AN EVALUATION OF THE POTENTIAL FOR DIETARY PROTEINS TO CONTRIBUTE TO SYSTEMIC DISEASES

September 1982

Prepared for
BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under
Contract Number FDA 223-79-2275
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edited by
Richard G. Allison, Ph.D.

LIFE SCIENCES RESEARCH OFFICE
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FOR EXPERIMENTAL BIOLOGY
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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was developed for the Food and Drug Administration (FDA) in accordance with the provisions of Contract No. FDA 223-79-2275. It was prepared and edited by Richard G. Allison, Ph.D., Senior Staff Scientist, LSRO, FASEB.

The LSRO acknowledges the contributions of the investigators and consultants who assisted with this study. The report reflects the opinions expressed by an ad hoc study group that met at the Federation on January 11-12, 1982. The study participants reviewed a draft of the report and their various viewpoints were incorporated into the final report. The study participants and LSRO accept responsibility for the accuracy of the report; however, the listing of these individuals in Section XI does not imply that they specifically endorse each study conclusion.

The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

September 17, 1982
(date)

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
SUMMARY

This report summarizes the discussions of an ad hoc review group concerning the potential contribution of dietary proteins to certain systemic diseases. Conflicting reports exist concerning the absorption of proteins from the gastrointestinal tract and the effects dietary proteins might exert on health by functioning as antigens or otherwise stimulating the immune system. This report considers the sources of dietary proteins, commercial food processing techniques, properties of dietary proteins, and their respective effects on absorption, digestion, and disposition of ingested proteins.

The small quantity of intraluminal dietary proteins that is incompletely digested and therefore available for transport or diffusion across the gastrointestinal epithelium is of possible immunologic rather than nutritional significance. Most available data support the hypothesis that membranous epithelial cells (M cells) are important sites for limited absorption and exposure of protein antigens to the mucosal immune system of human beings. The luminal surfaces of columnar epithelial cells appear to adsorb fewer types of antigens than do M cells. Conditions affecting the integrity and cell viability of the intestinal wall such as inflammation, ulceration, and malnutrition result in increased uptake of antigens. Hypersensitivity reactions to one food protein within the gastrointestinal tract may contribute to the nonspecific absorption of others. Factors favoring increased uptake of large, antigenically intact proteins include resistance to proteolysis, stability to acid conditions, high intraluminal concentration, and an ability to interact with the luminal surfaces of gut mucosal cells.

The presence of circulating antibodies to food antigens is presumptive evidence that antigens have traversed the mucosal epithelial cells and stimulated antibody production within the mucosal lymphatic system. A phenomenon termed oral tolerance or hyporesponsiveness characterizes an active suppression of subsequent responses of the systemic immune system to an antigen after that antigen has been ingested. Ingesting an antigen can promote an IgA response by the mucosal immune system and induce suppression of an IgG response by the systemic immune system. Several forms of suppression appear to be involved in oral tolerance depending on the nature of the antigen and the test system used. Investigations on the mechanisms of tolerance induction are of potential importance in understanding the immunologic and physiologic responses to food proteins. Additional research is required to define a role, if any, for oral unresponsiveness in normal protein digestion or in adaptation to new food sources.
Dietary antigens in complexes with any of several immunoglobulin types may occur periodically in a significant proportion of serum samples from an otherwise healthy population. Some individuals develop abnormally high concentrations of immune complexes after ingesting a meal. Because circulating immune complexes often occur in extremely low concentrations, their antigen composition is difficult to determine using conventional physical and chemical techniques. The assay and identification of immune complexes is a controversial area and the ad hoc review group suggested caution in interpretation of experimental results until methodology is more firmly established. Both the possible role of immune complexes in diseases and the existence of immune complexes containing food antigens are open to question in some instances. Mechanisms for removing complexes from the systemic circulation may involve specific cell surface receptors (for Fc-IgG and C3) present on macrophages as well as a liver transport system that can transfer complexes containing dimeric IgA to the bile. In addition to the type of antibody in the immune complex, antigen structure itself can influence disposition of the complex. The ad hoc group concluded that there was still no firm evidence that immune complexes containing food proteins play a role in pathogenesis of systemic diseases.

Evidence from experimental animal studies and from patients with gluten-sensitive enteropathy, selective IgA deficiency, Berger's disease, various food sensitivities, and systemic sicca syndrome indicate that the interaction of antigenic materials including food proteins with the mucosal immune system may be an important component of certain diseases. However, with the possible exception of gluten-sensitive enteropathy, these interactions have not been shown to involve a specific protein.

Additional conclusions and suggestions for future research on topics discussed by the ad hoc review group are provided.
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I. INTRODUCTION

A. BACKGROUND

The Food and Drug Administration (FDA) is responsible for evaluating and monitoring the safety of foods, establishing regulations, and providing information on nutrition to consumers. The Food and Drug Administration (1981) has identified several emerging technologies that may affect future processing, distribution, and source of food proteins. The sources of proteins in the U.S. are diverse and provide a variety of antigenic materials. Both clinical and experimental observations suggest that age- and health-dependent interrelationships between nutritional and immunologic states influence appropriate physiologic response to ingested antigens (U.S. Department of Health, Education and Welfare, 1976). There are recognized numerous dietary substances that may inadvertently affect the immune system (Asher, 1978). Conflicting reports exist in the scientific literature concerning the potential for dietary proteins to contribute to certain systemic diseases through immunologic mechanisms. Because a rapidly expanding knowledge of immunologic mechanisms offered a promise that a review of available data and scientific opinion might resolve conflicting reports and assist the agency in ensuring the nutritional quality and safety of the food supply, FDA requested the Life Sciences Research Office (LSRO) to conduct such a review. LSRO convened an ad hoc review group on January 11th and 12th, 1982, to discuss the interaction of dietary proteins with the immune system in health and disease.

B. SCOPE OF THE STUDY

The primary objective of this study is to identify potential interrelationships of dietary proteins and systemic diseases, particularly those effects mediated by the immune system through immune complexes. If such interrelationships exist, then available data might suggest or provide evidence of antigen absorption, production of circulating specific antibodies, formation of antibody and antigen complexes, presence of antigens in tissues, and subsequent pathology at sites of antigen deposition. Each of these aspects represents a quite broad area of continuing research; original references and review articles cited under major headings should be consulted for additional detailed data and clarification. An attempt has been made to focus on the interaction of proteins with the mucosal immune system. Bienenstock and Befus (1980) have reviewed current understanding of the components and regulation of mucosal immunology, highlighting recent advances and concepts they believe merit additional development.

It is well recognized that a number of adverse reactions to foods and food constituents are acute or may involve processes other than immunologic responses to ingested proteins. Adverse
reactions to foods of an acute nature, which are referred to as food allergies, and possible long-term effects resulting from reduced nutrient bioavailability were considered outside the scope of this study.

In addressing the primary objective of this study, the following questions were discussed by the ad hoc review group:

- What is the current understanding of normal digestion and absorption of proteins and what effects do protein source and structure have on these processes?

- Are there significant differences in the digestion products of proteins from different sources?

- What role, if any, does the immune system play in normal protein digestion and absorption?

- Is there any basis for attributing adverse effects, however mild, to the protein constituents of common foods including grains, beans, milk, eggs, fish, and nuts?

- Do epidemiologic data show an association between dietary history of protein source and particular diseases? To what extent is knowledge of biological mechanisms congruent with these data?

- Does exposure to proteins of different sources during infancy influence development of the immune system or effect tolerance to dietary proteins in later years?

- When interacting with food proteins, what are the specific roles of gut-associated-lymphoid tissue (GALT) and other cells known to be important in immune responses?

Many of the issues identified through discussions by the ad hoc group participants are highlighted in the report; however, no attempt is made to develop comprehensive answers to each question. Where information is inadequate, suggestions for further research are developed.
II. SOURCES OF DIETARY PROTEINS

Infants ingest proteins from few sources, usually mother's milk or a commercial formula based on bovine milk or soybean proteins. The sources of proteins in the diets of older children and adults are diverse and continually changing. In the United States, dietary protein derives from meat, poultry, and fish (42.3%); dairy products (21.2%); flour and cereal products (18.6%); dry beans, peas, nuts, and soya products (5.4%); vegetables (6.2%); eggs (4.9%); and fruits and miscellaneous sources (1.4%) (U.S. Department of Agriculture, 1981). Thus, the majority of dietary proteins are from heterologous species and theoretically capable of eliciting immune responses (Parker, 1977).

Commercially processed proteins used as food ingredients are obtained from few sources: casein and whey from bovine milk, gelatin and collagen from animal hides and tendons, soy protein isolates from soybeans, and gluten from the flours of wheat, corn, and oats (Satterlee, 1981; Select Committee on GRAS Substances, 1975; 1978; 1979; 1980; 1981a,b,c). Functional, sensory, nutritional, and economic factors play primary roles in the selection of proteins for the majority of commercial food formulations. Immunologic considerations have increased importance when formulating infant formulas, enteral nutrient mixtures, hypoallergenic foods, and other foods for special dietary purposes.

New sources of proteins or proteins apt to receive wider distribution in the diet include those from yeasts and the oilseeds: soybean, peanut, rapeseed, cottonseed, sunflower, and safflower (Kinsella, 1982). For example, a stable "peanut milk" product has been produced (Willhite, 1979). Protein hydrolysates, manufactured by the enzymatic hydrolysis of oilseed proteins, are being used increasingly as functional ingredients (Select Committee on GRAS Substances, 1978; 1980; 1981c). Changes in food processing that may impact on the immunologic nature and biological effects of dietary proteins include the wider application of ultrafiltration as a method for processing and concentrating fluid foods and the ultra-high temperature processing of milk (Kosaric et al., 1981). Chemical modification of food proteins could introduce new antigenic structures as well as decrease digestibility (Brinegar and Kinsella, 1981; Feeney et al., 1982). If highly charged macromolecules interact with cells constituting the intestinal mucosal barrier, greater absorption could result. Experimental models indicate that both positively charged (Ryser et al., 1978) and negatively charged (Hardy, 1969) polypeptides enhance cellular uptake of macromolecules.

