MINIREVIEW

Intestinal Pathophysiology in Autism

JOHN F. WHITE[1]

Department of Physiology, Emory University, Atlanta, Georgia 30322

Autism is a life-long developmental disorder affecting as many as 1 in 500 children. The causes for this profound disorder are largely unknown. Recent research has uncovered pathology in the gastrointestinal tract of autistic children. The pathology, reported to extend from the esophagus to the colon, is described here along with other studies pointing to a connection between diet and the severity of symptoms expressed in autism. The evidence that there is impaired intestinal permeability in autism is reviewed, and various theories are discussed by which a leaky gut could develop. Lastly, some possible ways in which impaired gastrointestinal function might influence brain function are discussed. Exp Biol Med 228:639–649, 2003

Key words: autism; intestine; casein; celiac disease; endoscopy; gluten; glycosaminoglycan; immunoglobulin; secretin; vaccine

Autism is the most prevalent of a subset of disorders organized under the umbrella of pervasive developmental disorder (PDD). Autism is a serious developmental disorder characterized by profound deficits in language, communication, and socialization, resistance to learning, and displays of stereotypical behavior including perseveration. The disorder is accompanied by mental retardation in three out of four patients (1). Boys are three times more likely than girls to receive the diagnosis. One out of three autistic individuals experience epileptic seizures (2). The diagnosis of autism is usually conferred when the child is 2 to 3 years old after extensive evaluation according to the criteria of the Diagnostic Statistical Manual IV (DSM-IV) (3). The children either exhibit a failure to advance from birth or, after a period of apparently normal growth, suffer a loss of newly acquired skills (language, eye-to-eye contact, and sociability). The latter pattern, seen in about one-third of autistic children (4), is referred to in different terms, including "regressive autism" (5).

It is generally agreed that there are multiple causes for autism. Some of the causes of autism are well established, including a strong genetic link in the cases, of tuberous sclerosis, fragile X, and some other disorders (1). The view that there is a strong genetic basis for the disorder stems from the observation that siblings of autistic offspring have a higher incidence of autism than the general population. A strong genetic link has been particularly inferred from studies of autistic children born as twins. Thus, monozygotic (identical) twins demonstrate a concordance for autism exceeding 90% (6). The concordance of less than 100% has been interpreted as evidence that some other factor such as an environmental challenge must be congruent with the genetic susceptibility before the disorder is expressed (7). Recently, the apparently large strength of the genetic influence in autism was called into question when a database of information on a large cohort of families having at least two siblings with autism was evaluated. A remarkably high proportion of twin pairs was observed. For example, there was a 10-fold greater frequency of monozygotic twins than the highest population frequencies that could be expected. It was concluded that twins have an increased risk of autism. Accordingly, Greenberg *et al.* (8) suggested that earlier estimates of the role of genes in autism, predicated in part on the twin data, may have been overstated, i.e., it was not appreciated that the incidence is higher in twins. Furthermore, it was concluded that the influential genes may reside in the parents genome rather than that of the autistic offspring (8). For example, environmental conditions in the womb such as competition for nutrients may be the greater influence.

Notwithstanding these interesting new findings, the cause of the disorder for the great majority of autistic individuals has not been determined. The term "autistic syndrome" is intended to describe a pattern of similar behaviors produced by a variety of different insults. The need to understand the causes of autism and the underlying patho-

[1] To whom requests for reprints should be addressed at Department of Physiology, Emory University, Atlanta, Georgia 30322. E-mail: jfwhite@physio.emory.edu

1535-3702/03/2286-0639$15.00

physiology has become more acute since the number of diagnosed cases has risen markedly in recent years (9–11). Gillberg and Wing (12) reviewed studies originating from several different countries in the period from 1966 to 1997 and reported that 18 studies of non-U.S. populations showed a highly significant ($P < 0.001$) increase in prevalence of autism of 3.8% per year. In a more recent review, Wing and Potter (13) offered evidence that, historically, autism has been underdiagnosed. They attributed most of the rise in the incidence of autism to changes in diagnostic criteria and increasing awareness of autism spectrum disorders. However, they acknowledged the possibility "that there is a real and continuing rise" in the number of cases. Unfortunately, the number of well-controlled clinical studies of the autistic population that have provided clues to the etiology of the disorder are very limited. Possibly, a breakthrough will result after publication of several studies reporting new observations of significant gastrointestinal pathology in autism or in attention deficit hyperactivity disorder (14–17). In this review, the new observations, particularly those on the intestinal tract, are described, as well as earlier studies indicating a link between gastrointestinal function and autism. Possible scenarios that could produce the impaired intestinal function are discussed, and some possible ways in which altered gut function could influence behavior are considered. Recent efforts to identify potential susceptibility genes for autism are not discussed in this review. The hope of the author is that this review will serve to spur interest in the study of gastrointestinal function as it relates to autism. The ultimate goals are to understand the etiology of autism and to provide clinical remedies for this severely handicapping disorder.

Children with Autism Have Gastrointestinal (GI) Pathology

In a study published in 1998, 12 children diagnosed with autism (all of the regressive pattern) and exhibiting a variety of GI ailments, including abdominal pain, diarrhea, and bloating, were examined extensively (14). The GI symptoms had developed coincident with the onset of autistic behavior, according to the parents. Endoscopy revealed that 10 of the 12 children displayed ileal lymphoid nodular hyperplasia (LNH). Lymph nodules are encapsulated bodies lying within the submucosa of the intestinal wall. Lymph nodules contain lymphocytes and neutrophils. The fluid absorbed from the intestinal lumen by the action of the absorptive epithelial cells is filtered through the lymph nodes. Here, antibodies are formed. Of the 12 children, eight also displayed abnormalities in the mucosa, the region consisting of the absorptive epithelium, underlying connective tissue, and muscularis mucosae. Mucosal abnormalities included granularity, loss of vascular pattern, and patchy erythema (nonspecific colitis). The findings were supported by histological examinations of mucosal biopsies. Cerebral magnetic resonance imaging (MRI) and electroencephalography (EEG) revealed no neurological abnormalities in the children.

In a more recent publication, these same researchers reported their observations on an expanded group of 60 children affected with various developmental disorders (15). The subject group included 50 children diagnosed with autism (including the 12 children from the original study) as well as five with Asperger's syndrome (autism without retardation) and two with disintegrative disorder. All but one of the 60 children had GI symptoms, including abdominal pain, constipation, diarrhea, and bloating. Findings were compared with those from a group of 37 developmentally normal (nonautistic) children with similar GI symptoms (the control group). It was observed that ileal LNH presented in 93% of affected children and 14.3% of control children. Colonic LNH was present in 30% of affected children and 5.4% of control children. Hyperplasia of the intestinal lymph nodes was found in 88.5% of biopsies of affected children. Active inflammation of the ileum (ileitis) was observed in 8% and chronic inflammation of the colon (colitis) was seen in 88% of affected children. The authors characterized the pathology as "a subtle new variant of inflammatory bowel disease that lacks the specific diagnostic features of either Crohn's disease or ulcerative colitis." In a comment on the Wakefield paper, Sabra *et al.* (16) reported identical pathology (LNH) in the terminal ileum of two patients diagnosed with food allergies and attention deficit hyperactivity disorder.

