

## GASTROINTESTINAL FOOD ALLERGY AND ITS ROLE IN LARGE DOMESTIC ANIMALS

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### ABSTRACT

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The significance of food allergy as a primary cause for gastrointestinal disturbances in domestic animals, especially calves and piglets, is discussed. The immunological backgrounds and pathogenesis are described in some detail. The clinical and pathological manifestations in animals are related to those in man. Diagnostic possibilities, therapy and prevention, as far as known in animals, are mentioned and, based on human experiences, further extensions are proposed.

### INTRODUCTION

In pruruminant calves several investigations have been performed from which indications for gastrointestinal food allergy have been obtained (Sissons & Smith, 1976; Barratt & Porter, 1979; Kilshaw & Sissons, 1979a; Kilshaw & Sissons, 1979b; Kilshaw & Slade, 1980; Kilshaw, 1981; Fledderus *et al.*, 1985). Similar findings have been reported in piglets (Barratt *et al.*, 1978; Miller *et al.*, 1983; Newby *et al.*, 1983; Miller *et al.*, 1984).

In man, a large volume of data on adverse reactions to foods has accumulated since the beginning of the twentieth century but many problems are still unanswered (Businco *et al.*, 1984).

In discussing a topic as complex as food sensitivity precise definitions on terms are necessary to avoid confusion (Atkins & Metcalfe, 1984; Businco *et al.*, 1984; Panush & Webster, 1985). In accordance with the Committee on Adverse Reactions to Foods of the American Academy of Allergy and Immunology (Atkins & Metcalfe, 1984) the term 'food sensitivity' is used for any adverse reaction to an ingested food or food additive. 'Food allergy' is synonymous with 'food hypersensitivity' and is the term for those adverse reactions in which the immune system plays a prominent role.

'Food intolerance' refers to reactions in which the immune system does not participate. This category includes pharmacologic, metabolic, toxic and idiosyncratic food reactions; this latter response resembles a hypersensitivity reaction in that the food or food additive induces a quantitatively abnormal response which differs from its normally expected physiologic or pharmacologic effect and simulates an allergic disorder (Atkins & Metcalfe, 1984; Panush & Webster, 1985). These idiosyncratic reactions, which may be based on enzyme defects or unknown causes (Businco *et al.*, 1984) are also known as pseudo-allergy or false food allergy (Ferguson, 1984). These pseudo-allergies, often quantitatively abnormal, are only seen in some species or in some individuals of a given

species. This may be explained by genetic peculiarities. Moreover, qualitative deviations in responsiveness can occur which are also categorized as pseudo-allergic reactions (Dukor *et al.*, 1980). Food allergy comprises a wide spectrum of signs and symptoms partly located in the gastrointestinal tract (gastrointestinal food allergy) and partly elsewhere in the body, such as the respiratory system, skin or eyes (alimentary food allergy) (Taylor, 1975; Eade & Wright 1976; Walker-Smith *et al.*, 1984). These localizations may be combined in one patient, resulting in a vast range of clinical manifestations, from acute anaphylactic, generalized reactions, even leading to death, to relatively minor local signs like diarrhoea or abdominal pain (Walker-Smith *et al.*, 1984).

In man, gastrointestinal food allergy is most often found in the first months and years of life and decreases with age (Eastham & Walker, 1979; Businco & Cantani, 1984; Ferguson, 1984; Van Spreeuwel *et al.*, 1984; Walker-Smith *et al.*, 1984). Breastfed children suffer less frequently from alimentary and gastrointestinal allergy than children receiving artificial feeding, which suggests that adverse contact with antigen in the neonatal and early infancy period has an especially high probability of provoking damaging sensitization (Businco & Cantani, 1984; Soothill, 1984). Moreover, intrauterine sensitization appears to be possible in man and guinea pigs (Businco & Cantani, 1984). Sensitizing exposure to antigens may also occur in individuals subsequent to gastroenteritis and to loss of integrity of the functional mucosal barrier, whatever its cause may be (Van Spreeuwel *et al.*, 1984; Panush & Webster, 1985). Gastroenteropathy may also result in a disturbed immunological response such as the decreased activity of suppressor cells (Mowat, 1984) found in some viral and bacterial infections or food contamination with microbial products (Pahud & Schwarz, 1984). This may be due to an adjuvant effect (Pahud & Schwarz, 1984). From these observations the hypothesis may be formulated that food allergy may also play a significant role in large domestic animals, especially calves and piglets.

In this review the possible etiology, pathogenesis, clinical and pathological symptoms, diagnostic possibilities, therapeutics and prevention of gastrointestinal food allergy and its role in large domestic animals will be discussed.

## ETIOLOGY AND PATHOGENESIS

### *Uptake of antigens*

In spontaneous cases of food allergy it is often difficult to determine the provoking antigen. In general, the antigens are macromolecules which have permeated from the gastrointestinal lumen and are presented to immunocompetent cells. Normally, after the neonatal 'closure' of the epithelial cells, the uptake of macromolecules decreases and is restricted predominantly to specialized follicle associated epithelial cells, known as membranous (M) cells and found in the dome epithelium overlying the gut-associated lymphoid tissue (GALT) (Walker, 1981; Brandtzaeg, 1985; Egberts *et al.*, 1985; Elson, 1985; Vellenga *et al.*, 1985). The moment at which closure occurs varies in the different species (Walker, 1981; Vellenga, 1985). Before closure, in neonates of most domestic animals, the predominant route of uptake of antibodies is by absorption from the gut. However, when other macromolecules are presented (as in formula-feeding) they can also be absorbed and may function as antigens. In addition, antibodies to food macromolecules may be present in the colostrum and hence the neonate can become sensitized to the corresponding antigens (Businco & Cantani, 1984).

After closure, permeability to macromolecules can be increased by defects in the mucosal barrier (Mouwen *et al.*, 1983; Egberts *et al.*, 1984). Such damage can be caused by infectious agents or toxins but can also result from local hypersensitivity reactions in

the gut (Mouwen *et al.*, 1983; Businco & Cantani, 1984; Brandtzaeg, 1985). Enhancement of antigen uptake is also related to increased concentrations of the macromolecules in the intestinal lumen (Walker, 1981; Mouwen *et al.*, 1983; Egberts *et al.*, 1984). Furthermore, increased uptake can result from IgA immunodeficiency, possibly because intraluminal complex formation by the antigens with IgA and subsequent degradation are absent (Ferguson, 1984; Porter *et al.*, 1984; Soothill, 1984).

