nutritional performance

A variety of anti-nutritional factors have been identified in soya-bean protein products and technology has been advanced to eliminate these and improve their safety and nutritional performance. However, additional problems have required resolution and these fall into the category of antigenic sensitization.

Recent studies comparing the effects of soya protein in calf milk-replacers indicate a severe impact on nutritional performance as well as health and viability (Roy *et al.* 1977).

A relationship between the poor performance of young animals fed on diets containing soya protein and some component of the soya protein that was antigenic to the animal was first demonstrated by Van Adrichem & Frens (1965) and Van Leeuwen *et al.* (1969) through the production of high titres of antibody to the soya protein in the blood serum. The last named authors showed that there was a relationship between the type of soya protein used, the maximum antibody titres obtained and the degree of lowered nutritional performance.

0029–6651/79/3813–2106 $01.00

Further evidence for the involvement of soya-protein antigens in the nutrition of the young calf comes from the work of Smith & Sissons (1975, 1976). The effects of diets based upon soya protein on the rates of flow and composition of digesta were studied in the abomasum and intestine of fistulated pre-ruminant calves. The feeding of soya-supplemented diets resulted in an inhibition of abomasal emptying, decreased transit time of markers through the small intestine, abnormal water and salt exchange and decreased nitrogen absorption. No such disturbances occurred on the first introduction of soya diets; the changes were only exhibited in animals which had previously been fed on soya protein and generally coincided with demonstrable serum antibody, strongly suggesting an immunological basis for these interferences with physiological function.

The precise antibody mechanisms mediating the digestive disturbances following ingestion of soya protein in calves and piglets have been recently described (Barratt *et al.* 1978). These mechanisms and their significance are re-examined here as a demonstration of the role of immunology in nutrition, a theme that was first introduced at the European Nutrition Conference in Cambridge (Porter, 1973).

It is worthwhile emphasizing at this point that there has been a consensus of opinion that commercial extraction of soya-bean meal with hot aqueous ethanol is beneficial in reducing the apparent antigenic nature of the product. This view has been supported by observations of improved performance of animals fed on such products (Gorrill & Thomas, 1967; Nitsan *et al.* 1971; Smith & Sissons, 1976). However the investigations presently described were carried out with alcohol extracted protein preparations in which the harmful antigen clearly survives. Therefore it will be necessary to apply more rigorous immunologic criteria in the quality control of soya-bean processing in order to ensure that nutritional disturbances associated with such unwanted components are obviated and that nutritional quality of soya-protein concentrates can be optimized.

Immunology and the gut

The intestinal mucosa is affected by a variety of foreign substances, many of which can, under suitable circumstances, act as antigens. These substances will fall into three main categories; ingested foodstuffs, bacterial and viral antigens, and chemical agents ingested as drugs, food preservatives or contaminants.

Although insufficient whole dietary protein is absorbed from the intestinal tract to have any significance from a nutritional standpoint, sufficient intact macromolecules can be absorbed to induce both local and systemic immune responses. This state of immunity may manifest itself in a protective function, or a destructive function being mediated as a hypersensitivity reaction.

Depending upon the immune mechanism of tissue damage these hypersensitivity reactions can be differentiated into four main types; type 1 being initiated by antigen reacting with tissue cells passively sensitized by antibody produced elsewhere, type 2 initiated by antibody reacting with antigenic components of cells in the presence of complement, type 3 where antigen

precipitates with antibody again activating complement in the tissue spaces and type 4 which is mediated by the reaction of actively sensitized lymphocytes with specific antigen, (Gell & Coombs, 1968).

Local synthesis and secretion of immunoglobulin within the intestinal mucous membrane is primarily responsible for the prevention of the absorption of antigen.

Topical immunization of the intestine which stimulates the production of secretory immunoglobulins chiefly of IgA and IgM classes, can significantly interfere with the intestinal uptake of dietary antigens (Walker *et al.* 1973; Andre *et al.* 1974) resulting in a decrease in absorption of the specific antigen.

If this function of immune exclusion is defective in some way, whether due to genetic influences, disease states or immunological immaturity, one or more of the hypersensitive states outlined above could result (Stokes *et al.* 1975).

However, even in animals where a normal local secretory antibody system is functioning, prolonged exposure of the intestinal mucosa to low concentrations of ingested antigen may result in a gradual systemic sensitization (Rothberg *et al.* 1973). This is presumably due either to dissemination of antigen reactive lymphoid cells from the gut-associated lymphoid tissues, or exposure of systemic lymphoid tissues to immunogenic concentrations of absorbed antigen.