In yet another study, Horvath and coworkers (17) used endoscopy with biopsy to examine the upper GI tract of 36 children diagnosed with autism and experiencing abdominal pain, chronic diarrhea, bloating, nighttime awakening, or unexplained irritability. Abnormal findings included reflux esophagitis in 25 of the children, chronic gastritis in 15, and chronic duodenitis in 24. Low activity of intestinal carbohydrate digestive enzymes was observed in 21 children, whereas 27 exhibited increased exocrine secretion of pancreatic-biliary fluid after intravenous administration of the GI hormone secretin. Secretin, a peptide hormone released by endocrine cells within the duodenal mucosa, promotes sodium bicarbonate and water secretion by the pancreas. It is important to note that this study describes altered function in the upper GI tract of autistic children, whereas the lymphoid nodular hyperplasia described by Wakefield *et al.* (14, 15) was observed in the lowest portion of the small intestine, namely the ileum. The results of these different studies taken together suggest that significant and widespread GI pathophysiology may accompany autism, at least within a subpopulation of patients. As discussed below, the pathology may be central to the etiology of autism. Alternatively, it may simply be a secondary consequence of the disorder. In either case, it is possible that such widespread pathology plays a major role in the symptomatology of the disorder in the affected children. It is crucial that these endoscopic analyses be conducted in other laboratories. In the meantime, these limited reports constitute a potentially

important new avenue of research in the effort to understand autism, its causes, and symptomatology.

In contrast with the findings above, a very recent study in the United Kingdom, which examined the early medical records of 66 children who were later diagnosed with autism and 30 children later diagnosed with "possible autism," reported that they did not indicate the presence of GI inflammation, celiac disease, food intolerance, or recurrent GI symptoms at a higher rate than were reported in the early medical records of 449 (control) children who did not subsequently develop autism (18). The authors concluded that there was not a substantial association between GI illness in children and the development of autism. However, the authors acknowledged that some children may have had subclinical GI symptoms that were overlooked and, furthermore, that severe GI disease may be associated with autism in certain individuals.

Earlier Studies Point to a Linkage Between Gut Function and Autism

There have long been indications that autistic children had impaired GI tract function. Anecdotal reports by parents extending back more than 30 years described evidence that their autistic children suffered disturbed GI tract function and intolerance to certain foods (19). In a recent survey of 500 parents of autistic children, almost one-half reported that their children had loose stools or frequent diarrhea (20). Food intolerance was noted particularly for wheat and cow's milk. In one early study, an autistic child was coincidentally afflicted with celiac disease, a disorder characterized by marked atrophy of the intestinal villi caused by a response of the intestinal immune system to gliadin, a peptide in gluten (19). The villi, which are projections of the intestinal mucosa into the lumen of the intestine, become shortened and denuded of surface epithelium by dietary gluten in affected individuals. Gluten is a protein found in wheat and barley (21); celiac disease is treated by eliminating gluten from the diet. When the autistic child was reexposed to gluten in the diet after a period on a gluten-free diet, his autistic symptoms were observed to worsen. The observation inspired a study in which transcephalic direct current (TDC) potentials were measured in several autistic children with histories of episodes of colic, diarrhea, dehydration, and food intolerance but lacking the diagnosis of celiac disease (19). TDC potentials are slowly changing voltages recorded from the surface of the head and originating from the cortex of the brain. When the children were exposed to an oral dose (1 g) of gliadin, frontal voltage was significantly inhibited compared with that produced by 1 g of sugar. Normal children and normal siblings of autistic children did not show this response. This report suggests that gliadin or its metabolites gains access to and has a direct effect upon the central nervous system (CNS) in these children. A CNS effect of gluten is also suggested by a recent report that in (nonautistic) celiac patients, dietary gluten produces ataxia (lack of coordination) and is associated with reactivity of antigliadin antibodies with their cerebellar Purkinje cells (22). The incidence of ataxia in celiac patients is 6% to 10%. Ataxia is not a diagnostic indicator of autism. CNS effects of gluten (or gluten metabolites) in neurological disorders may be quite common. Thus, a recent text chapter on autism listed several reports that associated celiac disease with "an extraordinary range of psychiatric and neuropathologic conditions" (23).

In two separate studies involving a large number of autistic patients, it was noted that an improvement of social, cognitive, and communication skills occurred when they were placed on a diet free of gluten and cow's milk or a diet free of cow's milk alone. In the first study, several objective measures of behavior were reported to improve when autistic children were placed on an elimination diet free of cow's milk (24). Significant symptom improvement was exhibited by 36 autistic patients in five of seven objective behavior scales 8 weeks after placement on a cow's milk elimination diet. In another study, 15 subjects with autistic syndromes were placed on a diet for a period of 4 years that was reportedly free of gluten and casein (25). Casein is the major protein of milk. Previously, all of the children exhibited pathological urine patterns and increased levels of peptides in their urine. Differences in urinary peptides levels in autistic patients had been reported by others in earlier studies (26, 27). The same research group subsequently reported that some of the urinary peptides derived from gluten and casein (28). After 1 year on the diet, a statistically significant improvement in social, cognitive, and communication skills was observed. Also, urine patterns and urine peptide levels normalized. Further significant improvements in behavior were observed after 4 years on the diet. Unfortunately, neither study controlled for the possibility that the improvements were dependent upon parallel instructional and other behavioral interventions. Also, objective measures of the extent to which the diet was free of gluten and casein were not reported. Nevertheless, these structured studies as well as anecdotal reports by parents of perceived behavioral improvements after dietary restrictions have stimulated research to determine whether the intestinal mucosal barrier was incompetent in autistic individuals, allowing selected foods or their metabolic products, impermeant in the normal human gut, to gain access to the interstitial fluid and either initiate immune reactions or produce pathology.

The small intestinal mucosa normally acts as a barrier to prohibit many substances within the intestinal lumen from entering the blood (29). The luminal membrane of the simple columnar epithelial cells, which line the mucosal surface (primarily enterocytes and goblet cells), as well as the tight junctional complexes linking the epithelial cells to their neighbors are the principal barriers to the free movement into the blood of dietary foods and the products of their hydrolysis released during intraluminal digestion. These architectural features embody the "intestinal luminal barrier." A functional method of assessing the physical in-

tegrity of the luminal barrier is to measure the ability of small sugar molecules, orally administered, to gain entry into the blood and, eventually, to be excreted into the urine (30). The sugar permeability test involves the simultaneous oral administration of two sugars, usually mannitol and lactulose, followed by the measurement of the ratio of mannitol:lactulose recovered in the urine. Mannitol is a monosaccharide that is relatively poorly absorbed by the human intestine because it has no affinity for the glucose-galactose carrier protein molecules that reside in the apical (luminal) brush border membrane of small intestinal enterocytes. Mannitol molecules pass through the luminal membrane by way of aqueous pores in the brush border membrane (31). Lactulose, a larger dissacharide molecule, also lacks affinity for the carrier and is too large to pass through the pores. Lactulose molecules, which do reach the blood, do so by passing between the epithelial cells (i.e., the paracellular pathway) and through zones of cell extrusion at the tip of the villus. The paracellular pathway is also the presumed route of passage that peptides such as gliadin take through a damaged intestinal mucosa. Using the sugar permeability test, abnormal intestinal permeability has been found in patients with recognized intestinal disorders (32, 33). More recently, D'Eufemia *et al.* (34) applied the test to patients with autism. They reported that intestinal permeability was significantly increased (i.e., greater permeability to lactulose relative to mannitol) in nine of 21 high-functioning autistic patients (4-16 years of age) when compared with a group of 40 healthy, age-matched children (34). The change in the urinary mannitol:lactulose ratio in 43% of the autistic children was accounted for solely by an increase in the lactulose permeability; there was no difference in mannitol recovery between the groups. The authors proposed that the elevated lactulose permeability reflects damage to the tight junctions linking the intestinal epithelial cells in the affected autistic patients. These results, in conjunction with those implicating dietary gluten and casein causally in autistic symptoms, are integrated into the view that products resulting from the incomplete hydrolysis of dietary gluten and casein penetrate the mucosal barrier through abnormally leaky tight junctions.