### *Presentation of antigens*

When antigens have passed the mucosal barrier they can be carried free in solution or on the surface of antigen-presenting cells (APC's) to the regional lymph node. The relationship between M cells and APC's is unknown, although they are often found close together (Wilders *et al.*, 1983c; Brandtzaeg, 1985). APC's are a special type of macrophage-like cell with long cellular extensions; in such organs as the spleen, lymph nodes, Peyer's patches, skin and thymus they are found as interdigitating cells in T-cell areas, as dendritic cells in follicles and B-cell areas or between and under epithelial cells (Wilders *et al.*, 1983abc; Roitt *et al.*, 1985). In the lymph vessels APC's are found as veiled cells (Wilders *et al.*, 1983abc). The APC's present the antigen to T- and possibly also to B-cells. This presentation is MHC restricted (Elson, 1985; Roitt *et al.*, 1985) i.e. receptors on the T-cell can only recognize an antigen if it is presented on an APC together with a special determinant of the major histocompatibility complex (MHC). This MHC restriction may explain why some individuals become hypersensitive to a given antigen and others do not (Elson, 1985).

Dependent on the type of MHC determinants on APC cells, different T-cell subclasses can be activated, resulting in, for instance, either help to B-cells in their antibody response or inhibition of the B-cell response. These interactions between T and B-cells also determine the quality of the B-cell response with regard to the immunoglobulin class (isotype: IgE, IgA, IgG or IgM), the genetic marker (allotype) and the determinants in the variable region (idiotype) (Elson, 1985; Roitt *et al.*, 1985). Both B and T cells, primed to an antigen in the gut, can leave the (regional) lymph node and home in on the intestinal mucosa via the thoracic duct and blood circulation (Brandtzaeg, 1985).

In this way local allergic reactions in the intestinal mucosa may be evoked but also generalized or systemic reactions can occur when antibodies or sensitized lymphocytes are released into the circulation.

### *Types of allergic reactions to food*

In the human gut acute, intermediate and delayed onset allergic reactions to food are well-known (Kuitunen *et al.*, 1975; Taylor, 1975; Bloch *et al.*, 1981; Atkins & Metcalfe, 1984; Walker-Smith, 1984). The acute reactions are mediated by cytotoxic antibodies (reagins), especially IgE; the intermediate and delayed reactions are mainly immune-complex mediated (Type III) or cell mediated (Type IV), although IgE may play some role in these as well (Atkins & Metcalfe, 1984; Businco *et al.*, 1984). Very often, several immunological mechanisms are involved in one patient suffering from food allergy.

Schematically, in immediate gastrointestinal allergic food reactions, which occur within minutes to a few hours after food ingestion (Bloch *et al.*, 1981; Bock & May, 1981; Atkins & Metcalfe, 1984), antigen-specific IgE antibodies (and in some species, including man and dog (Parish, 1983; Willemse, 1984), a subclass of IgG antibodies) become bound to cell surface receptors for the Fc component of that antibody (Ferguson, 1984; Brandtzaeg, 1985). These Fc<sub>ε</sub> receptors are present, among others, on and in the mucosal

mast cells (MMC's), the main part of the mast cell population in the *lamina propria* of the gut (Brandtzaeg, 1985). MMC's differ from the connective tissue mast cells, for instance by their dependence on T-cell lymphokines and their different behaviour in tissue fixation (Brandtzaeg, 1985). When an antigen interacts with the MMC-bound IgE a sequence of intracellular events is started, resulting in degranulation of the mast cells and release of preformed and newly formed mediators such as histamine and other vasoactive amines, eosinophil chemotactic factor of anaphylaxis (ECF-A), prostaglandins and leukotrienes (Roitt *et al.*, 1985). Physiologically, these extremely potent substances contribute significantly to immune mucosal defence, particularly against helminths (Brandtzaeg, 1985). This is augmented by the arming effect of IgE on macrophages and eosinophils, which may also express Fc<sub>ε</sub> receptors (Brandtzaeg, 1985). Furthermore, the mediators may play a role in goblet cell mucus release (Bloch *et al.*, 1981). In acute food allergy, the IgE-MMC system is overreacting to certain antigens. The evoked inflammation damages the intestinal mucosa; even systemic anaphylactic reactions may occur. The resulting increase of mucosal permeability for macromolecules may contribute to a vicious circle (Brandtzaeg, 1985). In addition, adhesion and invasion of toxins and infectious agents may follow the initial mucosal damage.

Degranulation of mast cells can also be induced by several other immunological and non-immunological mechanisms. Examples of the latter are reactions to egg-white in cats and rats, to plasma expanders like dextrans, to curare, codeine and so on (Kallós & Kallós, 1980). These pseudo-allergic reactions are often only found in susceptible individuals and the phenomenon appears to be an undefined hereditary trait (Kallós & Kallós, 1980).

Various organs appear to have different histamine receptors, called H<sub>1</sub> and H<sub>2</sub> receptors, which can be selectively inhibited by specific histamine antagonists (Kallós & Kallós, 1980). H<sub>2</sub> receptor stimulation appears to be solely responsible for the gastrointestinal symptoms in immediate allergic reactivity (Kallós & Kallós, 1980), although H<sub>1</sub> receptors are generally believed to be most important in anaphylactic responses and H<sub>2</sub> receptor stimulation in secretion of gastric acid.

The immune-complex mediated (Type III) gastrointestinal food allergy can occur several hours to days after food ingestion (Atkins & Metcalfe, 1984) and in these cases it is an intermediate reaction (Bloch *et al.*, 1981). This type is less well defined than the Type I reactions. Raised blood levels of IgG and IgE, of IgG-antigen and IgE-antigen complexes and of complement C1<sub>q</sub> binding complexes have been described in men and animals with food allergy (Barratt *et al.*, 1978; Barratt & Porter, 1979; Kilshaw & Sissons, 1979a; Kilshaw, 1981; Ferguson, 1984). Although, in some inflammatory bowel diseases, immunoglobulins A, G, M and E and complement factors C1<sub>q</sub> and C3 are detected in the *lamina epithelialis*, the *lamina propria* and/or in the basement membranes of the intestine, these findings are not consistently demonstrable (Perkkiö *et al.*, 1981; Miller *et al.*, 1983; Ferguson, 1984; Jewell & Patel, 1985).