The cellular characteristics of all these reactions in the host are given in a schematic form in Figure 1, as a basis for examining the mechanisms of immune mediated nutritional disturbances associated with soya-protein antigens.

Kinetics of the immune response to soya-protein antigens

We carried out initial studies on 7-d-old calves fed diets containing up to 25% of alcohol extracted heated soya-bean protein, to establish whether these animals produced a humoral antibody response comparable with those previously reported in the literature (Van Adrichem & Frens, 1965; Van Leeuwen *et al.* 1969; Smith & Sissons, 1975). Serum haemaglutinating antibody responses were detected in all calves from about 7 d after commencing feeding the experimental diet, with peak titres occurring within 3 weeks. The levels of antibody demonstrated by us and the workers cited above were surprisingly high. In fact they were of an order that would be more in keeping with that expected from a hyperimmunization schedule rather than that to be expected from the casual response to dietary antigens.

Age and secretory immunoglobulins affecting the profile of the serum antibody response. The kinetics of the response of calves to soya-protein antigens was related to the age of the calf when first introduced to the soya-protein diet. Animals fed the soya diet from 1 week of age produced antibody titres 10–100-fold greater than in those first introduced to the diet at 4 weeks of age. Similar observations relating to cow's milk protein have been made in the human infant (Kletter *et al.* 1971). A possible explanation for this diminished response in older animals might relate to the previously cited mechanism of antigen exclusion by mucosal antibody.

In the very young calf the level of secretory immunoglobulin synthesis in the intestine is low (Allen & Porter, 1975), and with age this level gradually rises,

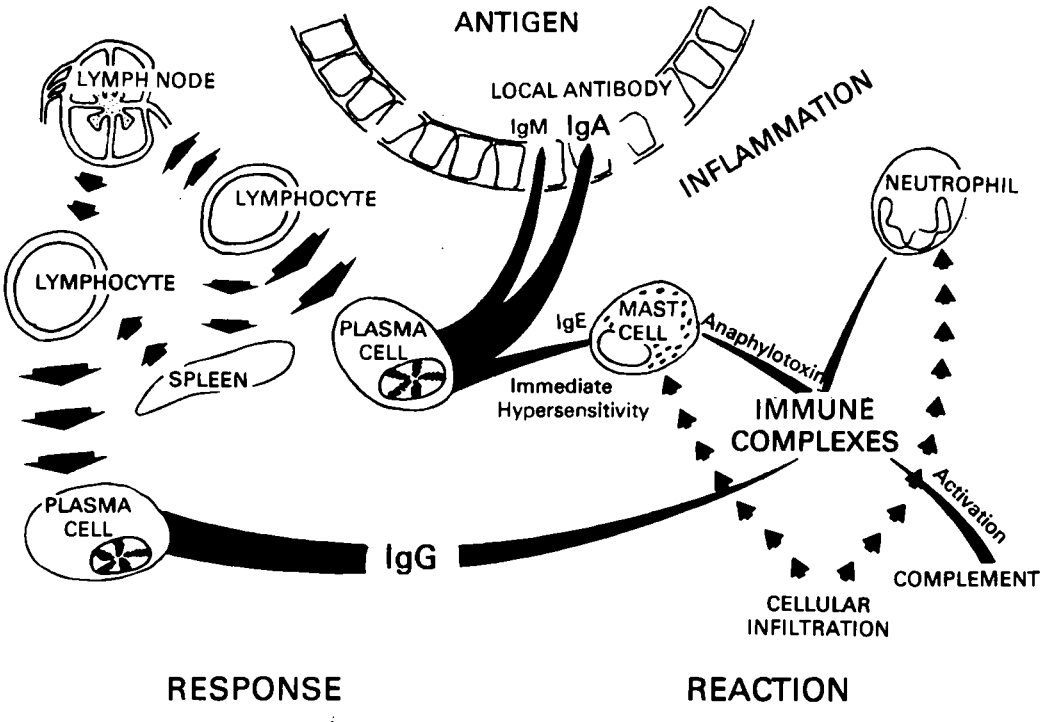

Fig. 1. Schematic representation of the mechanisms involved in the immune response to dietary antigens.

consequently complexing with the antigen and preventing its transmucosal transport.