An additional route by which dietary proteins such as gluten and casein may enter the submucosa is via the microfold (M) cell pathway (35). The immune system of the GI tract is organized in the lymph nodules, which, in the ileum, are aggregated in groups called Peyer's patches. As noted above, the lymph nodules lie below the epithelial cell layer, displacing the muscularis mucosae and forming folds in the mucosa. The epithelial cell layer contains, in addition to enterocytes and goblet cells, M cells. M cells are specialized to transport antigens and microorganisms transcellularly from the lumen into the subepithelial space. In the subepithelial space, the antigens and microorganisms interact with the cells of the immune system (35). The B cells of the lymph nodules respond to the presence of antigens in the diet by secreting antibodies, also called immunoglobulins (Ig's). Of the five classes of antibodies (IgA, IgM, IgG, IgD, and IgE), IgA antibodies are secreted by the digestive tract. Whether intact dietary protein molecules or their derivative peptides penetrate the mucosal barrier through the M cells or through and between the enterocytes, their interaction with the immune system is indicated by the fact that, in two reports, autistic patients reportedly exhibited significantly higher levels of IgA for dietary casein (24, 36), gluten (36), lactalbumin (24), and β-lactoglobulin (24). IgG was elevated for casein (24) and gluten (36); IgM was increased for casein (24). Removal of the dietary challenge reduced the immune reaction. This response of the immune system provides additional evidence that the autistic intestine is abnormally permeable to gluten and casein.

The Leaky Gut Hypothesis

The idea that the integrity of the intestinal mucosal lining, referred to as the intestinal mucosal barrier, is compromised in autism is embodied in the "leaky gut hypothesis." According to this hypothesis, the intestinal mucosa is abnormally permeable in autism. Digestion products of natural foods such as cow's milk and bread are able to enter the blood through the leaky mucosa and induce antigenic responses, as well as interfere directly with the central nervous system. It has been established that digestion of dietary gluten and casein in the lumen of the small intestine by the action of pancreatic and intestinal peptidases releases short chain peptides, which are structurally similar to endorphins. These products are called exorphins to reflect their dietary origin (37). Gliadomorphins are a family of exorphins released from the partial digestion of the wheat protein gliadin. Similarly, casomorphins are a family of exorphins released upon partial digestion of the milk protein casein (38). Casomorphins and gliadomorphins are potent psychosis-inducing factors (39). They are also very stable epitopes. Noting that schizophrenic patients had difficulty with dietary gluten, Dohan (40) hypothesized that there is a defect in the intestinal barrier of these patients that allows passage of neuroactive peptides of food origin into the blood and then into the cerebrospinal fluid to interfere directly with the function of the CNS. This hypothesis may be applicable to autistic patients as well because one member of the family of casomorphins, β-casomorphins-7, is reported to be elevated in the urine of autistic patients (28). Evidence that the exorphins are able to enter the mammalian CNS was reported by Hemming (41) who detected orally administered gluten fragments in rat brains. Furthermore, brain opiate receptors reportedly bind gluten exorphins (37). When infused into the blood stream of rats, β-casomorphin-7 activates an immediate early gene (c-Fos) in several regions of the rat brain, indicating that the exorphin not only gains access to the brain but activates brain cells as well (42).

Further supporting the notion that natural foods passing through a leaky intestinal mucosa play a role in producing the behavioral symptomatology of autism, the studies critiqued above reported that improvements in behavior, in-

cluding reduced incidence of seizures in autism, were seen after the introduction of a diet free of gluten (34) or a diet free of milk and gluten (25, 36). Schizophrenic patients, who likewise often exhibit elevated levels of IgA's for gliadin, casein, and β-lactoglobulin (43) and appear to have a higher than usual incidence of celiac disease (44), have also been reported to benefit behaviorally from a similar diet (45–47). Unfortunately, a gluten-free and casein-free diet (the "gfcf" diet), although reportedly ameliorating behaviors for many of the affected schizophrenic individuals, does not totally eliminate those behaviors. Hence, the diet does not represent a cure. Moreover, the failure to completely eliminate the behaviors raises the possibility that elevated exorphins cause permanent damage to the infant brain.

What is the Nature of the Change in Intestinal Permeability in Autism?

Pathological inflammation of the intestinal mucosa has long been recognized as a primary symptom in celiac disease and inflammatory bowel disease. The recent work detailed above indicates that there is also pathological inflammation of the ileum in autism (14–17), although there is no evidence to support the view that gluten or casein cause the inflammation. Intestinal inflammation can be regarded as the consequence of the disruption of the complex interaction between all of the cells of the mucosa (immune and nonimmune), as well as the extracellular matrix, the normal interactions being mediated by cell surface and paracrine molecules (48). The intestinal inflammation seen in Crohn's disease and ulcerative colitis is associated with disruption of the glycosaminoglycans that comprise vascular tissue, connective tissue, and the basal lamina of epithelia (49). Glycosaminoglycans are polysaccharides, which include heparin sulfate, dermatin sulfate, and chondroitin sulfate, as well as sialic acid residues. As highly anionic structural components of the connective tissue matrix and wall of blood vessels, they render the tissues electrostatically negative and thereby restrict blood proteins from exiting into the interstitial space and intestinal lumen. Deficiencies in the activity of the enzyme phenylsulfotransferase, which normally maintains sulfation, have been reported in autistic subjects (50, 51). The disruption of glycosaminoglycans and associated loss of tissue sulfation observed to occur in inflammatory bowel disease (52) may contribute to the generalized increase in intestinal permeability that occurs with leakage of protein and fluid across the wall of the intestine in that disease, although such an association is far from established. In Crohn's disease, blood proteins are lost into the intestinal lumen and are excreted into the feces; the extent of protein loss correlates positively with the length of the bowel that is inflamed (53). In the study by Wakefield *et al.* (14) examination of histological sections of intestinal tissue from autistic patients revealed a near doubling of the number of lymphoid follicles, as well as follicle enlargement and merging of adjacent follicles. More recently, Furlano *et al.* (54) reported that neutrophils and lymphocytes infiltrated the epithelium overlying the follicles; neutrophils also infiltrated the crypt epithelium that lies between the villi and provides a constant supply of new epithelial cells for the villi. Also, the intestinal epithelium was significantly more ulcerated and eroded in autistic versus normal subjects. Lastly, the basement membrane thickness was increased, whereas the density of sulfated glycosaminoglycans in the basement membrane and epithelium was greatly reduced. Taken together, these findings indicate pathological inflammation of the intestine in these autistic subjects. Although the findings would seem to provide evidence that the mucosal barrier is compromised in autism, no direct evidence of an alteration of the mucosal intestinal barrier, as could have been gained using the sugar permeability test, was sought in these studies. It remains to be seen whether a dietary antigen plays a direct role in altering intestinal permeability (see below) or simply gains access to the blood through a mucosa that has been made more permeable as a consequence of some other sequence of events.

