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Can the Pathophysiology of Autism be Explained by the Nature of the Discovered Urine Peptides?

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Opioid peptides derived from food proteins (exorphins) have been found in urine of autistic patients. Based on the work of several groups, we try to show that exorphins and serotonin uptake stimulating factors may explain many of the signs and symptoms seen in autistic disorders. The individual symptoms ought to be explainable by the properties and behavioural effects of the found peptides. The data presented form the basis of an autism model, where we suggest that exorphins and serotonin uptake modulators are key mediators for the development of autism. This may be due to a genetically based peptidase deficiency in at least two or more peptidases and, or of peptidase regulating proteins made manifest by a dietary overload of exorphin precursors such as by increased gut uptake.

Keywords: Autism; Peptides; Exorphins; Urine; Diet

INTRODUCTION

Several laboratories have found increase in urinary peptides in autism (Fig. 1–3 and Table I), and that some of these are opioids and also exorphins (Israngkun *et al.*, 1986; Reichelt *et al.*, 1986; 1991; Shattock *et al.*, 1990; Cade *et al.*, 2000; Shanahan *et al.*, 2000). The definite structure of these have been obtained by mass spectrometry and fragmentation mass spectrometry (Shanahan *et al.*, 2000; Remme *et al.*, 2001). The presence of the rare D-amino acid containing dermorphin has also been confirmed (Shanahan *et al.*, 2000). Furthermore increase of opioids has been found in serum and CSF (Gillberg *et al.*, 1985; LeBoyer *et al.*, 1994), and some of these are bovine casomorphins (Reichelt *et al.*, 1991; Reichelt and Reichelt, 1997; Cade *et al.*, 2000; Shanahan *et al.*, 2000). The time, therefore, seems ripe to see if we can explain the symptoms of the autistic syndromes as listed in Table II by referring to the properties of the isolated peptides. Below each symptom is probed for possible relationship to specific peptides found.

Social Indifference

The cardinal symptom of autistic syndromes is social indifference or aloofness. Panksepp demonstrated that opioids inhibit social bonding (Panksepp *et al.*, 1978) and found that opioids also casomorphins, caused social indifference and abrogation of separation distress calls in new-born animals. A chronic effect of casomorphins found in urine from autistic patients, as well as gliadinomorphin (Shanahan *et al.*, 2000) and glutemorphins, deltorphin and dermorphin could, therefore, explain this social indifference.

Intracranio-ventricularly injected opioids isolated from urine (Hole *et al.*, 1979) and casomorphine 1–7 injected IV in rats, induce acute, typical and similar effects (Sun and Cade, 1999) ranging from explosive motor behaviour, analgesia, wet dog shakes to later catatonia. Such acute changes are also seen when opioid drugs are used and could possibly explain periods of hyperactive agitation, aggressive and emotionally bizarre acting out behaviours as well as more catatonic phases. Furthermore, exorphins do

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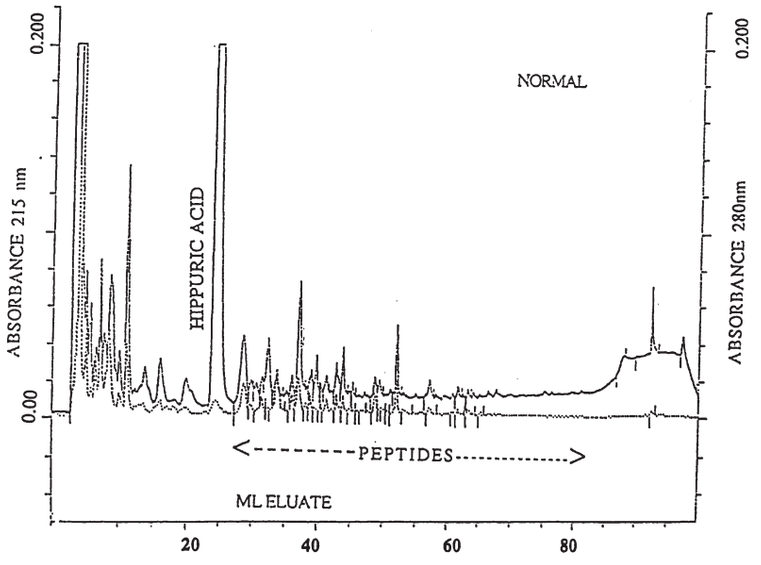