Deposition of these complexes in the intestinal mucosa or in other organs such as lungs or skin and sequential activation of complement factors may induce Arthus type-inflammatory lesions (Visakorpi, 1980; Lessof, 1983; Ferguson, 1984; Brandtzaeg, 1985). Moreover, C3a and C5a degradation split-off products, also known as anaphylatoxins, may release mediators from mast cells (Kallós & Kallós, 1980). The supposed inflammatory mucosal lesions will result in a similar disturbance of the mucosal barrier as described for the IgE-MMC system (Brandtzaeg, 1985). However, the pathogenic role of immune complexes in food allergies, although plausible from a comparative point of view, remains to be elucidated (Businco *et al.*, 1984; Jewell & Patel, 1985).

Cell mediated (Type IV) hypersensitivity, which is mostly a chronic process starting

one to several days after contact with an antigen, has been supposed to be involved in several gastrointestinal inflammatory diseases (Ferguson *et al.* 1981; Miller *et al.*, 1983; Atkins and Metcalfe, 1984; Businco *et al.*, 1984; Ferguson, 1984; Miller *et al.*, 1984; Jewell & Patel, 1985; Marsh, 1985). Increased density and sensitization of T-cells have been described in gluten-sensitive enteropathy (Marsh, 1985), ulcerative colitis and Crohn's disease (Jewell & Patel, 1985), cow's milk protein sensitive enteropathy (Ferguson, 1984) and in calves sensitized to soya protein (Barratt *et al.*, 1978). This T-cell activation may be involved in immunoregulatory control, lymphocyte cytotoxicity, and lymphokine production (Jewell & Patel, 1985) but, as in Type III hypersensitivity, the exact role in food allergies is unknown (Jewell & Patel, 1985). In man, the mean age at diagnosis is higher in patients with delayed onset hypersensitivity than in acute onset (Hill *et al.*, 1984).

### *Food allergens*

Food-associated antigens which may evoke alimentary allergy have been described mainly in man. Most common allergens are found in cow's milk (especially  $\beta$ -lactoglobulin), soya bean, chicken, beef, eggs (especially ovomucoid) and gluten from wheat, oats, rye and barley (especially gliadin) (Atkins & Metcalfe, 1984; Businco *et al.*, 1984; Soothill, 1984; Walker-Smith *et al.*, 1984). Food allergy has also been described after ingestion of fish, oranges, bananas, chocolates, rice, peanuts and food additives, but pseudoallergy cannot be excluded (Eade & Wright, 1976; Lessof, 1983; Atkins and Metcalfe, 1984; Businco *et al.*, 1984; Coombs & McLaughlan, 1984; Soothill, 1984; Walker-Smith *et al.*, 1984). In animals,  $\beta$ -lactoglobulin from cow's milk did not evoke an antibody response in calves (Kilshaw & Slade, 1980). Wheat gluten (Kilshaw & Slade, 1982) and ovalbumin (Kilshaw, 1981) can be immunogenic in intestines of calves. Soya bean proteins, especially glycinin and  $\beta$ -conglycinin (Kilshaw & Sissons, 1979b; Sissons *et al.*, 1982), are allergenic for about 50% of the calves (Sissons & Smith, 1976; Kilshaw & Slade, 1980; Kilshaw, 1981; Kilshaw & Slade, 1982). Both Type I (Kilshaw & Sissons, 1979a) and Type III (Barratt *et al.*, 1978) hypersensitivity have been suggested to occur (Kilshaw, 1981). Indications have been obtained that soya protein extract is also an intestinal allergen for piglets at three weeks of age (Barratt *et al.*, 1978). The relatively high antigenicity of soya proteins for calves presumably arises from the fact that proteolysis of these proteins in the abomasum is reduced, due to reduced secretion of abomasal acid, chymosin and trypsin and accelerated abomasal emptying (Roy, 1984). Soya bean flour is still more detrimental when the toxic soya bean oil is still present, the trypsin inhibitors and haemagglutinins have not been destroyed by steaming, toasting or treatment with alkali or acid or the oligosaccharides have not been removed (Roy, 1984).

### *Variation in susceptibility to food allergens*

A strong difference in immunologic response to orally administered antigens has been observed between species. Most rabbits produce high levels of antibodies against oral wheat gluten, in guinea pigs no such serum antibodies can be detected against this immunogen (but they can be after injection of gluten components) while rats react to an intermediate extent (Coombs & McLaughlin, 1984).

In piglets, brief pre-weaning exposure to small amounts of a conventional weaning diet or to a diet containing casein as a nitrogen source results in severe post-weaning diarrhoea, when the same diet is supplied after weaning (Miller *et al.*, 1983; Miller *et al.*, 1984). This phenomenon is interpreted as a Type IV hypersensitivity reaction because of

the histopathological findings described in piglets with post-weaning diarrhoea (Miller *et al.*, 1984). When piglets had not received any such diet before weaning or on the contrary had been exposed to a substantial amount of immunogenic diet continually during the pre-weaning period, no post-weaning diarrhoea was evoked with that immunogenic diet. This has been explained by assuming that a brief exposure to antigens is necessary for immunization and that continual administration of substantial amounts of antigen will lead to immunological tolerance (Businco & Cantani, 1984; Miller *et al.*, 1984; Weström *et al.*, 1984). This induction of tolerance may also explain why post-weaning diarrhoea stops after a short period (Miller *et al.*, 1984). Unfortunately, immunological parameters have not been determined.

The reason why about 50% of the calves were not sensitized to soya proteins (Kilshaw & Slade, 1982) has not been investigated or discussed, but may be linked to individual (MHC linked?) resistance to a possible damaging effect of soya lectins on the intestinal epithelium. In addition, incapacity for uptake or processing of soya antigens by enterocytes, M-cells or APC's may be involved.

