

26. Michalek SM, Kiyono H, Wannemuehler MJ, Mosteller LM, McGhee JR. Lipopolysaccharide (LPS) regulation of the immune responses: LPS influence on oral tolerance induction. *J Immunol* 1982;128:1992.
27. Roberts SA, Soothill JF. Provocation of allergic response by supplementary feeds of cow's milk. *Arch Dis Child* 1982; 57:128.
28. Wostmann BS, Pleasants JR, Bealmear P. Dietary stimulation of immune mechanisms. *Fed Proc* 1971;30:1779.
29. Shannon WR. Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea-pigs. *Am J Dis Child* 1921;22:223.
30. Kulangara AC. Demonstration of ingested wheat antigens in human breast milk. *IRCS Med Sci* 1980;8:19.
31. Colwell M, Meyer RR, Pazdernik TL, Halsey JF. Immunologic tolerance to dinitrophenylated human gamma globulin induced via colostrum. *Cell Immunol* 1980;52:229.
32. Jakobsson I, Lindburg T. Cow's milk as a cause of infantile colic in breast fed infants. *Lancet* 1978;2:438.
33. Miller DL, Hirovonen T, Gitlin D. Synthesis of IgE by the human conceptus. *J ALLERGY CLIN IMMUNOL* 1973;52:182.
34. Gerrard JW. Allergy in breast fed babies to ingredients of breast milk. *Ann Allergy* 1979;42:69.
35. Björkstén B. Does breast-feeding prevent the development of allergy? *Immunol Today* 1983;4:215.
36. Björkstén F, Saarinen UM. IgE antibodies to cow's milk in infants fed breast milk and milk formulae. *Lancet* 1978;2:624.
37. Juto P, Björkstén B. Serum IgE in infants and influence of type of feeding. *Clin Allergy* 1980;10:593.
38. Firer MA, Hosking CS, Hill DJ. Effect of antigen load on development of milk antibodies in infants allergic to milk. *Br Med J* 1981;283:693.
39. Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. *J ALLERGY CLIN IMMUNOL* 1984;73:265.

Allergen absorption in the intestine: Implication for food allergy in infants

W. Allan Walker, M.D. Boston, Mass.

An important but unappreciated function of the intestinal tract is to control the uptake of foreign proteins from the luminal environment, thus preventing immunologic reactions that may result in inflammatory or allergic diseases. In this review, mechanisms of antigen transport and factors controlling transport are discussed, with special emphasis on studies in humans. Of particular importance is the contribution that immaturity of mucosal host defenses in young infants makes in the expression of food allergic reactions. Although a considerable number of animal studies have been reported in the development of our understanding of the pathophysiologic conditions associated with antigen transport, these studies are beyond the scope of this review, and interested readers are referred to recent reviews on the subject.¹⁻³

TRANSPORT OF ALLERGENS

Several studies of allergen/antigen transport in human infants has been reported during the last 60 years. Lust⁴ reported that normal human infants did not absorb undigested beef or egg protein. However, in the presence of malnutrition and disease, egg protein appeared in the urine after

Abbreviation used

BSA: Bovine serum albumin

ingestion of this food. Schloss and Worthen⁵ were also unable to demonstrate the presence of cow's milk protein in the urine of normal infants after a similar feed.

Robertson et al.,⁶ using more sensitive methods, have recently evaluated macromolecular absorption in preterm and term infants. They quantitated the concentration of β -lactoglobulin in the serum of 47 neonates after feeding a standardized amount of a formula based on cow's milk. They found that preterm infants, particularly those of less than 33 weeks gestation, had higher serum concentrations of β -lactoglobulin than did term neonates given an equivalent milk feed, and concluded that the ability of the gastrointestinal tract to exclude antigenically intact food protein increases with gestational age.

In another recent study⁷ the urinary excretion of lactulose/L-rhamnose during continued infusion of milks containing both sugars was used as an index of intestinal permeability. Healthy infants of 31 to 35 weeks gestational age proved to have a period of enhanced permeability to lactulose during the first week of life, the lactulose/rhamnose excretion ratio being significantly higher on day 2 than on day 9 or 16, when a mature pattern of permeability could be seen. A note of caution is necessary here. Although it

From Harvard Medical School and the Combined Program in Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital and Children's Hospital Boston, Mass.

Reprint requests: W. Allan Walker, M.D., Children's Hospital, 300 Longwood Ave., Boston, MA 02115.