Possible Pathways by Which Intestinal Function May Become Impaired

Below, several hypotheses are described that have been advanced to account for the intestinal pathophysiology observed in autism. Evidence for each of these hypotheses is based upon a very limited number of basic and clinical research studies of varying rigor and conclusivity. None of the hypotheses has gained sufficient support to broadly influence clinical treatment of autistic patients.

Antigens in the Diet. Inasmuch as gliadin, the peptide derived from gluten, is known to extensively damage the intestinal mucosa in celiac disease and there is some limited evidence that autistic symptoms of many individuals are exacerbated by a diet that includes gluten, it is possible that gliadin produces the increase in intestinal permeability seen in autism. Children with cow's milk allergy also exhibit inflammation of the intestinal lamina propria and partial villous atrophy after ingesting milk; inflammation is reversed when milk is excluded from the diet (55, 56). Hence, milk proteins may also cause changes in gut permeability, accounting for the intolerance to this food in autism. The work of D'Eufemia *et al.* (34) described above offered some support for the view that gluten enhances intestinal permeability by increasing the permeability of the paracellular pathway, i.e., the route between the enterocytes. There was no evidence that the enterocytes themselves were rendered more permeable, as occurs in celiac disease, wherein a near total loss of the intestinal cell lining is manifest. In keeping with this distinction, the pathology reported to occur in the ileum of autistic subjects (14–17) is much more subtle than that observed in celiac disease. These considerations do not disallow a role for gluten and casein in intestinal hyperpermeability. For example, these proteins or their digestive products (gliadomorphins and casomorphins) may access the submucosa via the M cell pathway and produce antigenic responses that damage the mucosa. Two cytokines

released by mononuclear cells of the immune system upon exposure to antigens have been reported to increase intestinal permeability in experimental preparations. Thus, interferon-γ increased epithelial permeability in cultured cell lines of intestinal origin grown into confluent monolayers (57–59). Similarly, tumor necrosis factor α (TNF-α) released from mononuclear cells of infants with cow's milk allergy elevated tissue conductance and the transepithelial flux of the macromolecular marker horseradish peroxidase (HRP), as well as the flux of mannitol and sodium ions in a cultured cell line (60). The effect produced by TNF-α was potentiated by interferon-γ. The changes in intestinal permeability induced by the cytokines are suggestive of an increased transepithelial transit through the paracellular pathway. In a separate study, the effect of interferon-γ was seen to operate through elevation of cytosolic levels of nitric oxide in the target tissues (57). The manner in which food antigens may alter intestinal function and produce intestinal pathology in allergic individuals was recently discussed (61). Lacking is any evidence that gliadin and milk proteins, or their products, produce these cytokines in the autistic gut.

Vaccines. The intestinal pathology noted in the study of Wakefield *et al.* (14), namely, the hyperplastic lymph nodes in the ileum and colon, suggests that the immune system of the gut has been seriously challenged in the autistic patients. Although Wakefield *et al.* did not draw a conclusion as to the cause of the pathology, they noted parental reports that the children had received the trivalent measles-mumps-rubella (MMR) vaccine before undergoing behavioral regression. Likewise, Fudenburg (50) and Gupta (52) noted in separate reports a close temporal association between the administration of the MMR vaccine and the onset of autistic symptoms in patients under their care. The genomic RNA of the vaccine strain of measles virus was detected in peripheral mononuclear cells of three out of nine autistic patients examined (62). Very recently, 75 of 91 patients with developmental disorders and diagnosed with ileal LNH and enterocolitis were reported positive for measles virus in their intestinal tissue compared with five of 70 control patients (63). The measles virus was localized within cells of the immune system of hyperplastic lymph follicles. Previously, Lewin *et al.* (64) and Miyamoto *et al.* (65) reported detection of measles virus in tissue from patients with Crohn's disease. In contrast, Iizuka *et al.* (66) found no evidence of measles virus in a population of Crohn's patients. The reason for these very different results is unclear and underlines the need for further investigations. Parenthetically, after measles immunization of children, the primary cytokine produced is interferon-γ (67). The effect of interferon-γ to increase permeability of monolayers of cultured intestinal cells was discussed above. The studies suggesting that the MMR vaccine may be an etiological factor in autism have enormous implications for public health. Hence, they have prompted others to exam retrospectively several epidemiological databases of different populations to exam the relationship between vaccinations and the prevalence of autism. Fully five separate studies have failed to find any significant correlation between these variables (9, 11, 68–70). For example, after reviewing the case history of 473 autistic children in London (UK) over a 20-year period starting from 1979, Taylor *et al.* (11) found no significant change in the proportion of children with developmental regression or bowel problems before and after introduction (in 1988) of the MMR vaccine. Similarly, Dales *et al.* (9) found no correlation between the upward trend of early autism caseload in the period of 1980 to 1994 and childhood MMR immunization rates in California school children given the vaccine by the 17th or 24th month of age. Hence, this approach affords little support for a role of the MMR vaccine in producing autism. However, it is noteworthy that Edwardes and Baltzan (71) reported that the data of Dales *et al.* (9) reveal a positive correlation between MMR vaccination rates and autism incidence when the data for the children vaccinated at 17 months are examined in isolation. Nevertheless, Edwardes and Baltzan felt that immunization should be maintained. Without question, the MMR vaccine has been very efficacious in limiting childhood measles, mumps, and rubella infection. Vaccination against these diseases is central to the establishment and maintenance of the public health. For this reason, it is paramount that concerns regarding the safety of the MMR vaccine be fully alleviated as may only occur after gastroenterologic measurements, including endoscopy, are performed before and after vaccination.

Pharmaceutical formulations of selected infant vaccines have contained small amounts of an antimicrobial preservative, thimerosal ([{o-carboxyphenyl}thio] ethylmercury sodium salt), a compound consisting largely of the organomercurial ethylmercury. The role of mercurials in producing damage to the CNS has been documented (72). In addition, the organomercurial methyl mercury and inorganic $HgCl_2$ have been shown to increase the ionic conductance and the mannitol permeability of isolated segments of rat colon (73). There is also evidence that $HgCl_2$ can influence the immune system of the gut. Thus, a single large oral dose of $HgCl_2$ given to rats that were immunized against ovalbumin caused a significantly enhanced immune response to ovalbumin (elevated serum IgE and IgG) and damaged the DNA in the intestinal epithelial and lymph node cells (74). $HgCl_2$ may act in part to increase the intestinal permeability to ovalbumin. Additional studies are needed to determine whether methylated mercurials in lower doses produce similar effects as $HgCl_2$. These observations notwithstanding, there is no objective evidence directly implicating thimerosal in adverse responses to vaccines. The phaseout underway in thimerosal usage as a preservative in pharmaceutical vaccine formulations will reduce the exposure of children to this toxin.