## CLINICAL FEATURES

The clinical manifestations of food allergy can be divided into two main categories, i.e. an immediate or a delayed response (Taylor, 1975; Atkins & Metcalfe, 1984; Businco *et al.*, 1984). Both responses may be gastrointestinal or generalized (Taylor, 1975; Eade & Wright, 1976). Most signs and symptoms of the immediate response can be explained by the effects of mast cell degranulation (Kallós & Kallós, 1980). In man, acute gastrointestinal signs and symptoms often start with oedema and pruritus of lips, oral mucosa and pharynx, followed by abdominal pain, vomiting, nausea and diarrhoea which may be haemorrhagic (Eade & Wright, 1976; Atkins & Metcalfe, 1984; Businco *et al.*, 1984). The acute generalized reactions are very variable and include eczema, itching angioedema, acute urticaria, asthma attacks and allergic rhinitis, sometimes laryngeal oedema, dyspnoea, flushing, hypotension, shock and possibly 'cot death' (Eade & Wright, 1976; Atkins & Metcalfe, 1984; Businco *et al.*, 1984). The delayed allergic reactions to food are characterized by malabsorption with diarrhoea and weight loss, steatorrhoea and sometimes peripheral blood eosinophilia (Atkins & Metcalfe, 1984); also abdominal pain, headache, fatigue and depression have been mentioned but these associations remain unproven (Atkins & Metcalfe, 1984; Farah *et al.*, 1985). In man, several more or less circumscribed disease entities have been placed in this group (Walker-Smith *et al.*, 1984), such as food sensitive colitis, eosinophilic gastroenteritis, infantile colic, irritable colon syndrome, Crohn's disease and a group of food-sensitive small intestinal enteropathies (cow's milk sensitive enteropathy, soya-sensitive enteropathy, transient gluten intolerance and others). The antigens involved are mostly the same as those acting in the immediate response (Eade & Wright, 1976; Walker-Smith *et al.*, 1984). In large domestic animals, such as preruminant calves and pigs, gastrointestinal signs of food allergy appear to consist mainly of diarrhoea, weight loss, poor growth and occasionally death (Barratt *et al.*, 1978; Kilshaw & Sissons, 1979a; Kilshaw, 1981; Kilshaw & Slade, 1982; Miller *et al.*, 1984). Generalized signs of alimentary food allergy have not been described in large domestic animals.

## PATHOLOGICAL FEATURES

Morphological alterations in gastrointestinal food allergy have mostly been studied in gastrointestinal biopsies of children and experimental animals (Kuitunen *et al.*, 1975;

Barratt *et al.*, 1978; Eastham & Walker, 1979; Keren, 1980; Kilshaw & Slade, 1982; Sutanto, 1982; Newby *et al.*, 1983; Miller *et al.*, 1984; Sissons *et al.*, 1984).

In immediate responses in which Type I reactions are involved, morphological changes are minimal. There is often only mast cell degranulation, hyperemia, some oedema, some increase of mucus production and patchy desquamation of villous epithelium (Bock & May, 1981; Bloch *et al.*, 1981; Miller *et al.*, 1983; Parish, 1983; Atkins & Metcalfe, 1984; Walker-Smith *et al.*, 1984). Increased numbers of mucosal mast cells and eosinophils may occur (Ferguson, 1984).

In delayed gastrointestinal responses of man and animals, in which Type III and IV reactions dominate, morphological changes are more obvious (Kuitunen *et al.*, 1975; Taylor, 1975; Barratt *et al.*, 1978; Visakorpi, 1980; Ferguson *et al.*, 1981; Kilshaw & Slade, 1982; Stern *et al.*, 1982; Lessof, 1983; Miller *et al.*, 1983; Atkins & Metcalfe, 1984; Katz *et al.*, 1984; Stokes *et al.*, 1984). In general, more or less severe villous atrophy and increased numbers of intraepithelial lymphocytes are found, but these are not characteristic for allergy only. In addition, several other incidental findings have been described including peripheral blood eosinophilia and eosinophilic infiltration of the intestinal wall (Kuitunen *et al.*, 1975; Taylor, 1975; Eade & Wright, 1976; Harms, 1982; Sutanto, 1982; Atkins & Metcalfe, 1984; Ferguson, 1984; Katz *et al.*, 1984, but not in Eastham & Walker, 1979; Vitoria *et al.*, 1979; Brandtzaeg, 1985), bleeding in the *lamina propria* or in the gastrointestinal lumen (Taylor, 1975; Barratt *et al.*, 1978; Eastham & Walker, 1979; Businco *et al.*, 1984), erosion of the tips of villi (Parish, 1983), elongated crypts, especially in Type IV reactions (Vitoria *et al.*, 1979; Ferguson *et al.*, 1981; Stern *et al.*, 1982; Sutanto, 1982; Miller *et al.*, 1983; Katz *et al.*, 1984; Miller *et al.*, 1984), a lowered ratio between the villous epithelial cell height and the crypt epithelial cell height (Vitoria *et al.*, 1979), increased mitotic rate of crypt cells (Ferguson *et al.*, 1971), increased numbers of polymorphs, plasma cells and lymphocytes in the *lamina propria* (Vitoria *et al.*, 1979; Keren, 1980; Harms, 1982; Sutanto, 1982) and of mast cells (Bloch *et al.*, 1971; Keren, 1980), sometimes increased numbers of IgA, IgG, IgM and IgE containing cells in the *lamina propria* (Keren, 1980; Perkkio *et al.*, 1981; Harms, 1982; Stern *et al.*, 1982; Savilahti, 1983; Spreeuwel *et al.*, 1984), increase of goblet cells (Barratt *et al.*, 1978) and oedema in the *lamina propria* (Barratt *et al.*, 1978; Eastham & Walker, 1979). Degeneration of small mucosal blood vessels points to Type III reactions (Parish, 1983). None of the biopsy findings is specific for food allergy (Keren, 1980; Ferguson, 1984) but patterns of sign and symptom-complexes have been described in children as more or less characteristic for allergy for defined food components such as cow's milk or gluten (Harms, 1982; Stern *et al.*, 1982; Walker-Smith *et al.*, 1984).

## DIAGNOSIS

The classical criteria to confirm the diagnosis of milk protein allergy in children have been as follows:

1. Symptoms subside after dietary elimination of the antigen.
2. Symptoms recur within 48 hours after challenge.
3. Reactions to three such challenges are positive and last for similar durations with similar clinical features (Harms, 1982; Minford *et al.*, 1982).

Evidence that intolerance of a foodstuff is due to immunological reactions is often difficult to obtain (Taylor, 1975). The above criteria have been redefined as:

- a. identification of the antigen.
- b. demonstration of a relationship between exposure of the antigen and occurrence of the reaction and

c. discovery of the immunological mechanism involved (Atkins and Metcalfe, 1984).