FIG. 1 Shows the HPLC separation of peptides from a normal boy, based on the principles published (Bøhlen *et al.*, 1980). C-18 reverse phase column is used and absorbtion read at 215 and 280 nm giving us a purity index. That most of the material after hippuric acid is peptidic has been discussed extensively (Reichelt *et al.*, 1998), and is based on amino acid release by hydrolysis and also peptidase treatment followed by amino acid analysis.

pass the blood–brain barrier (Ermisch *et al.*, 1983; Nyberg *et al.*, 1989) and are extremely psychosogenic as seen in postpartum psychosis (Lindstrøm *et al.*, 1984). Exposing the blood–brain barrier to opioids during early growth in rats permanently alters the permeability to opioids in these membranes (Banks *et al.*, 1996). The exorphins show a bell shaped dose response curve called hormesis (Reichelt and Reichelt, 1997). This may well explain the varied response to naloxone or naltrexone, ranging from good, to no effect and even worsening (Campbell *et al.*, 1996) depending on the level of opioids in any given patient.

Poor Habituation

If palmar skin conductance is measured in autistic children, the conductance at rest fluctuates considerably more than in controls and most show exaggerated response to auditory stimulation with very poor habituation (Bernal and Miller, 1971). This indicates an increased sensory and autonomic

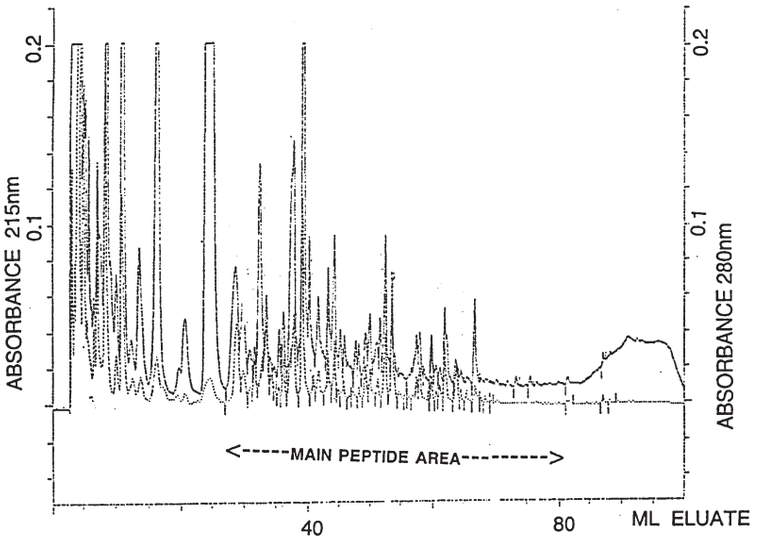

FIG. 2 The urine profile from a 6 year old boy with autism. Comparing this with Fig. 1, the difference is striking. Both runs are based on urine volumes equivalent to 250 nmol creatinin and the compounds eluting after hippuric acid have again been shown to be peptides. In the figure, the peptide bond is measured at 215 nm. And aromatic compounds at 280 nm.