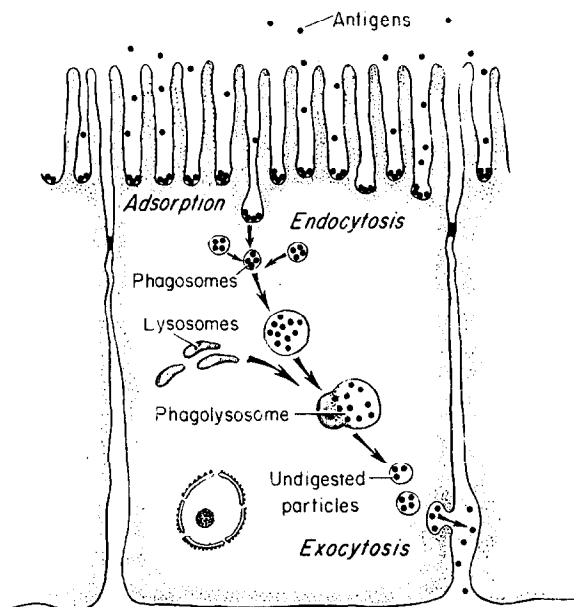


FIG. 1. General mechanism for uptake and transport of macromolecules by the intestine. After absorption and endocytosis by the microvillus membrane, macromolecules are transported in small vesicles and larger phagosomes. Intracellular digestion occurs when lysosomes combine to form phagolysosomes. Intact molecules that remain after digestion are deposited in the intercellular space by reverse endocytosis (exocytosis). (From Walker WA, Isselbacher KJ. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders. *Gastroenterology* 1974;67:531.)

is reasonable to assume that increased permeability to a disaccharide such as lactulose correlates with increased permeability to larger protein molecules, this remains to be demonstrated. Data from these animal and human studies support the concept that there is increased permeability of the intestine of human infants to macromolecules early in life.

It has not been resolved whether immunoglobulins from human breast milk are absorbed intact across the intestine of the suckling human. Data from studies in the 1960s support the notion that intact immunoglobulins are not absorbed and transported intact into the circulation of human newborns.⁸⁻¹⁰ However, several investigators have recently opposed this view and suggest that the human newborn infant may indeed absorb antibodies from mother's milk. Iyengar and Selvaraj¹¹ examined serum immunoglobulin concentrations from birth to 5 days of age. Levels of serum IgA, IgM, and IgG on the fifth day of life were significantly higher in colostrum-fed newborns than in infants who did not receive colostrum. More recently, Vukavic¹² studied two groups of newborn human infants to assess the intestinal absorption of IgA. Group A consisted of 30 mature newborn infants who were breast-fed. On the third day of life IgA was detected in the serum of all 30 infants (429 ± 365 mg IgA/ml). Pasteurized human breast milk that had no immunoreactive IgA was fed to group B infants matched for

gestational age. Serum IgA was not detected in the blood of any of these infants on the third day of life. In a third study Ogra et al.¹³ administered human colostrum rich in IgA antibody against poliovirus in a single feed to newborn infants 18 to 72 hours after birth. Thereafter, antipoliovirus antibody was detected in the blood of three of seven infants whose cord blood had been negative for antibody against poliovirus. This observation, like the previous two, suggests that uptake and transport of intact immunoglobulins across the human intestine does occur in the immediate neonatal period.

TRANSPORT MECHANISMS

There are at least four mechanisms by which macromolecules can cross intact cell membranes. Large molecules may gain entry into an intestinal cell by receptor-mediated endocytosis (Fig. 1). This is the process by which cells ingest extracellular materials by trapping them within inward foldings of the plasma membrane that pinch off from the surface to form intracellular vesicles. With the advent of the electron microscope this process of endocytosis has been extensively studied. Receptor-mediated endocytosis is initiated when the macromolecule (ligand) binds to a receptor on the surface membrane of the enterocyte.¹⁴ This stimulates the clustering of additional receptors in a coated pit area of the cell. This clustering is followed by invagination and internalization of the coated pit to form a coated vesicle. The ligand still attached to the receptor may be protected from intracellular degradation.¹⁴⁻¹⁶ The contents of the coated vesicle may then be discharged across the basolateral surface into the intercellular space, and the receptors utilized again through recycling.¹⁴ Recently, two types of receptors in cell membranes have been identified.¹⁷ In one type, binding of the ligand to the receptor (class I receptor) leads to change in cell behavior or metabolism. The binding of a ligand to a second type of receptor (class II receptor) results in ligand internalization, which does not in (and of) itself lead to alterations in cell behavior. Class II receptors are characterized by the ability of the cell continually to reutilize the receptor, whereas class I receptors are not reutilized, or at best are reutilized only a limited number of times.¹⁷ Several recent reviews have discussed in detail receptors and receptor-mediated endocytosis.^{14, 17, 18}