As no single test is available to confirm or refute the presence of food allergy, a tentative diagnosis must be supported by information from testing procedures and by ruling out other causes (Atkins & Metcalfe, 1984). Many testing procedures have been proposed including direct skin testing with food antigens (Katz *et al.*, 1984; Amlot *et al.*, 1985), or the Prausnitz-Küstner test (Taylor, 1975), oral challenge if not too damaging and elimination diets (Minford *et al.*, 1982; Ferguson, 1984). Nasal provocation tests have been shown to be a sensitive measure of Type I allergy to defined foods in patients with a positive skin prick test (Amlot *et al.*, 1985). Furthermore, a number of supporting *in vitro* tests have been described such as release of histamine from sensitized basophils or gastric biopsies after exposure to antigen (Reimann *et al.*, 1985), determination of serum levels of IgE, IgG, IgA and IgM antibodies and of circulating immune complexes or activated complement factors (Ferguson, 1984). The radioallergosorbent test (RAST) or an enzyme linked immunosorbent assay (ELISA) are often used for determination of IgE (Atkins & Metcalfe, 1984; Coombs & McLaughlan, 1984; Katz *et al.*, 1984).

Tests for cell-mediated immunity based on secretion of the lymphokine Leucocyte migration Inhibition Factor (LIF) have been proposed but tests for proliferative activity of lymphoid cells in response to food allergens are not useful (Ferguson, 1984). Gastro-intestinal biopsies with subsequent morphometric and immunohistochemical studies (Vitoria *et al.*, 1979; Perlman, 1980; Atkins & Metcalfe, 1984; Ferguson, 1984; Katz *et al.*, 1984; Forget *et al.*, 1985) are often appropriate. For local tissue hypersensitivity isolated intestinal epithelium can be used in an Ussing chamber; on challenge with specific antigen under short circuit current conditions a transient inward flowing current can be measured (Coombs & McLaughlan, 1984). Finally, a possible role as a diagnostic tool has been suggested for disodium cromoglycate which appears to be effective in some individuals with food allergic disease (Walker-Smith *et al.*, 1984).

The diversity of tests may indicate the difficulties for the diagnostician; most tests are not reliable individually, in that they may be positive in controls as well or they are positive in only some of the patients. The best method to establish a diagnosis of food intolerance is said to be repeated intestinal biopsies before and twenty-four hours after an acute challenge (Vitoria *et al.*, 1979) but, apart from ethical considerations, the immunological character of the reaction cannot be proved in this way (Ferguson, 1984). Furthermore, there is often no correlation between the severity of histological damage of the mucosa and the clinical symptomatology, although possibly such mucosal damage may result in malabsorption in due time (Liu *et al.*, 1977; Vitoria *et al.*, 1979; Lessof, 1983; Ferguson, 1984). Mucosal lesions may result in loss of membrane bound digestive enzymes such as disaccharidases (Holzel, 1975; Vitoria *et al.*, 1979; Harms, 1982; Lessof, 1983; Katz *et al.*, 1984). The resulting insufficient digestive capacity may also be used for diagnosis although it is certainly not specific (Kuitunen *et al.*, 1975; Ferguson, 1984; Fisher *et al.*, 1984; McDonald *et al.*, 1984).

In large domestic animals intestinal biopsies (Kilshaw & Slade, 1982; Sissons *et al.*, 1984) and several immunologic methods have been used to make a diagnosis of food allergy. In preruminant calves most work has been done to show serum antibodies (IgG, IgA, IgM) directed against soya constituents using the passive haemagglutination test (Barratt *et al.*, 1978; Barratt & Porter, 1979; Kilshaw & Sissons, 1979a, b; Kilshaw, 1981) or enzyme immunoassays (Kilshaw & Slade, 1980; Kilshaw, 1981). The Prausnitz-Küstner test for passive cutaneous anaphylaxis was used to detect IgE against soya (Barratt & Porter, 1979; Kilshaw & Sissons, 1979a, b; Kilshaw, 1981).

## THERAPY AND PREVENTION

Food allergic diseases may be prevented at four stages (Soothill, 1984). Firstly, avoidance of antigens to which an individual has been sensitised; secondly, the entrance of antigens may be prevented by management of the mucosal barrier; thirdly, the damaging state of sensitization may be modified by hyposensitization, although this has not been proven for food allergy in controlled trials; and lastly, the damaging sensitization may be avoided by preventing primary contact with an antigen in a period of high risk (eg the neonatal period or during attack of viral or bacterial gastro-enteritis).

In practice, the most important measure is the strict avoidance of the allergen or the food component which is known to be the cause of the allergy (Atkins & Metcalfe, 1984; Businco *et al.*, 1984; Ferguson, 1984). In immediate allergic reactions the identification of the allergens is more often possible than in intermediate or delayed reactions (Atkins & Metcalfe, 1984). Another possibility is to manipulate the allergenic food in such a way that the allergenic determinants are lost or to use hypo- or non-allergenic components for the diet (Atkins & Metcalfe, 1984; Businco *et al.*, 1984; Coombs & McLaughlan, 1984). In animals diminished allergenicity of soyabean flour has been achieved by extraction with hot aqueous-ethanol at 65–80 °C (Kilshaw & Sissons, 1979b; Sissons *et al.*, 1982).

For symptomatic treatment of food allergy, various drugs have been used including steroidal and non-steroidal anti-inflammatory agents and antihistamines (Lessof, 1983; Atkins & Metcalfe, 1984). Steroids may be indicated in severe cases of chronic food allergy (Eade & Wright, 1976; Harms, 1982; Atkins & Metcalfe, 1984; Panush & Webster, 1985). The effect of non-steroidal anti-inflammatory drugs such as aspirin is presumably non-specific, working by inhibition of prostaglandin synthesis (Lessof, 1983; Hill *et al.*, 1984; Panush & Webster, 1985). Antihistamines have been widely used in food allergy, especially disodium cromoglycate (Lessof, 1983; Atkins & Metcalfe, 1984; Businco *et al.*, 1984; Panush & Webster, 1985).

Beneficial effects of cromoglycate have been described for alimentary (Eade & Wright, 1976; Lessof, 1983; Atkins & Metcalfe, 1984; Businco *et al.*, 1984; Hill *et al.*, 1984) and gastrointestinal food allergy (Taylor, 1975; Lessof, 1983; Stefanini *et al.*, 1986) but its effect is not generally accepted for the gastrointestinal form (Atkins & Metcalfe, 1984; Panush & Webster, 1985). Cromoglycate can prevent the release of mediators from connective tissue mast cells in many but not all species (Kallós & Kallós, 1982). It appears to be ineffective in preventing mediator release from mucosal mast cells in rats (Pearce *et al.*, 1982) but it reduces *in vitro* mast cell degranulation in human jejunal mucosa after antigenic challenge (Selbekk, 1979). In calves with gastrointestinal allergy to soya bean protein no beneficial effect of cromoglycate has been recorded (Kilshaw & Sissons, 1979a). However, a decreased occurrence of gastrointestinal disturbances was noted in young veal calves after administration of cromoglycate during the fifth to twelfth day after arrival on a farm (Fleddéus *et al.*, 1985).