Studies in experimental animals have demonstrated that age and dietary history alter immunologic response to certain antigens. For example, soybean-based feeds have been associated with a number of nutritional and other problems in animal feeding.
(e.g., Melmed et al., 1976) and, in some instances, these problems had an immunologic component. Barratt et al. (1979) demonstrated that the soy protein antigen responsible for digestive disturbances in calves was resistant to protein processing involving hot ethanol extraction. This antigenic fraction produced an IgG1-immune mediated inflammatory response of the intestinal mucosa. The interaction of antigen with this antibody fixed complement, leading to intestinal dysfunction. Calves fed the soy diet during their first week of life produced greater antibody titers (concentrations) than those first receiving the diet at 4 weeks of age. The serum antibody response was increased with duration of exposure to the diet. Calves did not gain tolerance to this soy antigen. Animals removed from the soy diet for a period of time by feeding skim milk-based diets and later returned to the soy diet increased their antibody titers as though previous sensitization had occurred.

A well-characterized protein of soybeans, the Kunitz soybean trypsin inhibitor, reacted with a specific IgE antibody in one patient who experienced anaphylactic reactions after ingestion of soybean products (Moroz and Yang, 1980). The investigators suggested that low molecular weight, resistance to digestion, and stability to acid pH are properties that may enhance the absorption and interaction of proteins with the immune system. Soybean trypsin inhibitor fed to adult rats has been demonstrated by immunochemical methods to be adsorbed to the luminal surface of the brush border, particularly in the ileum (Wilson et al., 1978). On the other hand, IgE-mediated responses may exclude the specific antigen while enhancing non-specific absorption of others. Roberts et al. (1981) demonstrated this phenomenon in Hooded Lister rats that produced IgE antibodies against ovalbumin (OVA) and absorbed more β-lactoglobulin from a mixture with OVA than when β-lactoglobulin was administered alone.

The immunologic consequences from introducing new food protein sources (e.g., legume leaf protein concentrate), new processing technologies (food irradiation, chemical sterilization), and changes in traditional foods through emerging technologies (genetic engineering) cannot be predicted easily nor routinely measured. Antigenic properties of proteins depend on many factors. In addition to protein source and processing conditions, the composition of the total diet or of a particular product may influence immunologic response. For example, some pediatricians have questioned whether changes in nonprotein ingredients or processing procedures could inadvertently increase the antigenicity of infant formulas (Eastham and Walker, 1979).
III. ABSORPTION OF PROTEINS

The scientific community holds diverse opinions on mechanisms and quantitative aspects of protein absorption. Walker (1982) has reviewed evidence for the intestinal transport of macromolecules. Many investigators suggest that subtle, possibly adverse, effects result after absorption of intact proteins. Such effects might be exacerbated in infants or the elderly, and by drugs and illness, because of a reduced ability to respond to new or increased systemic doses of antigenic material. Clinical conditions associated with increased uptake of proteins include necrotizing enterocolitis, gastrointestinal allergy, dermatitis, inflammatory bowel disease, nephritis, and immune complex-mediated diseases (Walker, 1981).

Early reports (e.g., Van Alstyne, 1913) suggesting that antigenically intact proteins enter the blood and lymph through the healthy gut wall receive some support from modern immunological and radioisotopic methodology. However, suggestions that the extent of absorption approaches 40% of ingested protein (Hemmings and Williams, 1978) appear to be in sharp contrast with the widely held view that very small quantities, possibly only 0.01% of ingested proteins, are absorbed intact (Ho and Clifford, 1976; Udall et al., 1981a). There is increasing experimental and clinical evidence that incompletely digested intraluminal protein antigens may be transported across the epithelium into the lamina propria or systemic circulation (Walker, 1981). The quantities appear to be of possible immunologic significance, but of little nutritional importance in terms of protein status.

A. SITE OF ABSORPTION

Antigenic molecules are envisioned as potentially crossing the intact gut mucosal barrier at two possible sites: specialized cells of the epithelium overlying GALT and columnar epithelial cells of the villi.

The luminal surface of GALT, e.g., Peyer's patches, is covered with columnar epithelial cells and membranous epithelial cells (M cells). According to Cornes (1965a), the number of Peyer's patches in the human gastrointestinal tract varies from about 60 at 30 weeks' gestation to 240 at puberty. The patches are easily identified early in fetal life (poorly developed before antigen exposure) and tend to increase in both size and number until puberty although there is considerable individual variation in these parameters as well as distribution. Peyer's patches are situated on the anti-mesenteric border, one follicle thick, and intimately associated with the overlying, flat epithelium. After puberty, there is a rapid decline in the number of Peyer's patches to about 150 at age 30 with a further but more gradual decline with increasing age (Cornes, 1965b). With aging, patches become
flatter and gradually lose lymphoid follicles with reversion of the flattened epithelium to a papillary type. These age effects are not observed in some species of experimental animals, suggesting caution in selection of a model system for studying age effects. There is no evidence that the state of the Peyer's patches affects antigen absorption in elderly adults.

In section, M cells are recognized by shortened brush borders and underlying aggregates of lymphocytes within the epithelial cell layer. These cells are present also in the membranes of tonsils, appendix, and other lymphoid nodules of the gut in addition to Peyer's patches. Owen (1977) describes the M cells as extending between adjacent columnar cells of Peyer's patches, forming a membrane separating by approximately 0.3 μm the intestinal lumen from lymphocytes in the epithelial cell layer. Owen (1977) carefully documented with light and electron microscopy the presence of horseradish peroxidase (HRP) in lymphocytes of 2-month-old male, white Swiss mice after a specific absorption sequence: HRP adhered to the M cell surface (also to the surface of columnar cells); adhesion extended into surface "pits" in M cells; HRP appeared in vesicles within the M cells (but not columnar cells); and HRP was released into the extracellular space between the lymphocytes and M cells. The underlying lymphoid cells are medium-sized lymphocytes (possibly T cells), apparently not undifferentiated lymphoblasts or stem cells. Thus, M cells provide a specific site for antigens to pass from the lumen into the intestinal lymphoid system.

A second route of antigen absorption, via the columnar epithelial cells, is greatly influenced by both species and age. Columnar epithelial cells are the site of neonatal immunoglobulin uptake, a process particularly active in neonatal ruminants that receive maternal immunoglobulins by the oral route. The process is less active in rodents that receive immunoglobulins transplacentally and orally. Owen (1977) discussed this second route of absorption in relation to his work on M cells. After entering invaginations of the cell surface between microvilli, proteins or other materials that have been tested appear in vesicles or phagosomes via a tubular network in the apex of the cell. Lipid, HRP, ferritin, metal colloids, and adenoviruses are among the materials transported by this mechanism in experimental models. Lysosomes combine with these vesicles and hydrolyze their contents; however, homologous immunoglobulins are protected by an unknown mechanism. Other unhydrolyzed materials may be extruded from vesicles into extracellular spaces if lysosomal capacity is exceeded.

Some evidence indicates that the membranes of columnar epithelial cells are more selective or bind fewer different ingested materials than do the membranes of M cells. For example, reovirus type 1 was bound selectively to the surface of M cells as opposed to the luminal surface of goblet cells or absorptive, columnar epithelial cells of 10-day-old BALB/c mice (Wolf et al., 1981). The
investigators suggested that reovirus was likely to enter the host via adhering to the M-cell surface, traversing the cell in vesicles after endocytosis, and being released into the extracellular space enveloped by the M cell. Further studies (Wolf et al., 1982) indicate similar selective binding of reovirus type 1 in adult and suckling BALB/cJ and C3H/HeJ mice. In contrast, reovirus type 3 bound to the surface of all luminal epithelial cells. Both serotypes were transported by M cells, but only type 1 was disseminated to mesenteric nodes and spleen. Studies with recombinants suggested that adsorption of reovirus 3 to absorptive cells was a property of the viral hemagglutinin (protein σ1). Type 3 reovirus was found in apical tubules, vesicles, and lysosomes of absorptive cells but evidence of transport was not observed.

Surface invaginations and tubules of the columnar cells observed in neonatal animals probably do not contribute to macromolecular transport in adult animals. The very active, selective transport of immunoglobulin generally ceases after 1 or 2 days of age in ruminants and 2 to 3 weeks in rodents; it plays no prominent role in humans. However, this mechanism may transport immunologically significant amounts of material in adult animals. In the adult rat, vesicles formed from smooth membranes (pinocytotic vesicles) concentrate near the Golgi complex, migrate to the lateral membrane, and extrude their contents. Columnar cells near the distal end of villi of the ileum and jejunum have greater uptake than the less mature cells near the base (Cornell et al., 1971). In experimental animals, M-cell transport of macromolecules presents tubular uptake and vesicular transport more similar to that of neonatal rather than adult columnar cells (Owen, 1977).

The ad hoc group participants concluded most available data support the hypothesis that M cells are important sites for limited absorption of protein antigens in human beings. M cells represent a portal of antigen entry to the mucosal immune system. This "antigen sampling" may represent a source of stimulation necessary for effective proliferation of IgA-producing cells and of the mucosal immune system, but the role of the M cell in developmental sequelae requires further investigation. The route of entry of ingested antigens detected in the systemic circulatory system remains to be demonstrated through experimental observations.