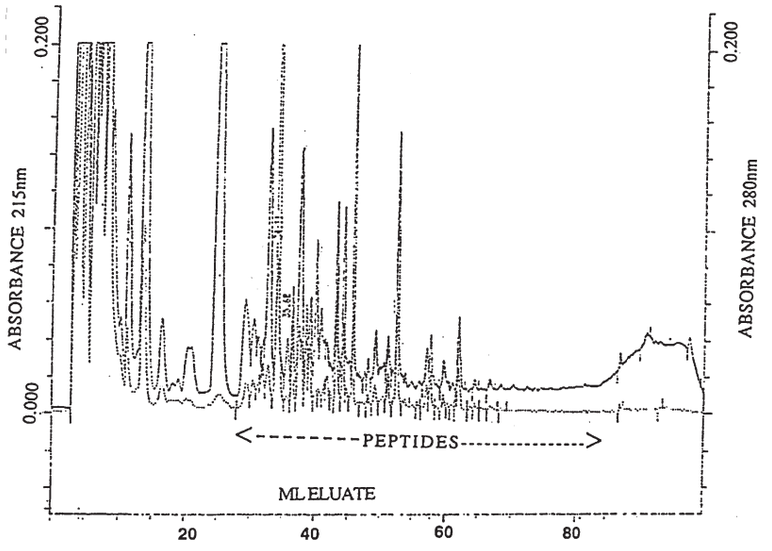

FIG. 3 Another boy with the same diagnosis and Childhood autism rating scale (CARS) score as the boy in Fig. 2. Notice the different but overlapping pattern in spite of having the same diagnosis, sex and age. This indicates that different enzyme defects are probably present in different families, but with overlaps.

arousal of the CNS and, or insufficient reactive inhibition (lack of habituation) (Mednick *et al.*, 1974). Lack of habituation would cause avoidance of new inputs and hence a strong preference for status quo and rituals. A chemical candidate for this reactive inhibition and habituation is serotonin. We found a peptide from autistic urines (pyroGlu-Trp-GlyNH2) that increased the uptake of serotonin into platelets (Pedersen *et al.*, 1999) and into synapses in the CNS (Persico *et al.*, 1998). Increased platelet serotonin is a frequently reported finding in autism (Rogeness *et al.*, 1992). PyroGlu-Trp-GlyNH_2 also increases the uptake into CHO cells transfected with the gene for the human serotonin transporter (Keller, 1997). Platelets are a much used pharmacological model for the serotonergic synapses. Increased uptake should decrease the level of serotonin in the synaptic cleft. It is well known that hypo-serotoninergic states such as seen in carcinoid, causes excessive sensory responses and also poor habituation, sleep problems and impulse dominated behaviour. It is reasonable that lack of adaptation and stronger than usual reaction to sensory and emotional stimuli, will cause withdrawal from these and insistence on sameness (Mednick *et al.*, 1974).

TABLE I The total peptide levels eluting after hippuric acid in pre-puberty autistic children

	Autism	Normal
Age range	2–14	3–14
N	315	143
Peptide level (mean)	720	346
SD	471	108
95% CI		
Lower value	560	329
Higher	773	365

The units are Absorption in μm^2 under the UV 215 nm trace of the peaks eluting after hippuric acid and based on urine = 250 nmol creatinin. Only patients who have been diagnosed by certified psychiatrists are included. Ratio of UV 215/UV 280 was used to check of purity of individual peaks. Many drugs have high UV 280 nm peaks due to aromatic ring structures. The autistic children are different from controls with a $p = 0.0001$. Samples from 8 different countries and 16 MD's did not differ statistically indicating that diagnosis has become quite standardized probably through the use of DSM III–IV.

Stereotyped and Repetitive Behaviour

These are also dependent on lack of reactive inhibition (habituation), seen under point 2. However, increase in exorphins, which inhibit the uptake of dopamine into synaptosomes (Hole *et al.*, 1979), would when dependent on meals, cause a fluctuating dopaminergic hyper-function *in vivo*. Dopaminergic hyper-function caused by amphetamine stimulation in animals, shows stereotypy as a typical feature. This dopaminergic hyperactivity would therefore be reinforced by simultaneous decrease in

TABLE II Symptoms and signs to be explained by the properties of the found peptides

1. Social indifference	9. Early and late onset subtype
2. Poor habituation	10. Genetic basis
3. Stereotypy and repetitive behaviour	11. Immune changes
4. Insisting on sameness	12. Sleep disturbances
5. Increase in epilepsy with age	13. Increased incidence in immigrants
6. Varying trophic changes	
7. Varying analgesia	14. Effect of diet
8. Language problems	15. Gut to brain axis

serotoninergic activity, because it is the ratio of functional dopamine to serotonin that seems important (Rogeness *et al.*, 1992) in different brain areas. The decreased habituation is demonstrable by the changes in auditory brainstem evoked responses (Rosenblum *et al.*, 1980) and decrease in post-rotational nystagmus in infantile autism (Ritvo *et al.*, 1969). This may explain why many autistic children are endlessly fascinated by rotating movement and show great ability to rotate most objects and if possible also themselves.