A second type of macromolecular transport may occur when extracellular proteins are trapped nonselectively in fluid droplets, which are internalized by cells through invaginations of noncoated regions of the cell.¹⁴ This type of uptake may be occurring in the specialized M cells overlying Peyer's patches. Owen and Jones¹⁹ and Owen²⁰ have demonstrated that macromolecules can traverse the epithelial barrier of the distal small intestine via specialized M cells overlying Peyer's patches. Electron microscopy of these cells indicates that they have a paucity of microvilli, a poorly developed glycocalyx, and an absence of lysosomal organelles. Using electron microscopy, they observed M cell uptake and processing of horseradish peroxidase. The marker is first taken up by these specialized cells by endocytosis and then extruded into the extracellular space, where it is phagocytosed by lymphoid cells circulating through Peyer's

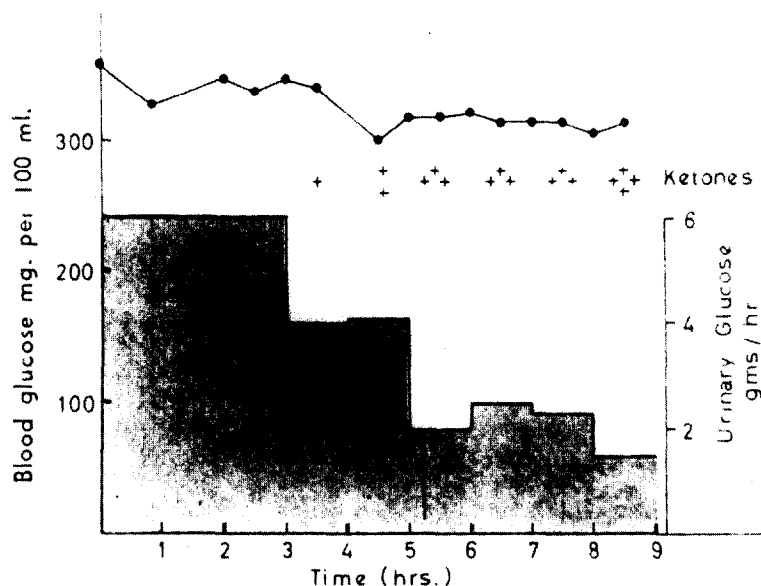


FIG. 2. Response of urinary glucose excretion and blood glucose to fasting, and after withdrawal of daily maintenance insulin for 4 days. Note appearance of ketone bodies in urine. (From Crane CW, Luntz GR. Absorption of insulin from the human small intestine. *Diabetes* 1968;17:625.)

patches.^{19, 20} This type of antigen absorption in the gut appears to represent an important access route for ingested antigen to reach lymphoid tissues and thereby stimulate the local and distant immune system.

Third, some bacterial and plant toxins and certain viruses appear to enter cells by direct penetration through the cell membrane. However, the direct penetration of physiologic molecules such as protein hormones has not been demonstrated.¹⁴

Last, leakage of large molecules through the intercellular junction may also occur, but the extent to which this happens is unknown.

FACTORS AFFECTING ANTIGEN TRANSPORT

Gastric acidity. A number of factors affect macromolecular transport across the intestinal epithelium. Kraft et al.²¹ reported increased circulating BSA antibodies in adults with achlorhydria or hemigastrectomies, and suggested that these individuals do not adequately hydrolyse the food antigen BSA in the stomach; the antigen may therefore be present in greater concentrations for absorption by the small intestine. Bloch et al.²² demonstrated increased macromolecular transport when bicarbonate was administered with antigen (BSA). It is presumed that the neutralization of gastric pH retarded the initiation of protein digestion by pepsin and increased amounts of antigen were emptied into the small intestine. Because gastric acid output is decreased in the newborn period,²³ increased amounts of intact macromolecules may escape degradation and be available for transport across the intestine of newborn infants.

Intestinal proteolysis. Proteolytic activity in the small intestine also determines the extent of intestinal macromolecular transport. The breakdown of ¹²⁵I-BSA by jejunal gut sacs from rats with ligated pancreatic ducts is signifi-

cantly decreased compared with that in sham-operated controls.²⁴ The increased amounts of intact BSA would therefore be available for transport across the intestine. In addition, treatment with pancreatic extracts before removal of the gut sacs of rats with ligated pancreatic ducts led to an increase in digestion of ¹²⁵I-BSA by gut sacs in vitro. These findings suggest that pancreatic enzymes adsorbed to the surface of the intestine contribute to proteolysis of antigens.