B. ESTIMATION OF PROTEIN UPTAKE

Several in vivo methodologies have been used to estimate the extent of intact protein absorption across the intestinal epithelium. The largest estimates derive from experiments in which radiolabeled proteins are instilled into the intestinal lumen followed by measuring plasma tungstic acid- or trichloroacetic acid-insoluble radioactivity (Hemmings and Williams, 1978). Udall et al. (1981a) demonstrated that digestive fragments of radiolabeled proteins can bind to serum proteins of the test
animal and thus produce overestimates of absorption by simulating macromolecular uptake. The method of radiolabeling can also produce labeled amino acids either free or in small peptides, thus promoting absorption of radioactivity. These investigators cautioned that great care be taken when interpreting absorption data based on extrinsically labeled proteins in comparison to data based on immunochemical detection of absorbed proteins.

The presence of circulating antibodies to specific food proteins indicates exposure rather than existence of a food sensitivity that will correlate with clinical symptoms. For example, normal infants fed (from birth to 112 days) either bovine milk, soy formula, or a formula containing both milk and soy proteins developed serum antibodies specific to the proteins in the corresponding formula and no adverse reactions occurred (May et al., 1980). The titer of antibodies produced to milk proteins by infants at 4 months of age appeared to be significantly greater than that produced to soy proteins. The occurrence of antibodies to milk proteins is 100% in serum of children ingesting milk if their immune systems are functioning normally (Bock, 1980). However, when carefully measured, elevated titers (above normal) of antibodies to milk proteins suggest intestinal disturbance from either food sensitivity or other disorders such as selective IgA deficiency or possibly aspiration of antigens into the lungs (Buckley and Dees, 1969; Lee et al., 1978).

C. THE GUT AS A BARRIER

LeFevre et al. (1979) characterized Peyer's patches as highly "absorptive," permitting the penetration of macromolecules and particulates. However, this penetration, presumably via M cells, is not necessarily complete in that such materials do not necessarily enter the systemic circulation by this route. LeFevre et al. (1979) concluded that most transported antigens are taken up by local macrophages within the Peyer's patches. The weight of evidence is compelling that there are mechanisms that severely restrict the absorption of antigenically intact dietary protein. Most experts agree that the low levels absorbed into the systemic circulation are cleared by normal processes and present no definable hazard (Parker, 1977). On the other hand, evidence from clinical and experimental models of abnormal metabolism should be examined because it may provide insight into potential adverse effects possibly arising from absorption of small amounts of dietary antigens by healthy persons.

In most instances, the combining of intestinal antibodies with antigens in the lumen of the gastrointestinal tract effects decreased antigen absorption (Walker and Isselbacher, 1977). A major exception to this is an Fc receptor-assisted uptake of antigens by the columnar epithelial cells in neonates of certain animal species (Abrahamson et al., 1979). This latter mechanism is not important in humans, and secretory antibodies to ingested antigens
will probably aid exclusion of antigenic materials (bacteria, viruses, and macromolecules) from the systemic circulation. It is not known if factors similar to those that account for the normally enhanced absorption of antigens in neonatal rodents may promote uptake of antigens in human infants under unusual situations or in cases of disease. A relative deficiency of intestinal secretory IgA, an apparent enhanced affinity of antigenic materials and epithelial cells in the immature gut, enhanced pinocytosis of macromolecules, and decreased intraluminal proteolysis are among factors that could promote uptake (Udall et al., 1981b,c). Clinical evidence suggests that selective IgA deficiency may result in greater absorption of antigens (Ammann and Hong, 1971). However, the relative importance of the various immunoglobulin types in preventing absorption of antigens is not clear.

Four hours after bovine serum albumin was fed to breast- and bottle-fed infant rabbits, circulating plasma concentrations of antigenically intact antigen were significantly lower in breast-fed (6.12 ± 0.77 μg/ml) than in bottle-fed (9.19 ± 0.93 μg/ml) 1-week-old rabbits (Udall et al., 1981c). No difference was noted in tests with 2-week-old rabbits, suggesting that breast milk provided passive intestinal barrier factors or promoted earlier maturation of intestinal function.

Cruz et al. (1981) have found antibodies directed against food antigens in the breast milk of mothers who ingested foods containing these antigens. It is possible that breast milk secretory IgA, which is not readily degraded in the intestinal tract, reduces the absorption of ingested antigens by infants; however, data are inadequate on this point. This view presents a passive role for the antigen-antibody complex, but does not exclude additional physiologic effects resulting from absorption of a smaller quantity of antigen. In other words, secretory IgA may reduce the amount of antigen that would otherwise enter the gastrointestinal tract in a free or uncomplexed state, and thus modulate the types of immune responses to repeated ingestion of specific antigenic materials, e.g., favoring an IgA rather than an IgE response.

Secretory IgA antibodies bound to bacterial cell walls interfere with bacterial adherence and may be important in controlling pathogenic as well as normal colonization of the gut (Walker, 1981). Antigens combining with antibodies in the glycocalyx would be restricted from reaching the enterocyte membrane where they could be taken up by pinocytosis. The release of goblet-cell mucus in response to antigen-antibody complex formation within the intestinal lumen may lower or prevent interaction of antigens with the mucosal surface (Walker, 1976). Local antibody response to ingested antigens is thus an important component in limiting the amount of antigenic material absorbed from the lumen of the gastrointestinal tract.
D. GUT ABNORMALITIES

Several conditions associated with gut inflammation and ulceration lead to increased passage of antigens through the gut into the systemic circulation. This is also known to occur in patients having jejunoileal bypass surgery and possibly others with gut permeability defects (Utsinger, 1980). It is generally thought that any condition affecting the integrity and viability of the intestinal wall will result in increased uptake of antigens by nonspecific diffusion rather than pinocytosis (Walker, 1981). This effect has been observed, for example, after ionizing irradiation treatment, administration of antimetabolites, and osmotic loading via tube feeding mixtures.

A number of animal models have been developed that demonstrate gut absorption abnormalities. In one model, rats after jejunoileal bypass, both enhanced absorption and liver dysfunction occur. It has been suggested that malnutrition plays a relatively more important role than does absorption of hepatotoxins produced by bacterial overgrowth in developing this liver dysfunction (Vanderhoof et al., 1980). General malnutrition, as has been shown for protein-deficient rats, could alter the viability of intestinal epithelial cells and the integrity of their junctions, facilitating absorption of antigens via diffusion into intercellular spaces of the epithelium and lamina propria (Worthington and Syrotuck, 1976). In a recent study (Rothman et al., 1982a), malnourished rats transported approximately twice the amount of tritium-labeled large molecular weight fragments of bovine serum albumin compared to controls when transport was expressed relative to body weight. However, when expressed relative to intestinal DNA, the results suggested to the investigators that the mechanism for macromolecular uptake saturated at a lower antigen concentration. Clearance of intravenously injected bovine serum albumin was similar in malnourished and control rats (Rothman et al., 1982b). Thus, there is support for the hypothesis that malnutrition may increase intestinal permeability to macromolecules.

Bloch et al. (1979) have measured nanogram amounts of transported antigens in animals in which intestinal anaphylaxis and flattening of villi have been induced. Intestinal inflammation from infestation with a nematode parasite (Nippostrongylus brasiliensis) or systemic anaphylaxis increased uptake of immunoreactive bovine serum albumin in adult rats. Murray et al. (1971) have suggested that interepithelial spaces permitted proteins from interstitial fluids of the lamina propria to enter the lumen of rats infected with N. brasiliensis.

Bloch and Walker (1981) observed that the association of intraluminal proteins with the wall of the duodenum and other parts of the small intestine was enhanced nonspecifically by local intestinal hypersensitivity, and postulated that such proteins might induce an IgE antibody response and thus increase the range
of anaphylactic sensitivity. Such observations provide evidence of a mechanism by which an IgE sensitivity to one food antigen might contribute to the development of sensitivities to other antigens by mediating change in gut permeability, increasing uptake of antigens from the lumen, induction of additional IgE reactions, and elaboration of local sensitivities.
IV. ORAL TOLERANCE

The presence of circulating antibodies to food antigens is presumptive evidence that antigens have traversed the mucosal epithelial cells and stimulated antibody production within the lymphatic system. In experimental animals, a phenomenon termed oral tolerance or hyporesponsiveness actively suppresses the development of systemic antibodies against soluble protein antigens that have been ingested as a single, large dose or repeated feedings of smaller doses (Tomasi, 1980). Experimental evidence provided by studies such as those by André et al. (1975) and Chalon et al. (1979) supports the hypothesis that circulating suppressor factors can produce systemic tolerance to specific antigens after immunization by the digestive route. Although the mechanisms controlling tolerance remain undescribed, experimental results demonstrate differences in the immune response after enteric and parenteral exposure to antigens. The antigen-presenting cells in the spleen appear to function differently than those of the mucosal immune system (Woloschak and Tomasi, 1982). Enteric exposure to antigenic proteins is often associated with subsequent systemic unresponsiveness, whereas prior systemic exposure usually enhances systemic responsiveness. Conversely, prior parenteral sensitization may eliminate development of tolerance to subsequent oral administration of the same antigen.