## CONCLUSIONS

In man, especially in children, food allergy is an important cause of gastrointestinal disorders, of which diarrhoea, vomiting and abdominal pain are the most obvious symptoms.

In animals, research concerning gastrointestinal disorders has so far been directed mainly to infectious agents. These may participate in the aetiology of gastrointestinal disorders but in many cases no infectious agent can be defined as the primary cause.

In this review indications are presented that food allergy may play a primary role in gastrointestinal disorders in animals, especially in calves and piglets. The early substitu-

tion of artificial diets for mother's milk may enable the uptake of foreign, antigenic macro-molecules leading to allergic manifestations, impairment of gastrointestinal function and so to further uptake of antigens and possibly subsequent infections. Further investigations on food allergy in large domestic animals, including its significance in gastrointestinal disturbances, its pathogenesis and its therapy and prevention are needed.

## REFERENCES

- Atkins, F. M. & Metcalfe, D. D., 1984. The diagnosis and treatment of food allergy. *Ann. Rev. Nutr.*, **4**, 233–255
- Amlot, P. L., Urbanek, R., Youlten, L. J. F., Kemeny, M. & Lessof, M. H., 1985. Type I allergy to egg and milk proteins: comparison of skin prick tests with nasal, buccal and gastric provocation tests. *Int. Archs. Allergy Appl. Immun.*, **77**, 171–173
- Barratt, M. E. J. & Porter, P., 1979. Immunoglobulin classes implicated in intestinal disturbances of calves associated with soya protein antigens. *J. Immunol.*, **123**, 676–680
- Barratt, M. E. J., Strachan, P. J. & Porter, P., 1978. Antibody mechanisms implicated in digestive disturbances following ingestion of soya protein in calves and piglets. *Clin. Exp. Immunol.*, **31**, 305–312
- Bloch, K. J., Lake, A. M., Sinclair, K. & Walker, W. A., 1981. IgE-mediated alterations in intestinal permeability and mucus release. In: P. L. Ogra & J. Bienenstock (eds), *The mucosal immune system in health and disease. Report of the eighty-first Ross conference on pediatric research*, (Ross Laboratories, Columbus, Ohio), 109–114
- Bock, S. A. & May, C. D., 1981. True manifestations of sensitivity reactions to food and the associated immunologic findings. In: P. L. Ogra & J. Bienenstock (eds), *The mucosal immune system in health and disease. Report of the eighty-first Ross conference on pediatric research*, (Ross Laboratories, Columbus, Ohio), 114–118
- Brandtzaeg, P., 1985. Research in gastrointestinal immunology. State of the art. *Scand. J. Gastroenterol.*, **20** (supplement 114), 137–156
- Businco, L., Benincori, N., & Cantani, A., 1984. Epidemiology, incidence and clinical aspects of food allergy. *Ann. Allergy*, **53**, 615–622
- Businco, L. & Cantani, A., 1984. Prevention of atopy—current concepts and personal experience. *Clin. Rev. Allergy*, **2**, 107–123
- Coombs, R. R. A. & McLaughlan, P., 1984. Allergenicity of food proteins and its possible modification. *Ann. Allergy*, **53**, 592–596
- Dukor, P., Kallós, P., Schlumberger, H. D. & West, G. B., 1980. Introduction. In: P. Dukor, P. Kallós, H. D. Schlumberger & G. B. West (eds), *PAR. Pseudo-allergic reactions. Involvement of drugs and chemicals, vol. 1: genetic aspects and anaphylactoid reactions*, (Karger, Basel), ix–xiv
- Eade, O. E. & Wright, R., 1976. Dietary hypersensitivity: a gastroenterologist's view. *J. Hum. Nutr.*, **30**, 157–163
- Eastham, E. J. & Walker, W. A., 1979. Adverse effects of milk formula ingestion on the gastrointestinal tract. *Gastroenterology*, **76**, 365–374
- Egberts, H. J. A., Brinkhoff, M. G. M., Mouwen, J. M. V. M., Dijk, J. E. van & Koninkx, J. F. J. G., 1985. Biology and pathology of the intestinal M-cell. A review. *Vet. Quart.*, **7**, 333–336
- Egberts, H. J. A., Koninkx, J. F. J. G., Dijk, J. E. van & Mouwen, J. M. V. M., 1984. Biological and pathobiological aspects of the glycocalyx of the small intestinal epithelium. A review. *Vet. Quart.*, **6**, 186–199
- Elson, C. O., 1985. Induction and control of the gastrointestinal immune system. *Scand. J. Gastroenterol.*, **20** (supplement 114), 1–15
- Fárah, D. A., Calder, J., Benson, L. & Mackenzie, J. F., 1985. Specific food intolerance: its place as a cause of gastrointestinal symptoms. *Gut*, **26**, 164–168
- Ferguson, A. C., 1984. Food allergy. *Prog. Food Nutr. Sci.*, **8**, 77–107
- Ferguson, A., Mowat, A. & Logan, R. F. A., 1981. Mucosal cell-mediated immunity to dietary antigens. In: P. L. Ogra & J. Bienenstock (eds), *The mucosal immune system in health and disease. Report of the eighty-first Ross conference on pediatric research*. (Ross Laboratories, Columbus, Ohio), 128–134
- Fisher, S. E., Markowitz, J. & Lifshitz, F., 1984. Food intolerance in childhood. *Compreh. Therapy*, **10**, 5–11
- Fledderus, A., Dijk, J. E. van, Mouwen, J. M. V. M. & Holzhauer, C., 1985. Prevention of intestinal disturbances using disodium cromoglycate in veal calves. *Vet. Rec.*, **117**, 583–583