More recently, Saffran et al.²⁵ administered lysine vasopressin by gavage to rats simultaneously with the trypsin inhibitor aprotinin (Trasylol), and noted an increased anti-diuretic effect in these animals compared with that in control animals given lysine vasopressin alone. This finding extends Walker's earlier in vitro studies²⁴ and demonstrates that in vivo inhibition of pancreatic enzymes leads to increased amounts of vasopressin being transported across the intestine and into the bloodstream to exert the physiologic effect.

At least one study assessing the effect of pancreatic proteases on macromolecular absorption in humans has been performed. Crane and Luntz²⁶ reported a 37-year-old coal miner who underwent a total pancreatectomy because of chronic pancreatitis. To assess proteolytic activity of fluid from the small intestine, a nasojejunal tube was passed. Aspirated fluid was found to have no trypsin or chymotrypsin activity. Thereafter, exogenous insulin was withheld and blood and urine glucose and urinary ketones were monitored (Fig. 2). During the fast, blood sugar remained relatively constant, but a considerable amount of glucose spilled into the urine, which also became positive for ketones (Fig. 2). On a subsequent day, under the same conditions, the patient was given 100 U of crystalline insulin per kilogram body weight by nasojejunal tube; blood glucose decreased significantly and no ketones were lost. The same amount of insulin given by nasojejunal tube to a normal



FIG. 3. Levels of salivary and serum IgA with age, expressed as a percentage of the adult level. (From Tomasi TB. The development of the secretor. In: Osler AG, Weiss L, eds. The immune system of secretion. Englewood Cliffs, NJ: Prentice-Hall, 1976:45.)

subject had no effect on blood sugar. The investigators concluded from this study that in the absence of pancreatic proteases, intact protein absorption across the small intestine is enhanced.

Proteolysis of ingested proteins may be incomplete in the neonatal period because of the decreased conversion of trypsinogen to trypsin by the brush border enzyme enterokinase. Antonowicz and Lebenthal²⁷ have noted that enterokinase activity in full-term infants is only 17% of that found in children between 1 and 4 years of age. This observation is in agreement with the report that newborn infants have approximately one tenth the intestinal trypsin activity found in children aged 9 months and older.²⁸

Early enteral nutrition. We have shown that breast-fed rabbits given a physiologic amount of BSA by gavage will have significantly less immunoreactive BSA in their serum than age- and weight-matched bottle-fed animals.²⁹ This difference in antigen transport in breast-fed animals could not be explained by the presence of anti-BSA antibodies in breast milk of the lactating dose or in the plasma of the naturally raised animals. We hypothesized that natural feeding may simply accelerate gastrointestinal tract development. This hypothesis is supported by the observation of Widdowson et al.³⁰ and of Heird and Hanson,³¹ who have shown that breast-feeding is associated with increased small intestinal weight gain and increased intestinal protein and DNA synthesis early in life.

Mucosal humoral immunity. The adequacy of immune function of the gastrointestinal tract affects transport of intact macromolecules. IgA is the immunoglobulin present in the highest concentration in intestinal secretions.³²⁻³⁴ It has been postulated that this IgA suppresses the transport of antigens by complexing with them in the lumen, thereby impeding absorption.³⁵ The concentration of IgA in saliva, stool, and serum of newborn animals and humans³⁶⁻³⁹ is low

(Fig. 3), and it has been hypothesized that this transient deficiency in part accounts for the increased transport of macromolecules in newborn animals. This hypothesis is supported by studies of patients with selective IgA deficiency. These patients have circulating immune complexes and precipitating antibodies to bovine milk proteins.⁴⁰ In addition, serum of individuals with IgA deficiency studied for the appearance of complexes after milk ingestion has been reported to be positive in three of seven subjects at 120 to 150 minutes after the milk feed.⁴¹ In another three subjects there was a tendency toward formation of two peak concentrations of complexes, the first at 30 to 60 minutes and the second at 120 to 150 minutes after drinking milk. The circulating immune complexes found in some of these seven patients contained bovine milk proteins.