When OVA is fed to mice, an antigen-specific suppression of the systemic immune response is induced (Hanson et al., 1979a). This phenomenon is associated with suppressor T cells activated by the oral exposure to the antigen. When these cells are transferred to histocompatible mice that have not been fed OVA (adoptive transfer), the recipient mice are tolerized as well (Ngan and Kind, 1978; Richman et al., 1978; 1981; Titus and Chiller, 1981). Other aspects of oral tolerance have been revealed in normal mouse strains by feeding sheep red blood cells (SRBC). Oral exposure to this particular antigen also induced a profound suppression of subsequent systemic responses. In this model, no suppressor cells have been demonstrated in mice although they have been shown in rats receiving SRBC (Mattingly and Waksman, 1978). Induced factors suppressed antibody production both in vivo and in vitro (Chalon et al., 1979; Kagnoff, 1978b). However, Kagnoff (1978a) found that serum factors of SRBC-fed mice were incapable of transferring inhibition of delayed-type hypersensitivity reactions (DTH) to normal (unsensitized) mice. Spleen cells from the SRBC-fed mice suppressed the production of DTH in normal mice, but cells from mesenteric lymph nodes or Peyer's patches did not. Thus, different regulatory mechanisms have been found depending on the immune response under study; and, the role of suppressor T cells in tolerance development is yet to be fully elucidated.

Ngan and Kind (1978) found prefeeding OVA inhibited IgG and IgE antibody production in response to intraperitoneal immunization, and cells transferred this suppression. They also found
that Peyer's patch lymphocytes were more effective suppressors than spleen lymphocytes and suggested that Peyer's patches were the source of the spleen suppressor cells. This possibility was also supported by work of Mattingly and Waksman (1978) who fed rats SRBC. After 2 days of feeding, suppressor T cells were detected only in the Peyer's patches and mesenteric lymph nodes (MLN). Conversely, after 4 or more days of feeding the suppressor cells were no longer detectable in the Peyer's patches and MLN, but were found in the thymus and spleen, suggesting migration of suppressor cells formed in the GALT. Mattingly et al. (1980) described the production of at least two distinct antigen-specific suppressor factors in cultures of spleen cells obtained from mice fed SRBC for 5 days prior to sacrifice. Suppressor cells appear to mediate these effects through soluble mediator factors.

The mucosal immune system and the systemic immune system interact in the induction of systemic tolerance by oral antigen exposure (Challacombe and Tomasi, 1980). It appears, at least for antigens requiring the presence of T cells to elicit an immune response, that ingestion leads to stimulation of mucosal immune responses, but suppression of the systemic response to subsequent parenteral challenge. In studies of oral tolerance by Thomas and Parrott (1974) who administered bovine serum albumin to rats and by André et al. (1975) who gave SRBC to mice, low titers of circulating antibodies occurred in animals after the oral regime and prior to parenteral challenge. Serum from mice fed SRBC passively transferred tolerance to naive animals and suppressed plaque forming colonies (PFC) in vitro from animals immunized intraperitoneally. Mice primed parenterally with SRBC had an unimpaired systemic response to subsequent intragastric challenge, i.e., their immunologic response was similar to the primary response expected after enteric exposure. Later experiments by the same group (Chalon et al., 1979) suggested the tolerizing activity was primarily associated with a suppressive factor having the molecular size of IgG, however, monomeric IgA with bound SRBC determinants was not excluded.

Similar results have been obtained by Kagnoff (1978b). Although he did not detect circulating anti-SRBC, there were small increases in IgG and IgA splenic PFC in the initial weeks of feeding. After the third week, only background PFC were detectable, and these animals were markedly suppressed to subsequent parenteral challenge. This suppression was transferable with serum to in vivo and in vitro test systems. Adsorption studies were compatible with immune complexes being present and having a role in the suppression, but the suppressor factor(s) was of smaller molecular size when examined by gel filtration. Circulating suppressor substances have been shown for only a few antigens and they remain poorly defined.

Vaz et al. (1977) injected mice intravenously with an amount of OVA estimated as comparable to the amount absorbed into the blood after feeding. They observed priming for later antibody
response rather than tolerance. Hanson et al. (1979a,b) suggested that orally induced tolerance in OVA-fed mice was actively maintained by factors other than antibodies or serum factors. BDF$_1$ mice given single or multiple intragastric feedings of OVA were unresponsive to parenteral immunization with OVA in Al(OH)$_3$ adjuvant. The rates of clearance of radiolabeled OVA from the blood were similar for normal and tolerant animals although there was a slight increase in primary antigen binding in the serum of OVA-fed mice. Transfer of serum from tolerant to normal mice did not affect their subsequent response to parenteral immunization. Responsiveness of syngeneic spleen cells (normal or OVA primed) was less after transfer into tolerant recipients than saline-fed recipients. Hanson et al. (1979b) concluded that production of circulating orally induced antibodies may not be an obligatory step for induction of oral unresponsiveness.

Rubin et al. (1981) have investigated the importance of antigen configuration and distribution in determining eventual immunologic outcome after antigen exposure to the mucosal immune system. They studied in vivo cellular immune responses by measuring immunologic tolerance for delayed-type hypersensitivity responses to reovirus in female A/J and BALB/c mice. Oral administration of UV-inactivated reovirus type 1, but not type 3, induced tolerance secondary to generation of virus-specific suppressor T cells. The serotype-specific suppression was a property of the viral hemagglutinin encoded for by the S1 gene. The failure of reovirus 3 to induce suppression was a property of the µlc capsid protein encoded for by the M2 gene. This protein in type 3 reovirus is degraded by chymotrypsin and may be sensitive to other proteases of the GI tract, intestinal epithelial cells, and macrophages. The capsid protein in type 1 reoviruses is resistant to proteolysis. Thus, two proteins, hemagglutinin and µlc capsid proteins, are required to induce tolerance to the viral hemagglutinin. The inability of irradiated reovirus type 3 to generate suppressor T cells was ascribed to the destruction of the µlc capsid protein by proteases, which apparently changes the processing of the viral hemagglutinin (Rubin et al., 1981). This is an example of one protein affecting the ultimate immune response to a second protein by the immune system of the gastrointestinal tract.

Another form of suppression is illustrated by clonal inactivation or inhibition such as is thought to be involved in self-tolerance (Richman, 1979). Thus, there are several forms of suppression involved in oral tolerance. Antigen-presenting cells of the mucosal immune system are clearly important; more work is needed to identify the cell or cell types responsible. Suppression of humoral and cellular immunity appear to be mediated by a variety of as yet poorly defined suppressor cells and soluble factors which may be antibodies, modified antigens, anti-idiotypes, complexes, or various soluble cell-derived factors such as TsF1, and TsF2 (Sy et al., 1980; Takaoki et al., 1982). Generalization
concerning the properties of such reactions may be premature because too little is known of the mechanisms involved in oral unresponsiveness. Development of experimental techniques and appropriate model systems in this area of research have been difficult, and data from various laboratories are not directly comparable. The actual responses probably depend on many variables including experimental animal species, dosage, and properties of ingested antigens.

Investigations of the mechanisms of tolerance induction (oral and systemic) are of potential importance in understanding the immunologic and physiologic response to food proteins. Additional understanding of the processes by which ingested antigens affect gastrointestinal immune responses is required before ascribing a role to oral unresponsiveness in normal protein digestion or in adaptation to food sources.
V. CIRCULATING IMMUNE COMPLEXES

In otherwise healthy persons, absorbed antigens circulate as part of complexes with any of several immunoglobulin types. In most instances, the complexes are in very low concentrations, approaching the lower limits of current immunologic methodology used to detect them. Because they occur at µg, ng, or even lower levels, the complexes or their constituent antigens are seldom studied by nonimmunologic-based physical or chemical methods.

Physiologic roles attributed to circulating immune complexes containing ingested antigens include clearance of absorbed antigens, involvement in tolerance mechanisms, and stimulation of development of the immune system. However, there is a body of experimental evidence suggesting that absorbed antigens or their antibody complexes may contribute to systemic disease processes. In order to investigate hypotheses linking these processes, researchers require methods that establish the identity of the antigen and immunoglobulin either circulating or at a tissue site.

A. ASSAY AND IDENTIFICATION

Soltis et al. (1979a,b) investigated methodologies employed in the detection of circulating immune complexes in the sera of patients with chronic inflammatory bowel disease. Assays employed in many earlier studies may have given false indications of the presence of immune complexes. A variety of factors produce false-positive results. These investigators and many others have suggested that the best demonstration of immune complexes would require isolation of the specific antigens involved. On the basis of assay of sera from 51 patients with inflammatory bowel diseases and use of appropriate controls from healthy volunteers, Soltis et al. (1979a) concluded that circulating immune complexes are unlikely to play a role in the etiology and pathogenesis of this disorder. Members of the ad hoc review group considered this area very controversial, suggesting caution in the interpretation of results.

A confounding factor in available methods of assay for immune complexes is their response to immunoglobulin aggregates (Soltis et al., 1979a,b). These aggregates may result from heating sera (a technique used to inactivate certain enzyme systems) or represent idiootype-anti-idiootype complexes (the complex of an immunoglobulin with an antibody directed against it). Thus, both the role of immune complexes in diseases and their very existence are open to question in some instances. The identification of specific food proteins in immune complexes presents additional difficulty to this problem.
Lambert et al. (1978), in a study under the auspices of the World Health Organization, compared the ability of 18 methods to detect soluble immune complexes containing unknown antigens. The tests relied either on the ability of specific serum proteins such as complement to combine with the immune complex or on the ability of the immune complexes to react with specific cell surface receptors. The techniques differed in their capacity to detect circulating immune complexes of certain diseases. Each test displayed certain limitations because of its underlying principle and the propensity for other substances to interfere with the reaction. For example, tests that are dependent on the activation of complement by a complex would not detect aggregates containing IgD and IgE because these isotypes are not effective activators of the complement system. Tests relying on the receptor for the Fc of IgG can detect complexes containing IgG, but not those of IgA, IgD, or IgE. Many of these tests required radioisotope-labeled reagents. While not included in this comparative study, enzyme-linked assays can often replace radioisotope assays. The final report of this collaborative study (Lambert et al., 1978) stated that no method differentiates nonspecifically-agglutinated immunoglobulins from true immune complexes; there is a need for reference standards and reagents; and, greater emphasis is required on the isolation of antigens from immune complexes if their relationship to pathogenesis, diagnosis, and treatment of diseases is to progress. Similar needs exist for food antigen studies.