Impairment in Gut Development. Nelson *et al.* (75) recently reported that several neuropeptides (that inhibit or excite neurons) and neurotrophins (that alter neuron metabolism) were very significantly elevated in the blood of

69 children who developed autism compared with the blood of 54 children who developed normally. Measurements were performed on archived neonatal blood samples drawn routinely at birth from infants born in California. Among a group of eight neuroactive compounds measured by immunoaffinity chromatography vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), brain-derived neurotrophic factor (BDNF), and neurotrophin 4/5 (NT 4/5) were elevated. The blood levels of neuropeptides and neurotrophins did not correlate with the degree of mental impairment or with the presentation of seizures or with the subtype of autism (regressive versus nonregressive). Ninety percent of the autistic children had a concentration of VIP that exceeded the control range; similar percentages for the other compounds were: NT 4/5 87%, CGRP 81%, and BDNF 65%. In 94% of the autistic infants, both a neuropeptide (VIP or CGRP) and a neurotrophin (BDNF or NT 4/5) were elevated. The findings were not exclusive for autism—children diagnosed with mental retardation without autism exhibited similar blood patterns of neuroactive compounds. Hence, the altered levels of neuropeptides and neurotrophins may be a necessary, but not a sufficient, condition for impairment of gut development in autism.

VIP, a member of the VIP-glucagon-secretin family of neuropeptides, is a neurotransmitter and neuromodulator important in cerebral growth, neurogenesis, and astrocytogenesis. In addition, VIP is present in the peripheral nervous system, endocrine pancreas, and in the enteric nervous system (ENS) of the intestine, among other sites. The ENS is an independently acting nerve network within the walls of the gastrointestinal tract that regulates secretion and motility. Although the source of the elevated blood neuropeptides and neurotrophins is unknown, the enhanced secretion of VIP (an inhibitory agonist in the ENS) by enteric neurons may impair gut development, motility, and secretion and thereby play a role in producing the GI symptoms noted in autism. In this respect, it may be noteworthy that 20% of autistic children examined in a recent study had decreased serum levels of IgA, the Ig formed in the gut (76). Perhaps, in these patients, the immune system of the GI tract is inadequately developed, or less responsive to stimuli, leaving them more susceptible to antigens.

Hyperacidity of the Intestinal Luminal Contents Due to Hyposecretion of Secretin. As noted above, there is some limited evidence, as yet unverified, that secretion of the GI hormone secretin is impaired in autism (17). Well-documented functions of secretin include stimulation of pancreatic sodium bicarbonate and water secretion and inhibition of gastric acid secretion. These two effects aid in maintaining the pH of the intestinal luminal fluid near neutrality. The catalytic activity of pancreatic digestive enzymes is optimal at pH values of 7 to 8 and are lower under acidic conditions (77). Horvath *et al.* (17) observed esophageal reflux of gastric acid in autistic patients. This could be caused by gastric hypersecretion of hydrochloric acid. They also noted impaired intestinal hydrolytic enzyme activity, and, when secretin was infused intravenously, they observed hypersecretion of pancreatic fluid. These observations may be explained by a lower than normal release of secretin in the autistic patients upon stimulation of the secretin cells. Consequently, the reduced blood levels of secretin could allow gastric HCl secretion to increase abnormally due to relief from secretin inhibition of gastric acid secretion and could also reduce pancreatic alkali secretion. These separate effects would, in turn, increase acidity of the intestinal luminal contents. The greater luminal acidity could then elevate intestinal permeability excessively by altering the integrity of the tight junctional complexes. In this respect, it has been observed that exposure of the rat intestinal mucosa to acidic saline for 30 min resulted in severe injury to the tips of the villi and elevated lumen-to-blood passage of the serum protein albumin (78). If hyposecretion of secretin does occur in autism, then it might be clinically beneficial to elevate serum secretin in these patients. Interestingly, Horvath *et al.* (79) reported that within 5 weeks of receiving intravenous secretin infusions sufficient to elevate the pancreatic secretory rate, three patients exhibited marked dimunition of GI symptoms and improvement in behavior, including expansion of expressive language. This surprising result caused considerable interest. However, when several laboratories attempted to replicate their results in trials with large numbers of patients, a beneficial effect of secretin was not seen whether patients were given secretin in single doses (80–83) or in multiple doses (84). Hence, the great weight of evidence argues against a role for hyposecretion of the hormone secretin in autism.

How Might Impaired Intestinal Function Influence Autistic Behavior?

Autistic individuals frequently exhibit mental retardation as well as unusual behaviors such as perseveration. These are indications of impaired CNS function (1, 85). The studies described above provide evidence that, in autism, much of the GI tract, extending from the esophagus to the colon, possesses pathology (14–17). The possibility cannot be discounted that any GI pathology that exists is unrelated to the function of the CNS. However, the reports that, in some autistic children, a diet free of gluten and casein produces a dimunition of autistic behaviors is intriguing and raises the possibility that altered GI tract function may at least increase the severity of the behavioral symptoms (24, 25, 86). Indeed, impairment of brain development in very young children through gut-associated pathways may be irreversible. Hence, it is valuable to consider two potential scenarios by which impaired intestinal function may play a role in altering CNS function. In this respect, it is important to recall that, as noted above, the etiology of autism is multifactorial.

Metabolites of Gluten and Milk Interfere with Brain Function. According to this theory, it is proposed that gliadomorphins and casomorphins arising from the partial luminal digestion of dietary gliadin and casein, respec-

tively, are absorbed through a leaky gut, enter into the CNS, and interfere with normal brain function because their functional properties mimic the opioid hormone β-endorphin. This hypothesis developed from the pioneering work of Dohan (40), who proposed that schizophrenia was caused by a dietary overload of peptides from gluten and milk. Others have disputed the role of gluten in causing schizophrenia (87). Panskepp (88), in developing his opiate excess hypothesis of autism, noted that young animals exposed to low doses of opiate drugs displayed behavioral symptoms similar to those seen in autistic children. The influence of opioids on human brain function has been described (27). Gliadomorphins and casomorphins are not hydrolyzed by proteolytic enzymes, hence, are very stable families of compounds that can produce long-lasting effects on the CNS (89). Casomorphins are detectable in human cerebrospinal fluid (CSF) (90). One member of this family, β-casomorphin-7, caused behavioral changes when injected into rats (91).

If exorphins do interfere with CNS activity, it is reasonable to expect that autistic individuals might have greater levels of endogenous endorphins (which include enkephalins and β-endorphin) in their cerebrospinal fluid. Gillberg (92) reported that six of 20 autistic youths possessed significantly elevated levels of enkephalins in a specific fraction of the CSF obtained by spinal puncture (92). These six patients also exhibited decreased sensitivity to pain. The level of endorphins in lumbar CSF is considered to reflect the level of β-endorphin in the brain (93). In a subsequent study of 31 autistic children, Gillberg *et al.* (94) found that CSF β-endorphins were statistically lower than in a group of nonage-matched (adult) individuals. Contrasting with these reports, Nagamitsu *et al.* (93) found that levels of β-endorphin in the CSF of 19 autistic individuals did not differ significantly from the levels of β-endorphin in age-matched controls. Hence, there is conflicting evidence whether endogenous β-endorphins, at least, are elevated in autism. This does not preclude the possibility that dietarily derived exorphins play a prominent role in producing the behavioral symptoms of autism. Nevertheless, a great deal of work is needed to establish that exorphins are absorbed into the blood and produce changes in CNS function.

It is perhaps relevant to this topic that untreated celiac patients, who, like autistic patients, exhibit intestinal pathophysiology, commonly also exhibit psychiatric symptoms, including anxiety and depression (95–97). Imposition of a gluten-free diet produces a rapid elimination of these symptoms in celiac patients (95). Likewise, Dohan (44) has reported anecdotal evidence of a high incidence of celiac disease in schizophrenic patients. Hence, autism may be only the most recent of several pathological conditions discovered to possess a strong brain-gut connection (54).