Epithelial maturity. Columnar epithelial cells and their precursors form about 90% of the cell population in the small intestine⁴² and thus are of major importance to macromolecular barrier defense in the small intestine. Epithelial cell function has recently been assessed in newborn humans by Ducker et al.⁴³ These investigators used D-xylose absorption as a test of absorptive function. Thirty-five infants of differing gestational ages were given a standard dose of D-xylose per unit body weight on the second and sixth days of life. Preterm infants had a lower blood xylose level after the feed than did term infants. With increasing age, absorptive function as assessed by the D-xylose test improved. This study suggests that epithelial cells of the proximal small intestine may be functionally immature early in life, assuming that intestinal surface area per body weight does not change with gestational age.

Changes in membrane composition may determine whether bacteria or macromolecules bind to the cell. Studies of epithelial cell membranes have also been performed that indicate that cell membrane composition changes as the

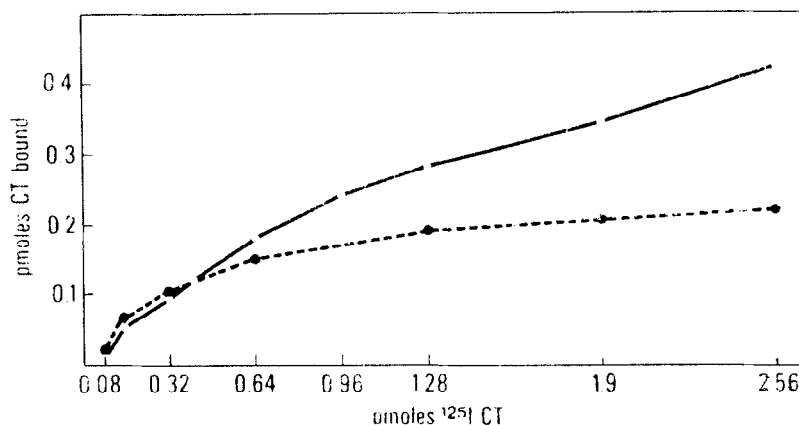


FIG. 4. Binding of ^{125}I -CT to adult (●) and newborn (○) membranes. Each point is the mean of at least eight experiments. CT binding to each preparation is significantly different beyond 0.96 pmol. (From Bresson JL, Pang KY, Walker WA. Microvillus membrane differentiation: quantitative difference in cholera toxin binding to the intestinal surface of newborn and adult rabbits. *Pediatr Res* 1984;18:984.)

epithelial cell migrates up the villus⁴⁴⁻⁴⁶ and as the animal matures.⁴⁷⁻⁴⁹

Bresson et al.⁵⁰ studied isolated microvilli membranes from the intestines of newborn and adult rabbits. They demonstrated that the membrane protein/phospholipid ratio is markedly decreased in newborn membranes compared with those of adults. In addition, they noted an increase in cholera enterotoxin binding to newborn membranes compared with that to adult small intestinal membranes (Fig. 4). These studies of the development of the intestinal surface may help us to understand why newborn infants absorb increased quantities of macromolecules and have a high incidence of some types of infectious diarrhea.

ANTIGEN TRANSPORT AND ALLERGIC DISEASE

Numerous studies of early feeding practices in humans have focused on the effect of breast- and bottle-feeding on the development of atopic dermatitis. Atopic dermatitis is a chronic, hereditary, and pruritic allergic cutaneous disorder frequently associated with asthma and allergic rhinitis.⁵¹ This disease may be the result of allergen absorption across the gastrointestinal or respiratory tract epithelium; however, the pathophysiology of the condition has not been elucidated. Most, but not all, patients with atopic dermatitis produce IgE antibodies in response to a variety of common food and inhalant allergens. The disease has a variety of clinical presentations. In infancy it usually appears as an acute eczematous eruption on the cheeks, and in childhood may occur as a chronic pruritic eczematous eruption confined to certain flexural areas (antecubital and popliteal fossae).

The influence of early feeding practices on the development of atopic dermatitis has been extensively studied. The first study was reported by Grulee and Sanford⁵² more than 40 years ago. These authors reviewed clinical records of more than 20,000 infants: 9749 breast-fed, 8605 partly breast-fed, and 1707 bottle-fed. The investigators noted that

the breast-fed infants has the lowest incidence of atopic dermatitis. The partly breast-fed and the bottle-fed infants had, respectively, two and seven times the incidence of atopic dermatitis found in the breast-fed infants. Although this report may be criticized because of the problems inherent in retrospective studies, it is the first to suggest that early feeding practices may influence the development of an allergic disorder.