The identification of specific antigens present in circulating immune complexes has seldom been accomplished. Circulating immune complexes from IgA-deficient patients have been shown to contain bovine serum and milk proteins (Cunningham-Rundles, 1981). IgA-deficient patients provide the best example of the occurrence of food proteins in such complexes.

An enzyme-linked immunosorbent assay (ELISA) using Raji cells (a lymphoblastoid B-cell line having high-affinity receptors for C3 but lacking surface immunoglobulin) can detect low levels of immune complexes (Cunningham-Rundles et al., 1980a). The extreme sensitivity of the ELISA for detecting specific antigens was demonstrated by detecting bovine κ-casein at levels of 2.0 ng/ml when the antigen is bound in the immune complex adsorbed onto Raji cells (Cunningham-Rundles, 1981). This method detects and identifies antigens present in IgG-containing immune complexes.

Firer et al. (1981) described an ELISA for the measurement of IgA, IgG, and IgM antibodies to bovine milk proteins. This work identified difficulties in some of the equipment used for assays, specifically the microtiter plates. ELISA assays are dependent on the binding of antigen to the wells of these plates. The amount of antigen detected varied among plates of different manufacturers, plates from the same manufacturer, and wells on individual plates, as well as with the antigen preparation used to coat the wells. The authors concluded that the lack of standardization of reagents
and equipment used in the ELISA system for analysis of antigens and antibodies in normal as well as hypersensitive individuals may make the general acceptance and interpretation of results difficult.

The development of assay systems whose results are more easily interpreted suggests that the etiology of certain "autoimmune" disorders may be clarified. For example, Numano et al. (1981) used a series of assay systems for circulating immune complexes to study the involvement of immune complexes in the pathophysiologic sequelae of Takayasu disease, a nonspecific arteritis affecting mainly young women. Takayasu disease had been attributed previously to an autoimmune mechanism. The mean level of circulating immune complexes by Raji cell assay was 30.4 ± 11.5 μg/ml compared with 3.5 ± 2.3 μg/ml in a control group. The investigators concluded that circulating immune complexes may accelerate or modify the sequelae but are not the primary causative factor.

Paganelli et al. (1981) have also reported a method for detecting specific antigens within circulating immune complexes. The importance of being able to identify antigens is underlined by the fact that all normal sera display antibodies to β-lactoglobulin (May et al., 1977) and such complexes are detectable by several methods (Brostoff et al., 1979). The assay developed by Paganelli et al. (1981) consists of dissociation of polyethylene glycol-precipitated immune complexes followed by adsorption onto the polystyrene tubes. The method permits comparisons of relative quantities of specific antigen between and within groups of sera. With this methodology, the investigators detected β-lactoglobulin in complexes from the sera of healthy and atopic patients after milk challenge. The level of β-lactoglobulin-containing immune complexes was highest in the atopic group, possibly reflecting increased absorption of these food antigens and higher titers of antibodies. In normal subjects, complexes containing food antigens were mainly of IgA immunoglobulin type, whereas in the atopic subjects the complexes contained IgG and IgE and were C1q-binding. The investigators interpreted their evidence as suggesting a physiologic role for serum IgA in the clearance of antigens from the circulation that might otherwise become complexed by IgE and IgG immunoglobulins forming complexes capable of tissue damage. Certain atopic patients absorbed lesser amounts of antigens and formed fewer immune complexes when treated orally with sodium cromoglycate (a drug that decreases IgE-mediated release of histamine) prior to feeding (Paganelli et al., 1979).

B. CLEARANCE AND DISPOSITION

Under normal conditions, the reticuloendothelial (RE) system presumably removes or clears immune complexes through the interaction of the immune complexes with specific cell surface receptors (Fc-IgG or C3) present on macrophages. It has been
hypothesized that defective RE system function may lead to prolonged circulation of immune complexes and thus contribute to tissue damage (Lawley, 1980). Increased levels of circulating immune complexes could overload homeostatic mechanisms and represent a health hazard in such instances (Finbloom and Plotz, 1979).

Some investigators now believe that dimeric IgA (two IgA molecules connected by a "J chain" polypeptide) may serve to scavenge absorbed macromolecules and eliminate them via a liver transport system that transfers the immunoglobulin and its complexes to the bile (Russell et al., 1981). IgA is of prime importance in the local response to ingested antigens and has been implicated in dermatitis herpetiformis (Katz and Strober, 1978) and the recurrent nephritis described by Berger and others (McPhaul, 1977). It has been suggested that immune complexes containing IgA and ingested antigens may be important in these diseases (Tomasi, 1976). Currently, evidence seems to support this contention for IgA nephropathy (Trascasa et al., 1980), but the evidence is controversial for dermatitis herpetiformis (Yancey et al., 1982). Any conditions that increase the quantity of antigens absorbed (e.g., irritated gastrointestinal mucosa, resistance to digestive process) or prolong survival of complexes in the circulation (e.g., viral infections, drug therapy) might possibly contribute to tissue pathology. Additionally, antigen structure itself is an important factor in the clearance of immune complexes under some circumstances (Finbloom et al., 1981). In C3H mice, blood clearances and hepatic uptake of orosomucoid-containing complexes with goat antibodies had a $T_{1/2}$ in excess of 300 minutes, whereas the $T_{1/2}$ for asialo-orosomucoid-containing complexes was 15 minutes.

1. **Glomerular deposits**

Gormly et al. (1981) described the association of IgA glomerular deposits and circulating immune complexes in a rat model of cirrhosis. Immunoglobulins eluted from the kidneys did not react with normal liver or kidney tissue. The investigators reasoned that because the liver sequesters antigens derived from the gastrointestinal tract and a large fraction of circulating IgA is derived from the gut, the origin of the deposited complexes might be the gut. Immune complexes in cirrhotic animals reached a level of 37.0 ± 25.4 μg/ml serum compared with normal levels below the limit of detection, 10 μg/ml. In rats made cirrhotic with carbon tetrachloride, the liver may be precluded from normal clearance of polymeric IgA which is normally removed to biliary secretion. The investigators concluded that increased concentrations of circulating immune complexes and IgA polymers common in cirrhosis were the cause of the associated IgA nephropathy.

Stachura et al. (1981) examined a series of 13 patients with IgA nephropathy (Berger's disease). Renal tissues contained IgA-producing cells and serum IgA was elevated. An abnormality in
IgA regulation was thought to contribute to the pathology in this renal disease through an inhibitory effect on clearance of both deposited and circulating immune complexes. These investigators highlighted the current controversy concerning whether pathogenic significance can be associated with the great array of immunologic abnormalities reported in a high proportion of patients with Berger's disease. In addition to their findings, they recognized that others have demonstrated elevated numbers of IgA-positive lymphocytes, suppressor cell defects, selective IgA hypergamma-globulinemia, and the presence of cold-reacting factor in the sera of such patients. The highly significant increase in the frequency of HLA-DRw4 (a specific histocompatibility antigen) in this form of glomerulonephritis may represent a genetic control of serum IgA levels, which may be selectively increased, leading to IgA nephropathy.

On the other hand, Sakai et al. (1979) concluded that a decrease in IgA-specific suppressor T-cell activity may not be under genetic control in cases of IgA nephropathy. In other words, the decrease in this activity might result from increased IgA-bearing peripheral lymphocytes and increased serum IgA levels rather than being a cause of these phenomena. More recently, Hall et al. (1982) have identified the presence of IgA-containing circulating immune complexes in patients with IgA nephropathy using a sensitive Raji cell radioimmunoassay.

Patients with idiopathic glomerulonephritis exhibit impaired clearance of the circulating immune complexes after ingesting a meal, and do not demonstrate abnormal absorption of food antigens. In patients examined by Cairns et al. (1981), the concentration of immune complexes reached higher levels and returned to normal levels at a significantly later time than was observed in normal controls. The change in circulating immune complex levels was detectable by latex agglutination inhibition but not by polyethylene glycol precipitation, solid phase C1q-binding assay, or 125I-C1q-binding assay. The types of complexes detected in sera were not related to those detected in renal biopsy material. It was presumed that the observed phenomena were due to impaired clearance of circulating immune complexes. The investigators argued that the delayed clearance in these patients was caused by a mechanism different from the delayed clearance observed in systemic lupus erythematosus and other immune complex diseases. In the latter conditions, it is presumed that an immune complex load results in blockage of the RE system which could theoretically be reversed by plasma exchange. Plasma exchange in the patients examined by Cairns et al. (1981) was without effect on the handling of circulating immune complexes. The investigators suggested that the primary defect may have involved complement abnormalities or T-cell defects, and that the persistence of circulating immune complexes may further modify the humoral and cellular immune function through interactions with cell receptors on immune cells.
2. **Vascular deposits**

Historically, a number of reports have suggested that milk proteins may be involved in the development of atherosclerosis and myocardial infarction. Some theories invoke properties of specific proteins such as xanthine oxidase (Carr et al., 1975). Briggs et al. (1960) reported myocardial infarction was twice as common in peptic ulcer patients on a milk diet as in controls or ulcer patients not treated with milk. Annand (1967) suggested that the ingestion of heated milk protein might be related to the development of arteriosclerotic heart disease because populations consuming no milk protein or only raw milk were essentially free of arteriosclerotic vascular disease. Of interest in regard to this possibility is that β-lactoglobulin, denatured by either cooking or pasteurization, appears to react with lactose, and its allergenicity increases 100-fold (Bleumink and Young, 1968). Its relatively low molecular weight (about 36,000) as well as resistance to denaturation at pH 2 and to hydrolysis by both trypsin and chymotrypsin might predispose to absorption of immunologically reactive β-lactoglobulin from the gastrointestinal tract (Annand, 1972).