Increased Rate of Epilepsy with Age

In ordinary children the frequency of fits and epileptic attacks decreases with age. In autistic children, an increase in epilepsy and EEG abnormalities is seen with increasing age, and at the age of 20 about 1/4–1/3 of the patients show EEG changes and/or epilepsy (Deykin and MacMahon, 1979). The frequency of epilepsy is increased celiac disease (Chapman *et al.*, 1978; Gobbi *et al.*, 1992), and increased urine peptide secretion including opioids has been found in celiac disease (Reichelt *et al.*, 1998). In a group of children with autistic disorders and increased urinary peptide secretion, we unexpectedly saw a decrease in epileptic fits followed by a reduction of medication when the children were on diet (Reichelt *et al.*, 1990), and a disastrous relapse (status epilepticus) when the diet was broken. This may be explained by the fact that exorphins and other opioids do have convulsant properties (Siggins *et al.*, 1986) and also modify kindling.

It is furthermore, reported that acute exposure of celiac children below 7 years of age to gluten after a gluten free diet for 1 year, induced long standing EEG abnormalities in 72% of the children (Paul *et al.*, 1985). Given the demonstrated effects of exorphins on the CNS outlined earlier, it is reasonable that such opioids may explain both the epileptic tendencies and the EEG abnormalities found. Unpublished data indicate that casomorphine 1–4 amide is of special importance to epilepsy in autism (under study).

Trophic Changes

These changes are not dramatic, but with MR-imaging techniques reduced brain stem size and especially decreased cerebellar volume can be measured (Hashimoto *et al.*, 1992; Courchesne *et al.*, 1994). Volume reduction was also reported for corpus callosum (Egaas and Courchesne, 1995). However, some 6–12% show hypertrophy (Piven *et al.*, 1996). Furthermore, parietal volume reductions in 70–80%, as well as increases in the parietal cortex were found in about 10%.

Opioids inhibit brain maturation (Zagon and McLaughlin, 1987), and this involves the maturation of the dendrites and spines (Hauser *et al.*, 1989). Although pruning by apoptosis takes place continuously, it is to be expected that during the brains most proliferative phase (proliferation dominating apoptosis usually during the first 5 years of life), the effects of opioids would be mainly inhibitory. However, during the extensive pruning (a phase where apoptotic removal of synapses and cells dominates) which takes place at puberty, and as much as 30% of the neuropil is removed (Feinberg, 1982/83), hypertrophy would be expected. Opioids do inhibit pruning (Tenconi *et al.*, 1991), and inhibition of pruning will be seen as increased volume.

It is relevant to the dominant cerebellar changes in autism that gluten related ataxia mainly shows cerebellar changes (Hadjivassiliou *et al.*, 1998), and antibodies to gliadin seem especially to react with the Purkinje cells of the cerebellum (Hadjivassiliou *et al.*, 2002). In progressive myoclonic ataxia in celiac disease cerebellar damage is found (Bhatia *et al.*, 1995). Futhermore, cerebellar damage in celiac disease is well established (Kinney *et al.*, 1982). Relevant to these data are the found increase in antibodies to precisely gliadin and gluten of both IgG and IgA type in autism usually without transglutaminase increase indicating increased protein uptake from the gut (Reichelt *et al.*, 1991; Lucarelli *et al.*, 1995; Cade *et al.*, 2000).

IV injection of casomorphin 1–7 induces the immediate early gene Fos antigen immuno-reactivity in rat brain (Sun *et al.*, 1999), thus linking this opioid to the trophic effects. Post mortem morpholgy likewise point to disturbed cell numbers and relationships in the neuropil (Ritvo *et al.*, 1986; Bauman, 1991), and that is what would be expected if brains are exposed to opioids. Damage to most cells is critically dependent on growth phase and state.

Decreased functional serotonin in the synaptic cleft because of increased uptake would likewise interfere with synapse maintenance and formation (Chen *et al.*, 1980). Blocking the cortical serotonin availability may thus reduce the synaptic density by almost 30%.