The literature on the nature of the secretory immunoglobulins of the bovine small intestine are conflicting. Porter *et al.* (1972), Cripps *et al.* (1974) and Mach & Pahud (1971) found that the predominant local secretory immunoglobulins in the bovine intestine were IgM or IgA. This correlates with the measurements of in vitro synthesis in which IgA production was most pronounced in bovine small intestinal tissues (Butler *et al.* 1972). Curtain *et al.* (1971) and Newby & Bourne (1976) on the other hand concluded that IgG_1 was the major secretory immunoglobulin. We studied the specific secretory antibody to soya protein in Thiry-Vella loop preparations in calves and found that antibody to be predominantly IgM and IgA (M. E. J. Barratt & P. Porter, unpublished results). Although one would assume that the calf was therefore able to sequester antigen in the gut mucosal tissue, sufficient soya antigen obviously gains further access to the body tissues and stimulates a response similar to that of parenterally administered antigen. The fact that the serum antibody response increases with time of exposure to the diet would suggest that the local immune mechanism is only partially successful in blocking access of the antigen.

https://doi.org/10.1079/PNS19790019 Published online by Cambridge University Press

Maternally acquired antibody has been shown in infants (Levi *et al.* 1969) and in baby pigs (Segre & Kaeberle, 1962) to enhance the immune response of the offspring. We have found that the presence of antibody to soya protein is common in the neonatal calf and this antibody can be shown to originate from the colostrum. Our results clearly showed an enhanced response in animals that had detectable antibody prior to feeding the soya-protein antigen.

The absence of tolerance. Calves that were fed for periods on soya-protein based milk-replacers were maintained for further periods on skim-milk based diets before being returned to the soya-protein feeding schedule. Examination of their serum antibody titres showed that each return to the soya diet resulted in an increase in the level of specific anti-soya antibody suggesting that previous sensitization had taken place. There was no evidence of the tolerogenic properties which have previously been demonstrated for other orally administered antigens such as albumin (Thomas & Parrott, 1974; Thomas *et al.* 1976) or sheep erythrocytes (Andre *et al.* 1973). Therefore, although we have demonstrated an age effect on the antibody response to soya protein in the diet it is clear that there is no evidence of tolerance to repeated soya feeding periods. Possibly merely the rate of antibody production is determined by the time of the initial introduction to soya into the diet of the young calf. Further periods of feeding soya-containing diets simply resulting in further stimulation of an already primed immune system.

Characteristics of the antibody response. IgG is the dominant immunoglobulin in the blood serum; its primary biological function is the opsonization of micro-organisms making them more receptive to phagocytic cells, and the neutralization of bacterial toxins. Many cell types display surface receptors capable of binding to IgG. Complexes of IgG and antigen will activate the complement system. IgM antibodies are produced early in the immune response; they also can activate the complement system and because of their high valency are extremely efficient agglutinating and cytolytic agents. IgA is present in serum in low concentrations but is the predominant antibody of sero-mucous secretions. IgE is also present mainly in sero-mucous secretions and is the antibody class involved in immediate hypersensitivity states.

In calves fed diets containing soya protein the predominant anti-soya antibody in the serum has been shown to be of the IgG_1 class, (Barratt *et al.* 1978). This antibody was shown to precipitate with soya-protein extracts and to activate complement. Specific IgA and IgM antibodies were detected at the level of the intestinal mucosa but these were obviously of low avidity as they were not effective in preventing the absorption of soya-antigen across the gut mucosa and stimulating the systemic immune system. The antibody-antigen interaction occurring in calves fed on soya protein is thus ideally suited to result in tissue-damaging reactions, of the type 3 complex-mediated hypersensitivity class. Here complexes between antigen and humoral antibody activate the complement system and aggregate platelets, leading to chemotaxis of leucocytes, release of vasoactive amines and proteolytic enzymes and a general inflammatory reaction.

Immunochemical analyses of serum antibody in soya fed calves provided the

essential criteria for this inflammatory mechanism in the majority of animals examined. On the other hand the classic hypersensitivity mechanism mediated by IgE was rarely encountered (Table 1).

Table 1. *Immunological properties of antibody produced by calves to dietary soya protein*

Reactions	Animals positive %	Antibody class
In vitro tests (*n* 547)		
Passive haemagglutination	100	IgG_1
Complement fixation	78	IgG_1
Precipitation	48	IgG_1
In vivo tests (*n* 80)		
Active anaphylaxis	5	IgE
Passive anaphylaxis (calf)	~1	IgE

Physiological and morphological disturbances associated with dietary soya protein

Having characterized the molecular features leading to the biologic disturbance the essential involvement in the tissue inflammatory response remained to be demonstrated. With this objective simple fistula were prepared in the duodenum and ileum of young calves, and biopsies taken for histological examination before, and at intervals after, the feeding of soya-containing diets. Certain changes became apparent after 7 d or more of feeding these diets. Most consistently, the villi became shorter and broader, and there was some disturbance in the regularity of the villous epithelial cells. There was also a conspicuous mononuclear cellular infiltration of both the villi and lamina propria, with some evidence of oedema and haemorrhage within the laminal tissue. These changes coincided with the period that serum antibody first became demonstrable.