Chandra,⁵³ in a prospective study, also evaluated the effect of breast-feeding on the incidence of atopic dermatitis. Newborn siblings of children with atopic disease who were exclusively fed breast milk or cow's milk formula for a minimum of 6 weeks were examined. The incidence of eczema, recurrent wheezing, and elevated serum IgE related to cow's milk was significantly lower in breast-fed infants compared with that in formula-fed matched controls. Saarinen et al.⁵⁴ and Saarinen and Kajosaari⁵⁵ have also found lower IgE levels in breast-fed infants compared with bottle-fed controls. However, they noted that the elevated IgE levels in the bottle-fed infants were not attributable to milk-specific IgE. Inasmuch as elevated IgE levels have been associated with an increased risk of subsequent allergic disease, Saarinen and colleagues concluded that their data provided circumstantial evidence in favor of breast-feeding as a method of reducing the prevalence of atopic dermatitis and allergic disease. Kramer and Moroz⁵⁶ have also examined the question of whether breast-feeding and delayed introduction of solid foods protects against the development of atopic eczema. These investigators were prompted to undertake their study because of methodologic flaws in earlier studies, including "insensitive study designs, non-blind observation and failure to control for confounding variables." To avoid these flaws they conducted a case-control study of 636 infants and children attending a dermatology clinic. The dermatologic condition of each patient was classified as (1) atopic eczema (case), (2) unrelated to atopy (control), or (3) of questionable relation to atopy (uncertain).

The feeding history was ascertained later, together with family history and demographic data, by an assistant blind to both the question under study and the case versus control status of each subject. The investigators found that breast-feeding was not associated with any reduction in the estimated relative risk for developing atopic dermatitis. Rasmussen and Provost⁵¹ agree with these investigators, and state that nearly 50% of infants and children with atopic dermatitis have no reaction to scratch tests with milk or other common foods, and a positive challenge with a suspect food produces hives not eczema. They believe that dietary restrictions and breast-feeding are of little, if any, benefit in the great majority of infants with atopic dermatitis.

One confounding factor not considered in any of these studies is maternal diet; this alone may account for the conflicting results. Saarinen and Kajosaari⁵⁵ have noted that trace amounts of antigens in human milk may be enough to sensitize an infant. Gamo et al.⁵⁷ found cow's milk proteins in human milk by the use of passive cutaneous anaphylaxis testing. Jakobsson and Lindberg⁵⁸ tested breast milk from 18 mothers ingesting a diet containing cow's milk. Thirteen of the 18 breast milk samples were positive for cow's milk protein by double-diffusion immunoprecipitation. Lake et al.⁵⁹ recently reported six breast-fed infants in whom inflammatory proctocolitis, evidenced clinically by bloody diarrhea and confirmed by biopsy examination, developed in the first month of life. All six infants had been born at term and had normal perinatal courses. No toxic, bacterial, viral, or parasitic cause was established. All infants responded clinically to initiation of feeding with a formula with either hydrolysed casein or soy protein base. Breast-feeding was subsequently resumed in five of the six infants; all experienced immediate recurrence of symptoms. Elimination of cow's milk protein from the maternal diet led to tolerance of breast-feeding in two of three infants. The authors concluded that cow's milk protein may be absorbed intact across the maternal intestine and may occasionally cause enterocolitis in exclusively breast-fed infants.