Vitamin B_{12} Deficiency Impairs Nervous System Development. According to this theory, impaired intestinal absorption of vitamin B_{12} produces a deficiency resulting in impaired nerve function. Dietary vitamin B_{12} is normally absorbed in the ileum of the small intestine. Pathology in the ileal mucosa of autistic patients, as observed to occur in the studies noted above, could interfere with the transport process for vitamin B_{12} in the ileal absorptive cells. If absorption is severely inhibited, the resulting lower blood vitamin B_{12} could interfere with the formation of myelin, the lipoprotein material surrounding the axon of myelinated nerve fibers (98). Myelin is necessary for normal conduction of the action potential in myelinated nerve fibers. Wakefield *et al.* (14) observed that, in conjunction with the intestinal pathology observed in his autistic population, vitamin B_{12} absorption was significantly reduced in all eight autistic individuals in which urinary methylmalonic acid excretion was measured. Methylmalonic acid is normally converted to succinyl coenzyme A by a vitamin B_{12} dependent mutase. Dietary vitamin B_{12} deficiency results in impaired mutase activity and spillover of methylmalonic acid into the urine. Wakefield *et al.* (14) proposed that nerve myelogenesis, which is dependent upon vitamin B_{12}, may be impaired in autistic children as a result. Direct evidence of vitamin B_{12} deficiency and impaired myelogenesis in autism is lacking at this time.

Future Directions for Research

This review has focused on recent reports of GI pathology in infantile autism. Collectively, the reports represent a potentially important new advance in our understanding of autism and related developmental disorders. However, there is very consideable controversy surrounding the published findings, as well as many of the related issues dealt with in this review. For this reason, there is a great deal that needs to be done. It is essential that others examine the GI tract of symptomatic autistic children to substantiate the findings of Wakefield and of Horvath and their respective coworkers. Research into the GI pathology must be extended to detail the alterations in morphology, metabolism, and transepithelial transport capacity at the cellular level and to evaluate the reputed pathology as a tool in the clinical diagnosis of autism. The extent to which the barrier functions of the small intestine and colon are affected needs intensive examination. The causative agent(s), whether exogenous or endogenous, must be identified. It is important to extensively evaluate the efficacy of gluten- and casein-free diets in ameliorating autistic symptoms and whether such diets ameliorate the gut pathology similar to the manner in which a gluten-free diet reverses the extensive mucosal pathology in celiac disease. All potential links between the GI pathology and the autistic symptoms must be given careful and rigorous examination. Even if it develops that the GI pathology is unrelated to the etiology of autism or its unique behavioral symptomatology, the discoveries appear to verify what parents and physicians have long suspected, namely, that many autistic children have abnormal GI function. Routine endoscopic examination of newly diagnosed autistic patients, coupled with an effective treatment to normalize GI tract morphology and function, may prove effec-

tive in lessening the severe impact this disorder has on the autistic child.

I am indebted to my son, Ted, for twenty-three years of daily instruction on what it means to be autistic.

1. Rapin I. Current concepts: autism. N Engl J Medicine **337:**97–104, 1997.
2. Deykin EY, McMahon N. The incidence of seizures among children with autistic syndromes. Am J Psychiatry **136:**1310–1312, 1979.
3. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), 4th ed. Washington DC, 1994.
4. Tuchman RF, Rapin I. Regression in pervasive developmental disorders: seizures and epileptiform electroencephalogram correlates. Pediatrics **4:**560–566, 1997.
5. Hoshido Y, Kaneko M, Yashima Y, Umashiro H, Volmar FR, Cohen DJ. Clinical features of autistic children with setback in their infancy. Japan J Psychiatry Neurol **41:**237–246, 1987.
6. Bailey A, LeCouteur A, Gottesman I, Bolton P, Simonoff E, Yuda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med **25:**63–77, 1995.
7. Trottier G, Srivastava L, Walker CD. Etiology of infantile autism: a review of recent advances in genetic neurobiological research. J Psychiatry Neurosci **24:**103–115, 1999.
8. Greenberg DA, Hodge SE, Sowinski J, Nicoll D. Excess of twins among affected sibling pairs with autism: implications for the etiology of autism. Am J Hum Genet **69:**1062–1067, 2001.
9. Dales L, Hammer SJ, Smith NJ. Time trends in autism and MMR immunization coverage in California. J Am Med Assoc **285:**1183–1185, 2001.
10. Kaye JA. Mumps, measles and rubella vaccine and the incidence of autism recorded by general practitioners: a time trend analysis. Br Med J **322:**460–463, 2001.
11. Taylor B, Miller E, Farrington CP, Petropoulos MC, Favot-Mayaud I, Li J, Wright PA. Autism and measles mumps and rubella vaccine: no epidemiological evidence for a causal association. Lancet **353:**2026–2029, 1999.
12. Gillberg C, Wing L. Autism: not an extremely rare disease. Acta Physiol Scand **99:**399–406, 1999.
13. Wing L, Potter D. The epidemiology of autistic spectrum disorders: is the prevalence rising? Mental Retardation Dev Disabilities Res Rev **8:**151–161, 2002.
14. Wakefield AJ, Murch SH, Anthony A, Linnell J, Casson DM, Malik M, Berlowitz M, Dillon AP, Thompson MA, Harvey P, Valentine A, Davies SE, Walker-Smith JA. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet **35:**637–641, 1998.
15. Wakefield AJ, Anthony A, Murch SH, Thomson M, Montgomery SM, Davies S, O'Leary JJ, Berelowitz M, Walker-Smith JA. Enterocolitis in children with developmental disorders. Am J Gastroenterol **95:**2285–2295, 2000.
16. Sabra A, Bellanti JA, Colon AR. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet **352:**234–235, 1998.
17. Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tildon JT. Gastrointestinal abnormalities in children with autistic disorder. J Pediat **135:**559–563, 1999.
18. Black C, Kaye JA, Jick H. Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database. Br Med J **325:**419–421, 2002.
19. Goodwin MS, Cowen MA, Goodwin TC. Malabsorption and cerebral dysfunction: a multivariate and comparative study of autistic children. J Autism Child Schizophrenia **1:**48–62, 1971.
20. Lightdale JR, Siegel B, Heyman MB. Gastrointestinal symptoms in autistic children. Clin Perspect Gastroenterol **1:**56–58, 2001.
21. Branski D, Troncone R. Celiac disease: a reappraisal. J Pediat **133:**181–187, 1998.
22. Hadjivassiliou M, Boscolo S, Davies-Jones GAB, Grunewald RA, Not T, Sanders DS, Simpson JE, Tongiorgi E, Williamson CA, Woodroofe NM. The humoral response in the pathogenesis of gluten ataxia. Neurology **58:**1221–1226, 2002.
23. Gualteri CT. Brain Injury and Mental Retardation: Psychopharmacology and Neuropsychiatry. Baltimore, MD: Lippincott, Williams and Wilkins, 2002.
24. Lucarelli S, Frediani T, Zingoni AM, Ferruzzi F, Giardini O, Quintieri F, Barbato M, D'Eufemia P, Cardi E. Food allergy and infantile autism. Panminerva Med **37:**137–141, 1995.
25. Knivsberg A, Reichelt KL, Nodland N, Hoien T. Autistic syndromes and diet: a follow-up study. Scand J Educ Res **39:**223–236, 1995.
26. Israngkun PP, Newman HA, Patel ST, Duruibe VA, Abou-Issa H. Potential biochemical markers for infantile autism. Neurochem Pathol **5:**51–70, 1986.
27. Shattock P, Kennedy A, Rowell F, Berney TP. Role of neuropeptides in autism and their relationships with classical neurotransmitters. Brain Dysfunct **3:**328–345, 1990.
28. Reichelt KL, Knivsberg AM, Lind G, Nodland M. Probable etiology and possible treatment of childhood autism. Brain Dysfunct **4:**308–319, 1991.
29. Wood NC, Hamilton I, Axon ATR, Khan SA, Quircke P, Mindham RHS, McGuigan K, Prison HM. Abnormal intestinal permeability. An etiological factor in chronic psychiatric disorders? Br J Psychiatry **150:**853–856, 1987.
30. Bjarnason I, Macpherson A, Hollander D. Intestinal permeability: an overview. Gastroenterology **108:**1566–1581, 1995.
31. Travis S, Menzies I. Intestinal permeability: functional assessment and significance. Clin Sci **82:**471–488, 1992.
32. Hodges S, Ashmore SP, Patal HL, Tanner MS. Cellobiose: mannitol differential permeability in small bowel disease. Arch Dis Child **64:**853–854, 1989.
33. Hamilton I, Hill A, Bose B, Boucher IAD, Forsyth JS. Small intestinal permeability in pediatric clinical practice. J Pediatr Gastroenterol Nutr **6:**697–701, 1987.
34. D' Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, Cardi E, Giardini O. Abnormal intestinal permeability in children with autism. Acta Paediat **85:**1076–1079, 1996.
35. Kerneis S, Bogdanova A, Kraehenbuhl J-P, Pringault E. Conversion of Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science **277:**949–952, 1997.
36. Reichelt KL, Ekrein J, Scott H. Gluten, milk proteins and autism: dietary intervention effects on behavior and peptide secretion. J Appl Nutr **42:**1–11, 1990.
37. Zioudrou C, Streaty RA, Klee WA. Opioid peptides derived from food proteins. The exorphins. J Biol Chem **254:**2446–2449, 1979.
38. Chang KJ, Su YF, Brent DA, Chang JK. Isolation of a specific μ-opiate receptor peptide, morphiceptin, from an enzymatic digest of milk protein. J Biol Chem **260:**9706–9712, 1985.
39. Lindstrom LH, Nyberg F, Terenius L, Bauer K, Besev G, Gunne LM, Lyrenas S, Willdeck-Lund G, Lindberg B. CSF and plasma β-casomorphin-like opioid peptides in post-partum psychosis. Am J Psych **41:**1059–1066, 1984.
40. Dohan FC. Hypothesis: genes and neuroactive peptides from food as cause of schizophrenia. Adv Biochim Psychopharmacol **22:**535–548, 1980.
41. Hemmings WA. The entry into the brain of large molecules derived from dietary protein. Proc Roy Soc London Ser B **200:**175–192, 1978.
42. Sun Z, Cade JR, Fregly MJ, Privette RM. β-Casomorphin induces Fos-like immunoreactivity in discrete brain regions relevant to schizophrenia and autism. Autism **3:**67–83, 1999.
43. Reichelt KL, Landmark J. Specific IgA antibody increases in schizophrenia. Biol Psych **37:**410–413, 1995.