Davies (1971), citing the earlier observations of Gunther et al. (1960; 1962), suggested that there was a decreased titer of antibodies to bovine milk proteins in the sera of individuals who were breast fed as infants. Davies et al. (1969; 1974), using a hemagglutination assay, reported an increased incidence of antibodies to dried bovine milk, and probably egg, in patients with myocardial infarctions. This was most notable in patients who died within 6 months of the infarction, and it appeared that the occurrence of such antibodies was often predictive of death, with a three-fold increase in mortality if either antibody was present (28% mortality in the presence of milk or egg antibodies, 10% if these antibodies were absent). The authors suggested a causal relationship through an immunologic mechanism acting directly via the formation of pathogenic immune complexes and/or activation of the complement system, or indirectly by effects on platelets or fibrinogen, leading to damage of the vascular endothelium and predisposing to atherosclerotic plaque formation.

In contrast, Toivanen et al. (1975), using an IgG and IgM immunoglobulin class-specific assay system, failed to find a significant difference between infarction patients and controls. More recently, Rzucidlo and Zikakis (1979) reported a correlation between intake of dairy foods (milkfat and whole milk) and the levels of serum antibodies that reacted with bovine milk xanthine oxidase.

If antibodies to bovine milk proteins play a role in the development of atherosclerosis by participating in the formation of pathogenic immune complexes, there must be absorption of immunologically reactive milk proteins from the gastrointestinal tract.
There has been evidence for over 60 years that antigenically intact milk protein may enter the circulation. In 1913, Van Alstyne reported that when milk was placed in the stomach of a dog, blood drawn 1-5 hours later contained enough immunologically recognizable milk protein to cause anaphylaxis in sensitized guinea pigs. Other studies have provided further evidence of the absorption of antigenically reactive milk proteins including bovine serum albumin (Rothberg, 1969), α-lactalbumin, β-lactoglobulin, bovine gammaglobulin (Carr et al., 1972), and casein (Carr et al., 1976). Furthermore, specific antibodies to these proteins have also been demonstrated in some instances concomitantly with the presence of the homologous antigen, indicating the presence of immune complexes (Carr et al., 1972; 1976).

Defects of Fc-receptor function of macrophages may be responsible for the decreased clearance of circulating immune complexes in patients with systemic lupus erythematosus and dermatitis herpetiformis (Frank et al., 1979; Lawley et al., 1981). Normal individuals without immune complexes who possess the HLA-B8 and DRw3 histocompatibility antigens have also been found to have an increased incidence of delayed Fc-mediated clearance. This is of particular interest because these histocompatibility antigens are associated with an increased incidence of a variety of autoimmune diseases. This Fc-receptor defect may play an important role in predisposing these individuals to autoimmune diseases. Massa et al. (1981) suggest that deposition of immune complexes, as indicated by immunofluorescence findings (detecting C3 and IgM) in diabetic dermal vessels, correlates with diabetic vascular complications, e.g., microangiopathy.

Thus, a body of circumstantial evidence has accumulated supporting the possibility that immune complexes may be instrumental in inducing a primary vascular lesion which subsequently develops into an atheromatous and finally an atherosclerotic plaque. However, no direct experimental evidence has ever been presented to support this possibility. That is, no studies have demonstrated conclusively suspect antigens, antibodies, or immune complexes in or underlying the atheromatous lesions.
VI. INVOLVEMENT OF MUCOSAL IMMUNITY IN DISEASE

Cochrane and Koffler (1973) concluded that immune complexes play an important pathogenic role in certain types of tissue injuries. Vasculitis, arteritis, glomerulonephritis, serositis, purpura, and other phenomena are examples of disorders associated with immune complexes where tissue injuries are typically present. Experimental serum sickness injures vascular membranes of the coronary arteries (McCluskey et al., 1960), glomerular capillaries (Dixon et al., 1961), and choroid plexus (McIntosh and Koss, 1974). Investigation of cellular and tissue pathology in various disease states provides information on the mechanisms of immune complexes that may be critical to experimental approaches for studying normal responses to food antigens. A role for circulating immune complexes containing endogenous or exogenous antigens in many of the examples which follow remains speculative. The particle size and composition of circulating immune complexes associated with specific diseases often characterize the observed tissue pathology.

A. IMMUNE DEFICIENCIES

Bovine milk proteins have been implicated in circulating immune complexes by the work of Cunningham-Rundles and her coworkers (1978a,b; 1981) who studied patients having selective IgA deficiency. IgA deficiency is associated with a higher incidence of autoimmune disease. This association is perhaps the best (but not incontrovertible) evidence that the normal gut immune system has mechanisms to prevent macromolecular absorption and any possible resulting autoimmune consequences. For example, three of four infants with physiologic IgA deficiency who were fed bovine milk had no detectable antigenemia but formed circulating immune complexes (Cunningham-Rundles et al., 1980a,b). After ingesting 100 ml of bovine milk, each of 13 IgA-deficient individuals formed circulating immune complexes as measured by Raji cell assay, and 11 of these subjects had antigenemia. Antigenemia was demonstrated by microimmunodiffusion using antisera to \( \kappa \)-casein, \( \alpha \)-lactalbumin, and \( \beta \)-lactoglobulin; sensitivity was estimated at 0.05 mg/ml of the antigens. On the basis of similar studies on a small number of patients with sex-linked agammaglobulinemia in which hyperabsorption was not demonstrated, these investigators concluded that secretory IgA deficiency may be an insufficient reason for excessive gastrointestinal absorption of food proteins. It was suggested that an additional unidentified immunologic mucosal abnormality accounts for the observed absorption of antigens.

In 1969, Buckley and Dees examined a series of immunodeficiency states and attempted to establish a correlation with precipitating antibodies to milk proteins. Among the deficiencies examined were IgG, IgM, IgA and IgG, IgG and IgM, and IgA and IgM deficiencies, respectively. A significant correlation was found between the deficiency of serum IgA (10 mg/dl) and the presence of
milk precipitins. The occurrence of precipitating antibodies to dietary antigens in patients with IgA deficiency suggests these patients are a useful group for investigations directed at elucidating the effect of absorbed antigens on systemic diseases.

Ammann and Hong (1971) have reviewed the literature on selective IgA deficiency. They define selective IgA deficiency as less than 50 mg/dl of serum IgA with no deficiency of other immunoglobulins, normal cellular immunity, and normal humoral antibody production. The incidence of selective IgA deficiency in various populations is reported between 1 in 500 to 1 in 1000. Most individuals with selective IgA deficiency appear asymptomatic; however, clinically these patients may have repeated upper respiratory infections, allergic symptoms, and a variety of autoimmune disorders including rheumatoid arthritis and systemic lupus erythematosus. Cases have been reported in which IgA deficiency has been associated with multiple autoimmune disorders in the same individual (Hauser et al., 1981). Others complain of intermittent diarrhea. Patients with celiac disease and selective IgA deficiency show atrophic changes in the intestinal mucosa, abnormal D-xylose absorption, and respond to a gluten-free diet. Frequently, compensatory elevation of serum IgG and IgM or of IgM alone can be observed. In the majority of patients a deficiency of serum IgA (predominantly monomeric) corresponds to a deficiency of secretory IgA (dimeric). A few patients have demonstrated the deficiency of serum IgA with normal secretory IgA levels. There appears to be a relationship between the local and systemic IgA systems. The development of IgA-containing plasma cells lining the respiratory and gastrointestinal tracts appears to be dependent on antigenic stimulation because animals that are germfree from birth fail to develop immunoglobulin-containing cells. Ammann and Hong (1971) concluded that there may be an inability to respond appropriately to certain antigenic stimuli in IgA deficiency. They noted particularly a high incidence of abnormal kappa/lambda ratios and an increased incidence of elevated IgG and/or IgM serum values. They suggested that absorption of an inappropriate antigen has the potential of initiating immunopathologic reactions resulting in tissue damage, release of altered antigen, and resultant autoimmune reactions which they refer to as an immune imbalance.

Strober et al. (1976) described a patient in whom there was no secretory IgA, but normal IgA serum levels. Apparently the patient had a normal capacity to synthesize IgA, as determined by the production of IgA from lymphocytes cultured in vitro with pokeweed mitogen. The patient had no detectable free secretory component and the basis of the disorder was attributed to a defect in the preferential movement of IgA precursor cells to secretory sites or in the selective proliferation and differentiation of IgA cells at those sites. Normal serum contains predominantly 7S IgA, and saliva contains both 7S and 11S IgA. The 11S IgA is a dimer associated with secretory component. About 10% of normal salivas contain less than 20 ng/ml of secretory component, but none was
detectable in the patient described by Strober et al. (1976). Most patients with IgA deficiency have greatly elevated secretory component levels in the saliva. Secretory component is synthesized by mucosal epithelial cells rather than lymphoid cells. IgA precursor cells are normally elaborated in Peyer's patches or mucosal lymphoid tissue. Available information suggests that these cells enter the circulation and subsequently migrate passively or are actively attracted to mucosal tissues for selective differentiation and proliferation.