Varying Analgesia

Autistic children may hurt themselves deliberately or in accidents apparently without much pain (Frith, 1988). This analgesia seems to vary from day to day and different times of the day. Exorphins would depend on the dietary input and consequently vary in level over a 24 h period and from one day to the next. Self-destructive behaviour is one of the most frequent signs ameliorated by opioid antagonists (LeBoyer *et al.*, 1990), reinforcing a reasonable role for exorphins. The continuous fluctuation of opioid levels because exorphins must depend on feeding, would probably prevent permanent changes in receptor numbers and or sensitivity also in

the dopaminergic system, in spite of dopaminergic hyperactivity at times.

Language Problems

Children with autistic disorders show language deficits ranging from mutism to fluent speech, but often lacking in prosody. Grammatical abnormalities like pronoun reversal are known, and many show a tendency to use repetitive language.

To comprehend a series of words, it is necessary to keep these in the working memory, and the apparatus responsible for processing a single word must be sufficiently inhibited to make place for the next word. With lack of habituation it follows that words will tend to overlap, and be conceived as absurd clusters of sounds or words. Patients we have treated with diet, tell us that this was exactly the problems with sentences. They were conceived as overlapping series of sounds, and consequently quite meaningless.

Early and Late Onset Subtypes

We all take up peptides (Gardner, 1994) and proteins (Husby *et al.*, 1984; Gardner, 1994) from the gut and inhibition of peptidases increases the uptake (Mahe *et al.*, 1989). These dietary proteins can be demonstrated in mothers' milk (Kilshaw and Cant, 1984; Troncone *et al.*, 1987). Feeding babies also ingest human casomorphins in the mothers' normal milk. It is, therefore, conceivable that lack of peptidases or inhibited peptidases may cause problems pre-natally and definitely post-natally in early onset autism.

If the gut is made leaky at some later point in late onset autism or CPDD, this would easily induce such problems by increasing the post prandial overload of peptides. Recent work on Ileal-lymphoid-nodular hyperplasia in CPDD (Childhood onset pervasive developmental disorder) (Wakefield *et al.*, 2000; Furlano *et al.*, 2001) may indicate such a mechanism. The initially published data has been vastly expanded with essentially the same results. Upper intestinal lesions related to autism have also been reported (Horvath *et al.*, 1999; Torrente *et al.*, 2002), and increased low molecular gut permeability in autism is known (D'Eufemia *et al.*, 1996). It has been suggested that the latter could be due to decreased sulphation of aminoglycans in the gut (Waring and Ngong, 1993), but peptidase defects or inhibition also increases gut uptake of peptides (Mahe *et al.*, 1989). Late onset autism could also be caused by introduction of gluten containing foods from about 6 months and onwards.

Because peptiduria is usually caused by decreased peptidase activity (Watanabe *et al.*, 1993), we propose that the genetic disposition may probably be in peptidases or proteins controlling peptidases (Persico *et al.*, 2000), and that this could be the final common defect of all these states of increased permeability for whatever reason. A probable genetic marker for autism is reelin (Persico *et al.*, 2001), which is a serin proteinase and would fit a limited break down hypothesis.

Genetics

Solid evidence for a genetic disposition for autism has been presented (Bailey *et al.*, 1995), but the genes for this disorder have been difficult to pin down. As we and others find that different autistic children have different chain lengths of their exorphins in their urine (Reichelt *et al.*, 1997), this may indicate that different sets of peptidases are malfunctioning in different families. Thus using mass spectrometry, we find the following distribution of a random subset of 34 autistic children. Bovine beta casomorphin 1–8 or Cm 1–8 in 38.2%; Cm 1–7 in 29.4%; Cm 1–5 in 41.2%; Cm 1-4 NH2 in 94.1%; Glutemorphin A5 (G-Y-Y-P-T) in 32.4% and Glutemorphin B 5 (Y-G-G-W-L) in 64.7%. None of these 34 were without increase over controls in one or more opioids, but could be completely without some of the remaining opioids. Most peptides are found in "families" of different chain lengths. Opioids with different chain lengths would have very similar biological effects.