- Forget, P., Arends, J. W. & Zandvoort, R. van, 1985. Immunoglobuline E in dunne darmmucosa; een kwantitatieve analyse. *Tijdschr. Kindergeneesk.*, **53**, 56–59
- Harms, H. K., 1982. Die Kuhmilchproteinabhängige Darmkrankheit des jungen Säuglings, eine Form der Kuhmilchproteinintoleranz. *Klin. Pädiat.*, **194**, 375–380
- Hill, D. J., Ford, R. P. K., Shelton, M. J. & Hosking, C. S., 1984. A study of 100 infants and young children with cow's milk allergy. *Clin. Rev. Allergy*, **2**, 125–142
- Holzel, A., 1975. Cow's milk allergy—some clinical and immunological aspects. *Postgrad. Med. J.*, **51** (supplement 3), 71–75
- Jewell, D. P. & Patel, C., 1985. Immunology of inflammatory bowel disease. *Scand. J. Gastroenterol.*, **20** (supplement 114), 119–126
- Kallós, P. & Kallós, L., 1980. Histamine and some other mediators of pseudo-allergic reactions. In: P. Dukor, P. Kallós, H. D. Schlumberger & G. B. West (eds), *PAR. Pseudo-allergic reactions. Involvement of drugs and chemicals, vol. 1* (Karger, Basel), 28–55
- Kallós, P. & Kallós, L., 1982. Pseudo-allergic reactions due to disodium cromoglycate. In: P. Dukor, P. Kallós, H. D. Schlumberger & G. B. West (eds), *PAR. Pseudo-allergic reactions. Involvement of drugs and chemicals, vol. 3: Cell mediated reactions. Miscellaneous topics*, (Karger, Basel), 122–132
- Katz, A. J., Twarog, F. J., Zeiger, R. S. & Falchuk, Z. M., 1984. Milk-sensitive and eosinophilic gastroenteropathy: Similar clinical features with contrasting mechanisms and clinical course. *J. Allergy Clin. Immunol.*, **74**, 72–78
- Keren, D. F., 1980. *Immunology and immunopathology of the gastrointestinal tract*. (Educational products division, Amer. Soc. of Clinical Pathologists, Chicago), 61–73, 96–102
- Kilshaw, P. J., 1981. Gastrointestinal hypersensitivity in the preruminant calf. In: F. J. Bourne (ed), *The mucosal immune system. Current topics in veterinary medicine and animal science*, **12**, 203–223
- Kilshaw, P. J. & Sissons, J. W., 1979a. Gastrointestinal allergy to soyabean protein in preruminant calves. Antibody production and digestive disturbances in calves fed heated soyabean flour. *Res. Vet. Sci.*, **27**, 361–365
- Kilshaw, P. J. & Sissons, J. W., 1979b. Gastrointestinal allergy to soyabean protein in preruminant calves. Allergenic constituents of soyabean products. *Res. Vet. Sci.*, **27**, 366–371
- Kilshaw, P. J. & Slade, H., 1980. Passage of ingested protein into the blood during gastrointestinal hypersensitivity reactions: experiments in the preruminant calf. *Clin. Exp. Immunol.*, **41**, 575–582
- Kilshaw, P. J. & Slade, H., 1982. Villus atrophy and crypt elongation in the small intestine of preruminant calves fed with heated soyabean flour or wheat gluten. *Res. Vet. Sci.*, **33**, 305–308
- Kuitunen, P., Visakorpi, J. K., Savilahti, E. & Pelkonen, P., 1975. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. *Arch. Dis. Child.*, **50**, 351–356
- Lessof, M. H., 1983. Food intolerance and allergy—a review. *Quarterly J. Med.*, **52**, 111–119
- Liu, H. Y., Giday, Zs. & Moore, B. F., 1977. Possible pathogenetic mechanisms producing bovine milk protein inducible malabsorption: a hypothesis. *Ann. Allergy*, **39**, 1–7
- Marsh, M. N., 1985. Functional and structural aspects of the epithelial lymphocyte, with implications for coeliac disease and tropical sprue. *Scand. J. Gastroenterol.*, **20** (supplement 114), 55–75
- McDonald, P. J., Goldblum, M., Sickle, G. J. & Powell, G. K., 1984. Food protein induced enterocolitis: altered antibody response to ingested antigen. *Pediatr. Res.*, **18**, 751–755
- Miller, B. G., Newby, T. J., Stokes, C. R., Hampson, D. & Bourne, F. J., 1983. The role of dietary antigen in the aetiology of post weaning diarrhoea. *Annl. Rech. Vet.*, **14**, 487–492
- Miller, B. G., Newby, T. J., Stokes, C. R. & Bourne, F. J., 1984. Influence of diet on post weaning malabsorption and diarrhoea in the pig. *Res. Vet. Sci.*, **36**, 187–193
- Minford, A. M. B., MacDonald, A. & Littlewood, J. M., 1982. Food intolerance and food allergy in children: a review of 68 cases. *Arch. Dis. Child.*, **57**, 742–747
- Mouwen, J. M. V. M., Egberts, H. J. A. & Koninkx, J. F. J. G., 1983. The outermost mucosal barrier of the mammalian small intestine. *Dtsch. Tierärztl. Wschr.*, **90**, 477–482
- Mowat, A. McL., 1984. The immunopathogenesis of food-sensitive enteropathies. In: T. J. Newby & C. R. Stokes (eds), *Local immune responses of the gut*, (CRC Press., Boca Raton, Florida), 199–225
- Newby, T. J., Miller, B., Stokes, C. R. & Bourne, F. J., 1983. Hypersensitivity to dietary antigens as the predisposing factor in post-weaning diarrhoea. *Pig Veter. Soc. Proceed.*, **10**, 50–58
- Pahud, J. J. & Schwarz, K., 1984. Research and development of infant formulae with reduced allergenic properties. *Ann. Allergy*, **53**, 609–614