Many immunodeficiency states are not associated with abnormal absorption of food proteins. IgA deficiency, per se, does not cause increased absorption because increased absorption is not observed in X-linked hypogammaglobulinemia.

B. INFLAMMATORY BOWEL DISEASE AND GLUTEN-SENSITIVE ENTEROPATHY

Within the scientific community, there is general acceptance that inflammatory bowel diseases have an immunologic etiology. However, direct evidence to preclude nonimmunologic processes is not available. Rabin and Singh (1981) suggest that there are a minimum of three different etiologies or pathogeneses of inflammatory diseases of the colon such as ulcerative colitis and Crohn's disease. The mechanisms involve cell-mediated immune reactions against exogenous antigens localized in the colon, immune system reactions against autologous antigens of the colon, or deposition of immune complexes in the colon. Each of these mechanisms offers the hypothetical possibility of food antigen involvement (Falchuk et al., 1980; Fällström et al., 1978; Rabin and Herrington, 1980; Rabin and Rogers, 1980). For example, an inflammatory response may be initiated by an aberrant immune reaction to autologous colonic antigens, to one or more ingested antigens, or to a combination of ingested antigens with cell surface antigens.

In the case of gluten-sensitive enteropathy, gluten bound at the surface of certain histocompatibility antigens on the cell surface is the target of T- or B-cell-mediated mechanisms having adverse effects on gastrointestinal functions (Falchuk et al., 1980). The response might be to the histocompatibility antigen, to the gluten, or to the antigenic structure presented by the combination of the gluten and histocompatibility antigen. Gluten-sensitive enteropathy may in fact represent the result of a break in oral tolerance to dietary gluten (Strober, 1980).

Patients with celiac disease frequently have circulating antibodies to gluten, to gliadin, or to subfractions of these proteins. Adherence to a gluten-free diet eliminates or greatly reduces the titer of these antibodies. For example, Unsworth et al. (1981) examined the sera of 62 children having gastrointestinal disease. The sera were examined for IgA, IgG, and IgM antibodies to gliadin by two different methods, an immunofluorescent test and a mixed reverse passive antiglobulin hemadsorption test. Children
with active celiac disease had IgG- and IgA-type antibodies that disappeared with institution of gluten-free diets. When antibodies to gliadin were IgA, the children had severe small intestinal villous atrophy and almost always were diagnosed as having celiac disease. Children with IgG antibodies to gliadin were more frequently diagnosed as having transient gluten intolerance. IgM antibody to gliadin was not disease-specific and did not correlate with any clinical features. The investigators suggested that sera positive for gliadin antibodies of the IgA class were predictive of active celiac disease. Observations on patients with Crohn's disease showed IgG-type antibodies to gliadin in sera from 7 of 14 patients with small intestine involvement, but in only 1 of 6 patients was the disease confined to the large bowel. The authors expressed a need for further studies looking for IgA antibodies to other food proteins before definitive statements could be made about the role of IgA in the enteropathy termed inactive celiac disease.

Data are available on both sides of the question concerning the induction and pathogenesis of IgA-containing immune complexes in patients with gluten-sensitive enteropathy. Hall et al. (1981) examined the sera of 22 patients with gluten-sensitive enteropathy for IgA-containing circulating immune complexes using a Raji cell assay which was specific for IgA-containing complexes. The C1q-binding assay was used to measure IgG- or IgM-containing complexes. The presence and concentration of immune complexes did not correlate with disease activity in these gluten-sensitive patients. Five patients whose disease was controlled with a gluten-free diet were challenged with gluten and their sera examined for IgA-containing immune complexes. Such complexes did not develop or increase despite the induction of gastrointestinal symptoms. Complexes containing IgG and IgM were absent from the sera of these challenged patients. The authors concluded that immune complexes do not play a primary role in the pathogenesis of gluten-sensitive enteropathy. However, since there was an association of the level of IgA circulating immune complexes with morphological evidence of gastrointestinal changes, it is probable that circulating immune complexes containing IgA occur secondarily to the disease process. On the other hand, Zone et al. (1982) have reported the induction of IgA immune complexes in dermatitis herpetiformis patients who were fed gluten-containing meals.

C. ASPECTS OF AUTOIMMUNITY

Cunningham-Rundles et al. (1981) have suggested that the chronic excessive permeability of the gastrointestinal tract in patients with IgA deficiency permits the absorption of excessive food proteins that leads to the formation of antigen–antibody complexes and autoimmunity. Clinical autoimmune disorders are found in many types of immunodeficiencies besides selective IgA deficiency (Ammann et al., 1979). Autoantibody formation in those instances may be favored by partial B-cell or T-cell dysfunction or phagocytic dysfunction. Patients with regulatory cell defects, involving helper or suppressor T-cell populations or partial deficiencies
of both B- and T-cell immunity, may have enhanced autoantibody formation. The predominant view of immunologists is that cellular immune mechanisms, involving basic homeostatic processes of the immune system, are essential to autoimmune phenomena (Johnson, 1981).

The number of B cells having the capacity for producing autoantibodies may increase with age. Fong et al. (1981) have measured an age-related differential expansion of the human auto-reactive B lymphocytes for IgG and thyroglobulin. Johnson (1981) cites the example of B lymphocytes capable of producing IgM-rheumatoid factor being present in both rheumatoid arthritis patients and healthy individuals in whom such cells are apparently well controlled but whose cells can be induced to proliferate in vitro in response to Epstein-Barr virus or pokeweed mitogen. Thus, autoimmune responses could be expected in response to T-lymphocyte dysfunctions, possibly even in the absence of autologous antigen. Recently, Kumagai et al. (1982) have demonstrated that T cells from patients with active systemic lupus erythematosus are "defective" as measured by cell culture techniques that evaluated capacity to support the formation of B-cell colonies from normal individuals.

Evidence from studies of bacterial and viral infections, as well as of idiootype-anti-idiotype networks is consistent with an important role for the intestinal tract in the initiation of autoimmune processes (Johnson, 1981). For example, Van Snick (1981) described an age-related occurrence of serum anti-IgG autoantibody in normal 129/Sv mice, but not in germfree animals of the same strain. The autoantibody was produced earliest in the lymph nodes draining the intestinal tract, and was detected at that site before its occurrence in the spleen or bone marrow.

The importance of the bacterial antigenic load from the gastrointestinal tract in the etiology of rheumatoid arthritis, via chronic formation of immune complexes containing peptidoglycan, remains an area of considerable interest to clinical investigators (Bennett, 1978). An underlying theme is the cross-reaction of antigenic determinants on infectious agents with those of the host components. For example, Bennett (1978) has suggested that mycoplasma infections might persist because of immunologic similarity of surface antigens with host histocompatibility antigens. Viral coat proteins can be expressed as cell surface antigens as demonstrated by certain murine lymphoblastoid cells with the G\textsubscript{\text{IX}} marker and, in bacteria, the surface antigenic components, peptidoglycan structures, are recognized immunologically as being similar to the Fc component of IgG. In any of these possibilities, the proliferation in cross-reacting antigens might interfere with homeostatic control of autoantibody production. In genetically susceptible individuals, particular antigens might contribute to the mechanisms of chronic arthritis and other inflammatory processes; however, these antigenic determinants are unlikely to be present as dietary components.
Studies using a rabbit model of rheumatoid-like joint disease suggests the possibility that food proteins are important etiologic factors (Coombs and Oldham, 1981). Old English rabbits, a breed known to be susceptible to the development of joint lesions, were fed and/or injected with bovine milk proteins. In one experiment, four of six rabbits drinking milk for 12 weeks developed synovial lesions in their knee joints. The combined route of administration had less effect in this preliminary experiment. While there are few clinical studies of the role of food proteins in rheumatoid diseases of human beings, Tisserch (Swarbrick and Stokes, 1979) has commented that in one cohort of 86 patients 50% reported pain and/or joint swelling aggravated by specific foods.

A case report by Parke and Hughes (1981) concerns the response of certain clinical manifestations to the selective deletion of foods from the diet of a female patient with rheumatic disease. Challenge with milk and cheese products produced increases in synovitis and immune complexes, IgE antibodies, and heat-damaged red cell clearance rates. Parke and Hughes (1981) considered the change in concentrations of circulating immune complexes slight (25-50 µg/ml) and the change of clearance rate large (15->100 minutes). Even though clinical improvement was reported after the withdrawal of specific foods, there was little evidence to suggest that food proteins played a causative role in the rheumatoid arthritis.

Elkon et al. (1982) have examined the sera of patients with systemic sicca syndrome (primary Sjögren's syndrome with systemic manifestations). Their results indicated a general perturbation of IgA metabolism in these patients with a selective increase in the proportion of circulating polymeric IgA with rheumatoid factor activity (antibodies directed against sites on the Fc fragment of IgG). The salivary, lacrimal, and other exocrine glands of these patients are chronically inflamed, suggesting that IgA rheumatoid factor may be produced by local mucosal plasma cells and be related to the inflammatory processes. Noting that the source of human serum IgA is uncertain and that elevated levels of serum IgA or polymeric IgA occur in Henoch-Schönlein purpura, Berger's disease, and after oral immunization, Elkon et al. (1982) reasoned that mucosal events more than systemic diseases are responsible for altering serum IgA. Their data provide indirect evidence for a mucosal origin of the polymeric IgA rheumatoid factor in systemic sicca syndrome. These findings and others, such as those of Stachura et al. (1981) relating to Berger's disease, suggest that interactions in the gastrointestinal tract contribute to associated systemic diseases.
VII. CONCLUSIONS

It is important to realize that low concentrations of autoantibodies and immune complexes may occur periodically in a significant proportion of serum samples from an otherwise normal population. Under other conditions, an increased concentration of antigen-antibody complexes can be observed; however, their presence even at elevated values is not in all cases associated with specific tissue injury. The ability of immune complexes to produce tissue injury has been adequately documented and is exemplified by autoimmune diseases. Tissue injury is dependent on many factors including the size of the antigen-antibody complexes, their ability to fix complement, the avidity of each antibody for its antigen, the relative proportion of antigen and antibody in the complexes, the nature of the antibodies, and the properties of the antigens. Similarly, it is evident that food antigens are absorbed and can be found in the blood. They are present in low concentrations and do elicit formation of immune complexes.