REFERENCES

1. Walker WA. Intestinal transport of macromolecules. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*. New York, Raven Press, 1981:1271.
2. Walker WA, Bloch KJ. Gastrointestinal transport of macromolecules in the pathogenesis of food allergy. *Ann Allergy* 1983;51:240.
3. Udall JN, Walker WA. The physiologic and pathogenic basis for the transport of macromolecules across the intestinal tract. *J Pediatr Gastroenterol Nutr* 1982;1:295.
4. Lust F. Die durchlässigkeit des Magendarmkanales für heterologe Eiweiße bei ernährungsgestörten Säuglingen. *Jahrb Kinderheilk* 1913;77:243.
5. Schloss OM, Worthen TW. The permeability of the gastrointestinal tract of infants to undigested protein. *Am J Dis Child* 1916;11:342.
6. Robertson DM, Paganelli R, Dinwiddie R, Levinsky RJ. Milk antigen absorption in the preterm and term neonate. *Arch Dis Child* 1982;57:369.
7. Beach RC, Menzies IS, Clayden GS, Scopes JW. Gastrointestinal permeability changes in the preterm neonate. *Arch Dis Child* 1982;57:141.
8. Ammann AJ, Stiehm ER. Immune globulin levels in colostrum and breast milk, and serum from formula- and breast-fed newborns. *Proc Soc Exp Biol Med* 1966;122:1098.
9. Kenny JF, Boesman MI, Michaels RH. Bacterial and viral coproantibodies in breast-fed infants. *Pediatrics* 1967;39:202.
10. Kraehenbuhl JP, Campiche MA. Early stages of intestinal absorption of specific antibodies in the newborn: an ultrastructural, cytochemical and immunological study in the pig, rat and rabbit. *J Cell Biol* 1969;42:345.
11. Iyengar L, Selvaraj RJ. Intestinal absorption of immunoglobulins by newborn infants. *Arch Dis Child* 1972;47:411.
12. Vukavic T. Intestinal absorption of IgA in the newborn. *J Pediatr Gastroenterol Nutr* 1983;2:248.
13. Ogra SS, Weintraub D, Ogra PL. Immunologic aspects of human colostrum and milk. III. Fat and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. *J Immunol* 1977;119:245.
14. Goldstein JL, Anderson RGW, Brown MS. Coated pits, coated vesicles and receptor-mediated endocytosis. *Nature* 1979;279:679.
15. Rodewald R. Selective antibody transport in the proximal small intestine of the neonatal rat. *J Cell Biol* 1970;45:635.
16. Rodewald R. Intestinal transport of peroxidase-conjugated IgG fragments in the neonatal rat. In: Hemmings WE, ed. *Materno-fetal transmission of immunoglobulins*. New York, Cambridge University Press, 1976:137.
17. Kaplan J. Polypeptide-binding membrane receptors: analysis and classification. *Science* 1981;212:14.
18. Silverstein SC, Steinman RM, Cohn ZA. Endocytosis. *Ann Rev Biochem* 1977;46:669.
19. Owen RL, Jones AL. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology* 1974;66:189.
20. Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructure study. *Gastroenterology* 1977;72:440.
21. Kraft SC, Rothberg RM, Knauer CM, Svoboda AC, Monroe LS, Farr RS. Gastric acid output and circulating antiovine serum albumin in adults. *Clin Exp Immunol* 1967;2:321.
22. Bloch KJ, Bloch DB, Stearns M, Walker WA. Intestinal uptake of macromolecules. VI. Uptake of protein antigen in vivo in normal rats and in rats infected with *Nippostrongylus brasiliensis* or subjected to mild systemic anaphylaxis. *Gastroenterology* 1979;77:1039.
23. Grand RJ, Watkins JB, Torti FM. Development of the human gastrointestinal tract. *Gastroenterology* 1976;70:790.
24. Walker WA, Wu M, Isselbacher KJ, Bloch KJ. Intestinal uptake of macromolecules. IV. The effect of pancreatic duct ligation on breakdown of antigen and antigen-antibody complexes on the intestinal surface. *Gastroenterology* 1975;69:1223.
25. Saffran M, Franco-Saenz R, Kong A, Papahadjopoulos D, Szoka F. A model for the study of the oral administration of peptide hormones. *Can J Biochem* 1979;57:548.
26. Crane CW, Luntz GR. Absorption of insulin from the human small intestine. *Diabetes* 1968;17:625.
27. Antonowicz I, Lebenthal E. Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. *Gastroenterology* 1977;72:1299.
28. Zoppi G, Andreotti G, Pajno-Ferrara FA, Njai DM, Gaburro D. Exocrine pancreas function in premature and full term neonates. *Pediatr Res* 1972;6:880.