Thus diamino peptidase IV could be one of these enzymes involved (Shaw W personal communication), because the casomorphins start with Tyr-Pro (Y-P), and diaminopeptidase IV is also known to be an Adenosine deaminase binding protein. The deaminase may be involved in some cases of infantile autism (Persico *et al.*, 2000). Furthermore, glycosylation defects would also affect this enzyme as well as other peptidases and could also thus be one of the genetic causes. Another enzyme may be Tyr and Pro-aminopeptidase. For isolated peptides see Table III. Their isolation has been extensively described (1,5).

A parallel to our model is Føllings disorder. Even though phenyl-ketonuria is a genetic disease, it would never have become manifest in a protein environment containing very little phenyl-alanine. We think that a limited break down capacity (peptidases) would only become manifest if subjected to a dietary overload of peptides and, or proteins caused by increased uptake. A limited peptide break down ability could likewise explain recently published data on neuropeptide and neurotrophin increases in the blood of neonatal autistic children (Nelson *et al.*, 2001) as well as increase in oxytocin precursor peptides (Green *et al.*, 2001). Because peptides in general are good peptidase inhibitors (LaBella *et al.*, 1985) this makes sense.

TABLE III Some peptides found in autistic urine

Compound	Cochrom. HPLC	Antibody binding	Receptor binding	Correct comp	Mass. Sp MW
IAG	+			+	
CM 1–8	+	+	+	+	887 (887.1)
CM 1–7	+	+	+	+	789.9 (789.93)
CM 1–5	+		+	+	579.1 (579.6)
CM 1–4	+		+	+	nm
CM 1–4NH2	+			+	521.2 (521,3)
A4	+			+	nm
A5	+		+	+	599.6 (599.7)
GM	+		+	+	728.6 (728.8)

The preliminary receptor assay was carried out by Dr L. Terenius, Stockholm. CM is Casomorphin (bovine), IAG = indolyl acryloyl glycine, A4 and A5 are glutemorphins and GM: gliadinomorphin (Usually several peaks due to deamidation of glutamine: Shanahan *et al.*, 2000). Composit.: Is amino acid composition after acid hydrolysis and amino acid analysis × 3. IAG yielded only glycine; CM 1–8 gave: Y (0.8), P (4), F (0.9), G (1.3) I (1); CM 1–7: Y (0.7), P (3), F (0.8), G (1.3) I (1); CM 1–5: Y (0.7), P (2.2), F (0,7), G (1.5); CM 1–4: Y (0.7), P (2), F (0.8). The two glutemorphins came out with: A4: Y (1.8), P (1), G (1.4) and A5: Y (1.7), P (1), G (1.5), T (1). Found mass spectrometry weights and theoretical based on average istopic mass in parenthesis (Mono isotopic mass is usually lower). Mass spectrometry was run on the Pesciex AP 2000 MS/MS machine with samples dissolved in methanol/water (50% by volume) and 0.01 M formic acid and in the positive mode.

Immunological Changes

The extensive regulation of the immune system, by neuropeptides, has been reviewed by Singh (1995). Exorphins would easily react with opioid receptors on immuno-competent cells, and an effect of the antagonist naltrexone on the CD4/CD8 lymphocytes in autism has been found (Scifo *et al.*, 1996). A series of immunomodulating peptides are formed from casein (Migliore-Samour and Jollet, 1988). Thus depressed lymphocyte responsiveness in autistic children (Stubbs *et al.*, 1977) could easily be explained by a dietary aetiology and exorphins in particular.

An increased frequency of the mucosal IgA antibodies in serum against gliadin, gluten and casein has been found (Reichelt *et al.*, 1990; 1991; Lucarelli *et al.*, 1995; Cade *et al.*, 2000), usually without endomycium antibody increase. These immunological changes reflect an increased protein uptake in about 1/3 of the autistic children and again points to a dietary aetiology. It is also interesting that IgA antibodies against gliadin and gluten have a very strong affinity for cerebral blood vessel structures (Pratesi *et al.*, 1998) and may alter the permeability of these vessels. The recent data on antibodies against a protein in MMR vaccines (Singh *et al.*, 2002) dovetails nicely with Wakefields data and would of course through inflammatory mucosal changes cause increased uptake, and possibly explain the Th1 to Th 2 shift and cytokines increases such as interleukin 2, 12 and interferon-γ (Singh *et al.*, 2002)