There appears to be general agreement that tissue injury in the kidney, blood vessels, and possibly liver is more apt to result from immune complexes than from antigens or antibodies by themselves. There are many clinical examples of the former, but few of the latter. There is ample evidence that normal immune systems have a great capacity to compensate for wide variations in all of these factors, and that a genetic predisposition to certain diseases having an immunologic etiology does not necessarily mean an individual will develop clinically significant symptoms. Thus, even if food antigens as part of immune complexes are found in association with tissue damage, it may not be possible to assign a causative role for food antigens in particular systemic diseases.

Additional conclusions of the ad hoc group were as follows:

- Many commonly consumed diets contain large amounts of essentially undenatured antigens from single sources. It is probable that a sufficiently large amount of any one antigen would exceed the ability of even a fully developed secretory immune response to exclude that antigen in a highly effective way. In disease states, particularly those of the gastrointestinal tract, compensatory mechanisms may be further restricted and contribute to excessive absorption of antigens across the intestinal mucosal barrier.

- The route of exposure, type and amount of antigen, nutritional status, and the age of the individual when initially exposed are believed to be important factors in immune response to certain antigens and in the course of immune reactions in a variety of diseases.
The interactions of ingested antigens with the mucosal and systemic immune systems are not fully understood. Clinical and experimental evidence indicates individuals can develop a variety of immune responses to ingested antigens. Individuals may respond differently, possibly adversely, to dietary antigens because of inherent genetic deficiencies or acquired alterations in their ability to respond appropriately.

Ingested antigens differ in their ability to act upon the mucosal immune system and, if absorbed, in their ability to influence systemic responses. The chemical nature of the antigens and their resistance to digestion are important in these interactions.

Some individuals develop abnormally high concentrations of immune complexes after ingesting a meal. The ad hoc group concluded there was still no firm evidence that circulating immune complexes containing food proteins either cause or have a role in pathogenesis of systemic diseases. There is a lack of information regarding the structure, physiologic properties, and any possible adverse effects attributable to immune complexes containing specific food antigens.

The gastrointestinal tract is important in determining the nature of immune responses to a variety of antigens. The possibility exists for the cross-reaction of dietary antigens with histocompatibility antigens, other antigens of cells, or idiotypes. The ad hoc group participants suggested that the gastrointestinal tract may play a role in the pathogenesis of autoimmune diseases even though it has little to do with elaboration of immune complexes important in these diseases.

A widely accepted working hypothesis concerning inflammatory bowel diseases suggests that these conditions originate from abnormal responses to dietary constituents by the local immune system of the gastrointestinal tract. An equally attractive possibility is that the actual problem is associated with a local immune response (to dietary constituents) that may itself be initiated by some infectious agent, as yet unknown and unidentified.

Evidence from experimental animal studies and patients with gluten-sensitive enteropathy, selective IgA deficiency, Berger's disease, various food sensitivities, and systemic sicca syndrome indicates that the interaction of antigenic materials with the mucosal immune system may be an important component of certain diseases, either causative of an important manifestation or of the disease itself. However, with the possible exception of gluten-sensitive enteropathy, these interactions have not been shown to involve a specific protein and may involve groups of proteins.
VIII. SUGGESTIONS FOR FUTURE CONSIDERATION

• Most, if not all, otherwise normal human infants develop antibodies to certain ingested bovine milk proteins. Young experimental animals (mice, rats, minipigs) easily develop tolerance after oral exposure to similar proteins. Such animals are very resistant to immunization and induction of gastrointestinal lesions when challenged orally by the sensitizing antigens. To study this aspect of mucosal immune response in humans requires invasive technology that is difficult to justify on ethical grounds. Even when justified, it is difficult to obtain informed consent or permission from sponsoring agencies and institutions to do such studies. There is a need to develop additional experimental techniques and approaches that will facilitate responsible clinical studies on human subjects.

• The prevalence and range of observed adverse reactions to foods are not adequately documented. In addition to reporting food-borne disease, physicians should be encouraged to submit reports about adverse reactions to foods to a data collection center. Such a center, responsible for correlation and analysis of data, should be established. Current efforts of collecting similar data for drugs suggest possible value of extending the scope of collection to foods.

• A long-term commitment of time and financial resources to a prospective study of a large number of individuals, such as young children having adverse reactions to foods, could provide valuable data about the frequency of such reactions to foods and the association of food proteins with systemic diseases. Questions concerning the importance of tolerance development, repeated exposure, and delayed initial exposure to dietary proteins could be addressed by carefully designed long-term prospective protocols.

• After gastrointestinal exposure of the mucosal immune system, certain systemic immune responses are suppressed or prevented. Basic mechanisms interrelating mucosal and systemic immune responses are currently subjects of intense investigation, and rapid development of basic knowledge of these phenomena is anticipated. The immune system of the gastrointestinal tract can influence uniquely the systemic immune system, and this aspect should be examined more critically for possible clinical utility to patients with classical rheumatic diseases.
Further studies are warranted on the effects of different processing treatments, e.g., different heat treatments, on the antigenicity of food proteins. Such work should include investigation of systemic effects on experimental animals.
IX. GLOSSARY*

antibody: A protein molecule (immunoglobulin) produced in the body by lymphoid cells, particularly plasma cells, in response to stimulation by antigen.

antigen: A substance with which an antibody reacts in a specific manner; usually a substance that elicits a specific immune response when introduced into the tissues of the body.

anti-idiotype: Antibodies elicited by the unique antigenic features present in the combining site of another antibody referred to as an idiotype. The complexing of an anti-idiotype may block the binding of antigen by the idiotype.

atopy: A genetic tendency to develop sudden hypersensitivity states such as allergic asthma or hay fever.

autoantibody: An antibody appearing in an individual which reacts with normal body constituents of that individual.

B cell (bone marrow-derived cell): A lymphoid cell present in one of the lymphoid organs which originated in the bone marrow and matured independent of the influence of the thymus gland.

C1q: A component of the complement system.

cell-mediated immunity: Specific immunity which is mediated by small lymphocytes and is dependent upon the presence of a thymus gland at birth.

complement: A series of serum proteins which are activated by antigen-antibody and other complexes and which mediate important biological functions, such as engulfment of particles by specialized cells.

Fc (fragment crystallizable): Proteolysis of IgG by papain yields three fragments, two identical Fab fragments containing the antigen binding sites and one Fc fragment containing the C-terminal ends of the two heavy chains. Certain cells have surface receptors that bind the Fc portion of IgG.

glycocalyx: A glycoprotein- and polysaccharide-containing covering such as found on cells of the intestinal lumen.

**HLA histocompatibility antigens:** The cell surface histocompatibility antigens on human cells which are important in tissue transplantation and which are controlled by a single gene complex.

**Humoral immunity:** Immunity mediated by specific antibodies which are present in the blood serum and tissue fluids of the body.

**Hypersensitivity:** The state, existing in a previously immunized individual, in which tissue damage results from the immune reaction to a further dose of antigen. If tissue damage is severe, the condition may be referred to as one form of allergy.

**Idiotype:** Identifying feature of antibodies denoting the specific antigen against which the antibodies are directed. Idiotypic determinants are usually associated with the unique combining sites in the variable part of an antibody.

**IgA:** A class of immunoglobulins present in serum in low concentration (1.4–4 mg/ml) but representing the predominant immunoglobulin in body secretions such as saliva, human colostrum and milk, intestinal fluids, tears, and bronchial secretions.

**IgD:** An immunoglobulin molecule usually associated with lymphocyte surfaces and present at very low concentrations (0–0.4 mg/ml) in human serum.

**IgE:** A class of immunoglobulins present in mucosal secretions and occurring in very low concentrations (17–450 ng/ml) in normal serum. This immunoglobulin class is associated with the release of histamine through interaction with mast cells and basophils, making it important in allergy and hypersensitivity reactions.

**IgG:** The predominant immunoglobulin class in human serum (8–16 mg/ml), having a molecular weight of about 150,000. It enhances the interaction of phagocytic cells with microorganisms and functions to neutralize bacterial toxins. Complexes of certain subclasses of IgG with antigen will bind and initiate the complement system.

**IgM:** A high molecular weight (900,000) immunoglobulin class capable of activating the complement system. Antibodies of this class are efficient agglutinating and cytolytic agents. Normal serum concentrations are 0.5–2 mg/ml.

**Immunological tolerance:** Immunological unresponsiveness conferred upon an individual by prior contact with a given antigen.

**Lymphocyte:** A cell associated with all aspects of specific immunity. Lymphocytes are the chief constituents of lymphoid tissue.

**Macrophage:** Any of the diverse group of cells (except granulocytes) which have the capacity to engulf and destroy foreign material.
plasma cell: The predominant immunoglobulin-producing cell type of the lymphoid cell series.

prime: To expose the immune system to an antigen for the first time usually as a means of obtaining a greater immune response on reexposure to the same or cross-reacting antigen, e.g., immunization.

T cell (thymus-derived lymphocytes): Small lymphocytes which on (or after) residence in the thymus gland attain new immunologic capabilities.
X. LITERATURE CITED


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