29. Udall JN, Colony P, Fritze L, Pang K, Trier JS, Walker WA. Development of gastrointestinal mucosal barrier. II. The effect of natural versus artificial feeding on intestinal permeability to macromolecules. *Pediatr Res* 1981;15:245.
30. Widdowson EM, Colombo VE, Artavanis CA. Changes in the organs of pigs in response to feeding for the first 24 hours after birth. II. The digestive tract. *Biol Neonate* 1976;28:272.
31. Heird WC, Hanson IH. Effect of colostrum on growth of intestinal mucosa. *Pediatr Res* 1977;11:406.
32. Walker WA, Isselbacher KJ. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders. *Gastroenterology* 1974;67:531.
33. Chandra RK. Immunological aspects of human milk. *Nutr Rev* 1978;36:265.
34. Ogra PL. Ontogeny of the local immune system. *Pediatrics* 1979;64:765.
35. Walker WA, Isselbacher KJ. Intestinal antibodies. *N Engl J Med* 1977;297:767.
36. Allansmith M, McClellan BH, Butterworth M, Maloney JR. The development of immunoglobulin levels in man. *J Pediatr* 1968;72:276.
37. Selner JC, Merrill DA, Claman HN. Salivary immunoglobulins and albumin: development during the newborn period. *J Pediatr* 1968;72:685.
38. Haneberg B, Aarskog D. Human faecal immunoglobulins in healthy infants and children, and in some diseases affecting the intestinal tract or the immune system. *Clin Exp Immunol* 1975;22:210.
39. Burgio GR, Lanzavecchia A, Plebani A, Jayakar S, Ugazio AG. Ontogeny of secretory immunity: levels of secretory IgA and natural antibodies in saliva. *Pediatr Res* 1980;14:1111.
40. Cunningham-Rundles C, Brandeis WE, Good RA, Day NK. Milk precipitins, circulating immune complexes and IgA deficiency. *Proc Natl Acad Sci USA* 1978;75:3387.
41. Cunningham-Rundles C, Brandeis WE, Good RA, Day NK. Bovine antigens and the formation of circulating immune complexes in selective immunoglobulin A deficiency. *J Clin Invest* 1979;64:272.
42. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. *Am J Anat* 1974;141:461.
43. Ducker DA, Hughes CA, Warren I, McNeish AS. Neonatal gut function, measured by the one hour blood D(+) xylose test: influence of gestational age and size. *Gut* 1980;21:133.
44. DeBoth NJ, Van Der Kamp AW, Van Dongen JM. The influence of changing crypt cell kinetics on functional differentiation in the small intestine of the rat: nucleotide and protein synthesis. *Differentiation* 1975;4:175.
45. Raul F, Simon P, Kedinger M, Haffen K. Intestinal enzyme activities in isolated villus and crypt cells during postnatal development of the rat. *Cell Tissue Res* 1977;176:167.
46. Quaroni A, Kirsch K, Herscovics A, Isselbacher KJ. Surface-membrane biogenesis in rat intestinal epithelial cells at different stages of maturation. *Biochem J* 1980;192:133.
47. Lojda Z. Cytochemistry of enterocytes and of other cells in the mucous membrane of the small intestine. In: Smyth DH, ed. *Biomembranes*, vol 4A. Amsterdam: Excerpta Medica, 1974:43.
48. Toofanian F, Kidder DE, Hill FWG. The postnatal development of intestinal disaccharidases in the calf. *Res Vet Sci* 1974;16:382.
49. Etzler ME, Branstrator ML. Cell surface components of intestinal epithelial cells and their relationship to cellular differentiation. *Ciba Symposium No. 70: Development of mammalian absorptive processes*. Amsterdam: Excerpta Medica, 1979:51.
50. Bresson JL, Pang K, Udall J, Fritze L, Walker WA. Evidence for increased enterotoxin binding to newborn microvillus membrane: a possible explanation for enhanced toxigenic diarrhea in infancy. *Gastroenterology* 1980;79:1145.
51. Rasmussen JE, Provost TT. Atopic dermatitis. In: Middleton CE Jr, Reed CE, Ellis EF, eds. *Allergy: principles and practice*, vol 2. St. Louis: CV Mosby, 1978:1039.
52. Grulee CG, Sanford HN. The influence of breast and artificial feeding on infantile eczema. *J Pediatr* 1936;9:223.
53. Chandra RK. Prospective studies of the effect of breast feeding on incidence of infection and allergy. *Acta Paediatr Scand* 1979;68:691.
54. Saarinen UM, Kajosaari M, Backman A, Siimes MA. Prolonged breast-feeding as prophylaxis for atopic disease. *Lancet* 1979;2:163.
55. Saarinen UM, Kajosaari M. Does dietary elimination in infancy prevent or only postpone a food allergy: a study of fish and citrus allergy in 375 children. *Lancet* 1980;1:166.
56. Kramer MS, Moroz B. Do breast feeding and delayed introduction of solid foods protect against subsequent atopic eczema? *J Pediatr* 1981;98:546.
57. Gamo I, Yabuuchi H, Nishida M, Ikeda T, Ichihara H, Toyoshima K. Studies on prophylaxis of cow's milk allergy by digestive enzymes. *Acta Paediatr Jap* 1966;8:1.
58. Jakobsen I, Lindberg T. Cow's milk as a cause of infantile colic in breast-fed infants. *Lancet* 1978;1:437.
59. Lake AM, Whittington PF, Hamilton SR. Dietary protein-induced colitis in breast-fed infants. *J Pediatr* 1982;101:906.