44. Dohan FC. More on celiac disease as a model for schizophrenia. Biol Psych **18:**561–564, 1983.
45. Dohan FC, Grasberger JC. Relapsed schizophrenics: earlier discharge from hospital after cereal-free, milk-free diet. Am J Psych **130:**685–688, 1973.
46. Reichelt KL, Sagedal E, Landmark J, Sangvik BT, Eggen O, Scott H. The effect of gluten-free diet on urinary peptide excretion and clinical state in schizophrenia. J Ontomol Med **5:**223–239, 1990.
47. Singh MM, Kay SR. Wheat gluten as a pathogenic factor in schizophrenia. Science **191:**401–402, 1976.
48. Fiocchi C. Intestinal inflammation: a complex interplay of immune and nonimmune cell interactions. Am J Physiol **273**(Gastrointest Liver Physiol 36):G769–G775, 1997.
49. Murch SH, MacDonald TT, Walker-Smith JA, Levin M, Lionetti P, Klein NJ. Disruption of sulphated glycosaminoglycans in intestinal inflammation. Lancet **341:**711–741, 1993.
50. Fudenberg HH. Dialysable lymphocyte extract (DLyE) in infantile onset autism: a pilot study. Biotherapy **9:**13–17, 1996.
51. Alberti A, Pirrone P, Elia M, Waring RH, Romano C. Sulphation deficit in "low functioning" autistic children: a pilot study. Biol Psych **46:**420–424, 1999.
52. Gupta S. Immunology and immunologic treatment of autism. Proceedings of the National Autism Assn; 1996; Chicago: 455–460.
53. Beeken WL, Busch HJ, Sylwester DL. Intestinal protein loss in Crohn's disease. Gastroenterology **62:**207–215, 1972.
54. Furlano RI, Andrew A, Day R, Brown A, Garvey L, Thomson MA, Davies SE, Berelowitz M, Forbes A, Wakefield AJ, Walker-Smith JA, Murch SH. Colonic CD8 and γδ T-cell infiltration with epithelial damage in children with autism. J Pediat **138:**366–372, 2001.
55. Harrison M, Kilby A, Walker-Smith JA, France NE, Wood CB. Cow's milk protein tolerance: a possible association with gastroenteritis. Br Med J **19:**1501–1504, 1976.
56. Heymann M, Grasset E, Ducroc R, Desjeux JF. Antigen absorption by the jejunal epithelium of children with cow's milk allergy. Pediatr Res **241:**197–202, 1988.
57. Unno N, Menconi MJ, Smith M, Fink MP. Nitric oxide mediates interferon-γ-induced hyperpermeability in cultured human intestinal epithelial monolayers. Crit Care Med **23:**1170–1176, 1995.
58. Sanders SE, Madara JL, McGuirk DK, Gelman DS, Golman SP. Assessment of inflammatory events in epithelial permeability: a rapid screening method using fluorescein dextrans. Gastroenterology **106:**1514–1523, 1995.
59. Madara JL, Stafford J. Interferon-γ directly affects barrier function of cultured intestinal epithelial monolayers. J Clin Invest **83:**724–727, 1989.
60. Heymann M, Darmon N, Dupont C, Dugas B, Hirribaren A, Blaton MA, Desjeux JF. Mononuclear cells from infants allergic to cow's milk secrete tumor necrosis factor α, altering intestinal function. Gastroenterology **106:**1514–1523, 1994.
61. Heymann M, Desjeux JF. Cytokine-induced alteration of the epithelial barrier to food antigens in disease. Ann NY Acad Sci **915:**304–311, 2000.
62. Kawashima H, Mori T, Kashiwagi Y, Takekuma K, Hoshika A, Wakefield A. Detection and sequences of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism. Dig Dis Sci **45:**723–729, 2000.
63. Uhlmann V, Martin CM, Sheils O, Pilkington L, Silva I, Killalea A, Murch SB, Walker-Smith J, Thomson M, Wakefield AJ, O'Leary JJ. Potential viral pathogenic mechanism for new variant inflammatory bowel disease. Mol Pathol **55:**84–90, 2002.
64. Lewin J, Dhillon AP, Sim R, Pounder RE, Wakefield AJ. Confirmation of persistent measles virus infection of intestinal tissue by immunogold electron microscopy. Gut **36:**564–569, 1995.
65. Miyamoto H, Tanaka T, Kitamoto N, Fukuda Y, Shimoyama T. Detection of immunoreactive antigen, with a monoclonal antibody to measles virus, in tissues from a patient with Crohn's disease. J Gastroenterol **30:**28–33, 1995.
66. Iizuka M, Nakagoni O, Chiba M, Ueda S, Masamune O. Absence of measles virus in Crohn's disease. Lancet **345:**199, 1995.
67. Pabst HF, Spady DW, Carson MM, Stelfox HT, Beeler JA, Krezolek MP. Kinetics of immunologic responses after primary MMR vaccination. Vaccine **15:**10–14, 1997.
68. Miller D, Wadsworth J, Diamond J, Ross E. Measles vaccination and neurological events. Lancet **349:**730–731, 1997.
69. Taylor B, Miller E, Lingam R, Andrews N, Simmons A, Stowe J. Measles, mumps, and rubella vaccination and bowel problems or developmental regression in children with autism: population study. Br Med J **324:**393–396, 2002.
70. Fombonne E, Chakrabarti S. No evidence for a new variant of MMR-induced autism. Pediatrics **108:**E58, 2001.
71. Edwardes M, Baltzan M. MMR immunization and autism. J Am Med Assoc **285:**2852–2853, 2001.
72. Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a novel form of mercury poisoning. Med Hypotheses **56:**462–471, 2001.
73. Bohme M, Diener M, Mestres P, Rummel W. Direct and indirect actions of $HgCl_2$ and methyl mercury chloride on permeability and chloride secretion across the rat colonic mucosa. Toxicol Appl Pharmacol **114:**285–294, 1992.
74. Watzl B, Abrahamse SL, Treptow-van Lishaut S, Neudecker C, Hansch GM, Rechkemmer G, Pool-Zobel BL. Enhancement of ovalbumin-induced antibody production and mucosal mast cell response by mercury. Food Chem Toxicol **37:**627–637, 1999.
75. Nelson KB, Grether JK, Croen LA, Dambrosia JM, Dickens BF, Jelliffe LL, Hansen RL, Phillips TM. Neuropeptides and neurotrophins in neonatal blood of children with autism or mental retardation. Ann Neurol **49:**597–606, 2001.
76. Warren RP, Odell JD, Warren WL, Burger RA, Maciulis A, Daniels WW, Torres RA. Brief report: immunoglobulin A deficiency in a subset of autistic subjects. J Autism Dev Disord **27:**187–192, 1997.
77. Johnson LR. Gastrointestinal physiology: Digestion and absorption. In: Johnson LR, Ed. Essential Medical Physiology, 2nd ed. Philadelphia, PA: Lippincott-Raven, 1998.
78. Lundin P, Westrom BR, Pantzar N, Karlsson BW. Bidirectional small intestinal permeability changes to different-sized molecules after HCl-induced injury in the rat. Dig Dis Sci **42:**677–683, 1997.
79. Horvath K, Stefanatos G, Sokolski KN, Wachtel R, Nabors L, Tildon JT. Improved social and language skills after secretin administration in patients with autistic spectrum disorders. J Assoc Acad Minor Phys **9:**9–15, 1998.
80. Sandler AD, Sutton KA, DeWeese J, Girardi MA, Sheppard V, Bodfish JW. Lack of benefit of a single dose of synthetic human secretin in the treatment of autism and pervasive developmental disorder. N Engl J of Med **341:**1801–1806, 1999.
81. Chez MG, Buchanan CP, Bagan BT, Hammer MS, McCarthy KS, Ovrutskaya I, Nowinski CV, Cohen ZS. Secretin and autism: a two-part clinical investigation. J Autism Dev Disorders **30:**87–94, 2000.
82. Dunn-Geier J, Ho HH, Auersperg E, Doyle D, Eaves L, Matsuba C, Orrbine E, Pham B, Whiting S. Effects of secretin in children with autism: a randomized controlled trial. Dev Med Child Neurol **42:**796–802, 2000.
83. Lightdale JR, Hayer C, Duer A, Lind-White C, Jenkins S, Siegel B, Elliot GR, Heyman MB. Effects of intravenous secretin on language and behavior of children with autism and gastrointestinal symptoms: a single-blinded, open-label pilot study. Pediatrics **108:**E90, 2001.
84. Roberts W, Weaver L, Brian J, Bryson S, Emelianova S, Griffiths A, MacKinnon B, Yim C, Wolpin J, Koren G. Repeated doses of porcine secretin in the treatment of autism: a randomized, placebo-controlled trial. Pediatrics **107:**E71, 2001.
85. Gillberg C, Coleman M. The biology of the autistic syndromes. 3rd ed. In: Hart HM, Ed. Clinics in Developmental Medicine No. 153/4. London: MacKeith Press, 2000.
86. Knivsberg A, Wiig K, Lind G, Nodland M, Reichelt KL. Dietary intervention in autistic syndromes. Brain Dysfunction **3:**315–327, 1990.