Sleep Problems

These are common in early childhood in children with autism. Colic and screaming and apparent lack of tiredness after the briefest of naps are commonly reported. Non-autistic children showing similar behaviour are often helped by removal of cow's milk from the mother if nursing and from the diet, if the child if getting diluted cow's milk directly (Lucassen *et al.*, 1998), In normal children with these problems antibodies to beta-lactoglobulin have been demonstrated (Kahn *et al.*, 1987). If opioids are involved and with bell shaped dose response curves, all manner of gut and sleep problems are to be expected, especially since enkephalin is a transmitter for mucosal ganglion cells. Therefore, our hypothesis that exorphins are key elements in development of autism, is strengthened also by the data from normal sleepless infants. Low synaptic cleft serotonin should reinforce this state of affairs, because functional serotonin decrease causes insomnia in carcinoid disease.

Increased Incidence in Immigrants from Certain Countries

Immigrants from the Developing World to Western Europe have an increased rate of autistic syndromes (Gillberg and Gillberg, 1996). Generally these families move from a low grain, low milk area to an extremely high milk and grain consuming area, not the least because this is the cheapest food in Western Europe/USA. Therefore, such data are to be expected, if our model is correct.

Effect of Gluten and, or Casein Free Diet

It has been difficult to run double blind controlled dietary experiments, because the control group tends to quit after weeks without change. However, testing before and after intervention, and also testing without knowing who is on diet has been carried out (Reichelt *et al.*, 1990; 1991; Knivsberg *et al.*, 1995; Lucarelli *et al.*, 1995; Whiteley *et al.*, 1999; Cade *et al.*, 2000; Knivsberg *et al.*, 2002). It has been argued that placebo may account for some of the changes reported. It should be noted, though, that

the intervention periods in some of these projects are one year or more and in one of trial four years (Knivsberg *et al.*, 1995) (Table IV). Placebo has not been reported to last that long. Furthermore, an inquiry by family questionnaire (Rimland, 1988) and also a small sociological investigation (Shattock, 1995) as well as numerous identical anecdotal reports all point in the same direction; that diet ameliorates the disease process. As may be expected, dietary intervention works better the younger the child and the shorter the history. The spontaneous reports pointing out abstinence or withdrawal symptoms by many parents also reinforce a probable effect of diet. Rashes, itching, pupillary changes, diarrhoea and sleep problems that appear transiently are not easy to misjudge.

Gut–Brain Connection

It has been demonstrated that late onset and regressive autistic children often have regional nodular ileitis and colits of the upper colon (Wakefield *et al.*, 2000; Furlano *et al.*, 2001). Further evidence has been found of mucosal damage found also in the upper gastrointestinal tract (Horvath *et al.*, 1999; Torrente *et al.*, 2002). Mucosal damage would clearly entail increased gut permeability especially since the enterocytes form close to a monolayer. This might explain the increased uptake of protein measured as specific IgA antibody increases in serum (Reichelt *et al.*, 1991; Lucarelli *et al.*, 1995; Cade *et al.*, 2000) and increased low molecular weight permeability (D'Eufemia *et al.*, 1996). Several papers have furthermore, established that inflammatory gut disease regularly causes white matter lesions in the brain (Geissler *et al.*, 1995; Hart, 1998). Peptides may easily be seen to mediate such actions especially because of the opioid link also to epilepsy as outlined. Also in celiac disease increased peptide excretion is found (Reichelt *et al.*, 1998).

Animal Models

Animal not usually eating gluten ought to demonstrate effects of excess feeding of gluten and psychophysiological changes. Feeding gluten to cats causes profound changes in monoamines, the amino acids profile and dopamine beta-hydroxylase in the brain of these cats (Thibault *et al.*, 1988). Thus gluten can clearly have effects on the CNS. Excessive gluten fed to rats causes these animals to learn to attend to redundant stimuli usually ignored in a conditioned reflex paradigm (Harper *et al.*, 1997). This inability to differentiate essential and non-essential inputs is typical for the autistic state (Frith, 1988).