- Panush, R. S. & Webster, E. M., 1985. Food allergies and other adverse reactions to foods. *Med. Clin. North. Am.*, **69**, 533–546
- Parish, W. E., 1983. Intolerance and allergy to foods and food additives: its relevance to toxicology. In: D. M. Conning & A. B. G. Lansdown (eds), *Toxic hazards in food*, (Croom Helm Ltd., London & Canberra), 22–72
- Pearce, F. L., Befus, A. D., Gauldie, J. & Bienenstock, J., 1982. Mucosal mast cells. II. Effects of anti-allergic compounds on histamine secretion by isolated intestinal mast cells. *J. Immunol.*, **128**, 2481–2486
- Perkkiö, M., Savilahti, E. & Kuitunen, P., 1981. Semi-quantitative analysis of immunoglobulins and complement fractions 3 and 4 in the jejunal mucosa in coeliac disease and in food allergy in childhood. *Acta. path. microbiol. Scand. Sect. A.*, **89**, 343–350
- Perlman, F., 1980. Allergens. In: J. E. Liener (ed), *Toxic constituents of plant foodstuffs*, (Academic Press, New York), 295–327.
- Porter, P., Allen, W. D., Barratt, M. E. J., Davis, P. J. & Linggood, M. A., 1984. Relationships between the immune system and intestinal disease. In: R. M. Batt & T. L. J. Lawrence (eds), *Function and dysfunction of the small intestine*, (Liverpool University Press, Liverpool), 133–148
- Reimann, H. J., Ring, J., Ultsch, B. & Wendt, P., 1985. Intra-gastral provocation under endoscopic control (IPEC) in food allergy: mast cell and histamine changes in gastric mucosa. *Clin. Allergy*, **15**, 195–202
- Roitt, J. M., Brostoff, J. & Male, D. K., 1985. *Immunology*, (Gower Medical Publishing Ltd., London), ch. 4, 8, 10, 19
- Roy, J. H. B., 1984. Dietary sensitivities in the calf. In: R. M. Batt and T. L. J. Lawrence (eds), *Function and dysfunction of the small intestine*, (Liverpool University Press, Liverpool), 95–132
- Savilahti, E., 1973. Immunochemical study of the malabsorption syndrome with cow's milk intolerance. *Gut*, **14**, 491–501
- Selbekk, B. H., 1979. The effect of disodium cromoglycate on in vitro mast cell degranulation in human jejunal mucosa. *Allergy*, **34**, 283–288
- Selbekk, B. H., 1985. A comparison between in vitro jejunal mast cell degranulation and intra-gastric challenge in patients with suspected food intolerance. *Scand. J. Gastroenterol.*, **20**, 299–303
- Sissons, J. W., Pedersen, H. E. & Wells, K., 1984. Mucosal morphology in the alimentary tract of calves given heated soya bean flour. *Proceed. Nutr. Soc.*, **43**, 113A
- Sissons, J. W. & Smith, R. H., 1976. The effect of different diets including those containing soya-bean products, on digesta movement and water and nitrogen absorption in the small intestine of the pre-ruminant calf. *Br. J. Nutr.*, **36**, 421–438
- Sissons, J. W., Smith, R. H. & Hewitt, D., 1982. Prediction of the suitability of soya bean products for feeding to preruminant calves by an in-vitro immunochemical method. *Br. J. Nutr.*, **47**, 311–318
- Soothill, J. F., 1984. Prevention of food allergic disease. *Ann. Allergy*, **53**, 689–691
- Spreeuwel, J. P. van, Lindeman, J., Maanen, J. van & Meyer, C. J. L. M., 1984. Increased numbers of IgE containing cells in gastric and duodenal biopsies. an expression of food allergy secondary to chronic inflammation? *J. Clin. Pathol.*, **37**, 601–606
- Stefanini, G. F., Bazzocchi, G., Prati, E., Lanfranchi, G. A. & Gasbarrini, G., 1986. Efficacy of oral disodium cromoglycate in patients with irritable bowel syndrome and positive skin prick tests to foods (letter). *Lancet*, **8474**, 207–208
- Stern, M., Dietrich, R. & Müller, J., 1982. Small intestinal mucosa in coeliac disease and cow's milk protein intolerance: morphometric and immunofluorescent studies. *Eur. J. Pediatr.*, **139**, 101–105
- Stokes, C. R., Newby, T. J., Miller, B. G. & Bourne, F. J., 1984. The induction of gut damage as a result of a transient immune hypersensitivity to dietary antigens. *Proceed. Nutr. Soc.*, **43**, 117A
- Sutanto, A. H., 1982. Cow's milk protein sensitive enteropathy. Clinical and histological features in infants. *Paed. Indones.*, **22**, 65–69
- Taylor, K. B., 1975. Alimentary and gastrointestinal allergy. In: L. van der Reis (ed), *Immune disorders. Front. gastrointest. res.*, **1** (Karger, Basel), 1–11
- Vellenga, L., Mouwen, J. M. V. M., Dijk, J. E. van & Breukink, H. J., 1985. Biological and pathological aspects of the mammalian small intestinal permeability to macromolecules. *Vet. Quart.*, **7**, 322–332
- Visakorpi, J. K., 1980. Nahrungsmittelintoleranz im Säuglingsalter. *Monatsschr. Kinderheilkd.*, **128**, 166–169
- Vitoria, J. C., Camarero, C., Solaguren, R., Aranjuelo, M., Oliveros, R., Navajas, A. &

- Rodriguez-Soriano, J., 1979. Cow's milk protein-sensitive enteropathy. Clinical and histological results of the cow's milk provocation test. *Helv. paediat. Acta*, **34**, 309–318
- Walker, W. A., 1981. Intestinal transport of macromolecules. In: L. R. Johnson (ed), *Physiology of the gastrointestinal tract*, (Raven Press, New York), 1271–1289
- Walker-Smith, J. A., Ford, R. P. K. & Phillips, A. D., 1984. The spectrum of gastrointestinal allergies of food. *Ann. Allergy*, **53**, 629–636
- Weström, B. R., Svendsen, J., Ohlsson, B. G., Tagesson, C. & Karlsson, B. W., 1984. Intestinal transmission of macromolecules (BSA and FITC-labelled dextrans) in the neonatal pig. *Biol. Neonate.*, **46**, 20–26
- Wilders, M. M., Sminia, T. & Janse, E. M., 1983a. Ontogeny of non-lymfoid and lymfoid cells in the rat gut with special reference to large mononuclear Ia-positive dendritic cells. *Immunology*, **50**, 303–314
- Wilders, M. M., Drexhage, H. A., Weltevreden, E. F., Mullink, H., Duyvestijn, A. & Meuwissen, G. M., 1983b. Large mononuclear Ia-positive veiled cells in Peyer's patches. I. Isolation and characterization in rat, guinea-pig and pig. *Immunology*, **48**, 453–460
- Wilders, M. M., Sminia, T., Plesch, B. E. C., Drexhage, H. A., Weltevreden, E. F. & Meuwissen, S. G. M., 1983c. Large mononuclear Ia-positive veiled cells in Peyer's patches. II. Localization in rat Peyer's patches. *Immunology*, **48**, 461–467
- Willemsse, A., 1984. *Investigations on canine atopic dermatitis*. (Ph.D. Thesis, State University Utrecht).

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