These morphological disturbances are reminiscent of those seen in gluten-sensitive enteropathy in humans (Fontaine & Navarro, 1975) and transient intolerance to soya protein in infants (Mendoza *et al.* 1970; Ament & Rubin, 1972). Although Smith & Sissons (1976) failed to detect any similar histological lesions in the intestines of two calves fed on soya-protein diets, gross intestinal disorders were suggested by the appearance of what appeared to be partially digested blood clots in the ileal contents. Similarly, Roy *et al.* (1977) reported that in two calves that were on soya-containing diets and had to be killed in extremis there was evidence of villous atrophy in the intestine, the duodenum and jejunum being particularly affected.

A direct association between the serological immune response and physiological disorders has been implied (Smith & Sissons, 1975). These observations have been corroborated in studies on flow through Thiry-Vella loops prepared in piglet intestine (Barratt *et al.* 1978). There was no evidence of an inhibition of flow in perfusion experiments prior to the local stimulation of intestinal antibodies. Thereafter there was a significant reduction of flow through the intestinal loop on

each experimental introduction of soya-protein extract (Fig. 2). In the presence of antibody, the introduction of antigen into the loop resulted in a significant drop in flow rate; removal of the antigen caused the flow to regain normal values.

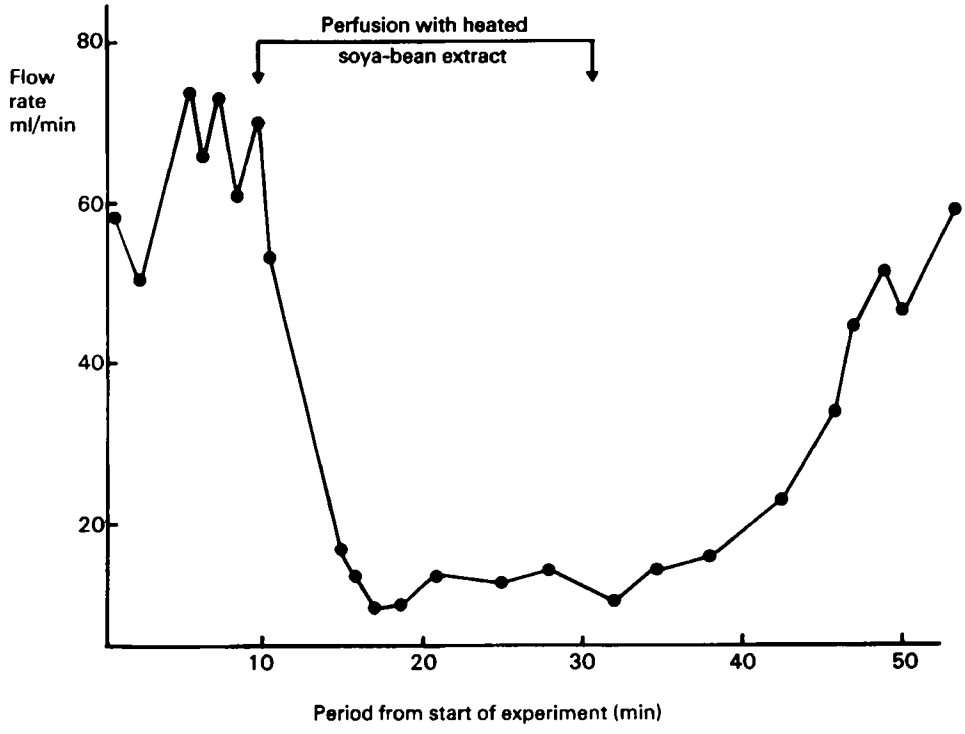

Fig. 2. Flow rates through Thiry-Vella intestinal loops prepared in piglets. The effect of perfusion of an aqueous extract of heated soya protein through a loop prepared in an animal already sensitive to soya protein.

Conclusion

A soya-protein antigen which is resistant to conventional processing by hot ethanol extraction is responsible for digestive disturbances in calves. This is attributable to immune mediated inflammatory responses within the intestinal mucosa largely associated with an IgG_1 class of antibody which precipitates with antigen and fixes complement. Intestinal disfunction occurs when antibody has been synthesized and antigen enters the lumen of the gut.

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Printed in Great Britain