87. Hallert C, Astrom J. Psychic disturbances in adult coeliac disease. II. Psychological findings. Scand J Gastroenterol **17:**21–22, 1982.
88. Panksepp J. A neurochemical theory of autism. Trends Neurosci **2:**174–177, 1979.
89. Brantl V, Teschemacher H, Blasig J, Henschen A, Lottspeich F. Opioid activities of β-casomorphins. Life Sci **28:**1903–1909, 1981.
90. Nyberg F, Liebermann H, Lindstrom LH, Lyrenas S, Koch G, Terenius L. Immunoreactive β-casomorphin-8 in cerebrospinal fluid from pregnant and lactating women: correlation with plasma levels. J Clin Endocrinol Metab **68:**283–9, 1989.
91. Sun Z, Cade JR. A peptide found in schizophrenia and autism causes behavioral changes in rats. Autism **3:**85–95, 1999.
92. Gillberg C, Terenius L, Lonnerholm G. Endorphin activity in childhood psychosis. Arch Gen Psych **42:**780–783, 1985.
93. Nagamitsu S, Matsuishi T, Kisa T, Komori H, Miyazaki M, Hashimoto T, Yamashita Y, Ohtaki E, Kato H. CSF β-endorphin levels in patients with infantile autism. J Autism Dev Disorders **27:**155–163, 1997.
94. Gillberg C, Terenius L, Hagberg B, Wit-Engerstrom I, Eriksson I. CSF β endorphins in childhood neuropsychiatric disorders. Brain Dev **12:**88–92, 1990.
95. Benson GD, Kowlessar OD, Sleisenger MH. Adult celiac disease with emphasis upon response to the gluten-free diet. Medicine (Baltimore) **43:**1–40, 1964.
96. Cooke WT, Smith WT. Neurological disorders associated with adult coeliac disease. Brain **89:**683–722, 1966.
97. Hallert C, Derefeldt T. Psychic disturbances in adult coeliac disease. I. Clinical observations. Scand J Gastroenterol **17:**17–19, 1982.
98. Dillon MJ, England JM, Gompertz D, Goodey PA, Grant DB, Hussein HA, Linnell JC, Matthews DM, Mudd SH, Newns GH, Seakins JW, Uhlendorf BW, Wise IJ. Mental retardation, megaloblastic anaemia, homocysteine metabolism due to an error in B_{12} metabolism. Clin Sci Mol Med **47:**43–61, 1974.