CONCLUSION

Based on the above data, we may present our simple model. The genetic fault is believed to be primarily found in at least two or more peptidases or peptidase regulating proteins. Increased gut permeability/uptake and subsequent peptide increase, which may also be caused by peptidase defects, overwhelm limited break down capacity and make borderline states burst into full blown syndromes. We think that the enzymes involved must be different pairs or more of enzymes (peptidases) in different families, because the opioids found differ in chain length from one patient to the next. The exorphins and other isolated peptides can explain a large part of the symptomatology of the autistic syndromes with its many manifestations. Exposing animals to peptides early during their development can have long lasting effects, and in rats be detectable after 3 months (Gschanes and Windisch, 1999). This could probably be due to trophic effects on the brain and all peptides must therefore be treated with considerable care. This includes also secretin.

With varying chain length of the opioids found as seen for the casomorphins, and varying levels of opioids and bell shaped dose response curves

TABLE IV Changes due to dietary intervention (Knivsberg *et al.*, 1995)

Test	Initial score	1 year change	4 years change	*N*	*p*
C-Raven:	6.8 ± 2.8	+8.6 ± 2.8	+8.6 ± 3.2	12	0.005
ITPA:	25.7 ± 5.5	+2.7 ± 2.5	+6.1 ± 2.8	10	0.005
Tafjord scheme:					
1: Social interaction	53.7 ± 15.2	+12.1 ± 5.9		14	0.005
2: Language	71.0 ± 15.2	+8.7 ± 6.5		14	0.005
3: Structure ability	56.7 ± 17.1	+9.1 ± 5.4		14	0.001
4: Sensory/Motor	72.9 ± 12.3	+7.2 ± 4.5		14	0.001
DIPAB:					
A: Social isolation	8.5 ± 3.3	−6.1 ± 2.7		14	0.005
B: Bizarre traits	5.3 ± 2.2	−5.3 ± 1.22		14	0.005

Notice that Raven C reaches a maximum level after 1 year while Illinois test of psycholinguistic ability (ITPA) improves even more after 4 years, probably reflecting the more complex nature of advanced learning (language). DIPAB = Haracopos scheme: "Diagnosis of Psychotic Behaviour in Children"). Peptide levels decreased and followed the decrease in symptoms. Tafjord is a Norwegian scheme for registering play, interaction, structural ability and sensory motor behaviour during play. For details see Knivsberg *et al.* (1995). Change is measured as delta increase or decrease.

(Reichelt and Reichelt, 1997), it is not surprising that symptoms, abilities, morphology and EEG data are so varied in the autistic syndromes.

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References

Bailey, A., Le Coneur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E. and Rutter, M. (1995) "Autism is a strongly genetic disorder: evidence from a British twin study", *Psychol. Med.* **25**, 63–77.

Banks, W.A., Kastin, A.J., Harrison, L.M. and Zadina, J.E. (1996) "Perinatal treatment of rats with opiates affects the development of blood–brain barrier transport system PTS-1", *Neuroendocrinol. Teratol.* **18**, 711–715.

Bauman, M. (1991) "Microscopic neuroanatomic abnormalitites in autism", *Pediatrics* **87**(Suppl.), 791–796.

Bernal, M.E. and Miller, W.H. (1971) "Electrodermal and cardiac responses of schizophrenic children to sensory stimuli", *Psychophysiology* **7**, 155–165.

Bhatia, K.P., Brown, P., Gregory, Manji, H., Thompson, P.D., Ellison, D.M., *et al.* (1995) "Progressive myoclonic ataxia associated with coeliac disease. The myoclonus is of cortical origin, but the pathology is in the cerebellum", *Brain* **118**, 1087–1093.

Bøhlen, P., Castillo, F., Ling, R. and Guillemin, R. (1980) "An efficient procedure for the separation of peptides from amino acids and salts", *Int. J. Pept. Protein Res.* **16**, 306–310.

Cade, R.J., Privette, R.M., Fregly, M., Rowland, N., Sun, Z., Zele, V., Wagemaker, H. and Edelstein, C. (2000) "Autism and schizophrenia: intestinal disorders", *Nutr. Neurosci.* **2**, 57–72.

Campbell, M., Schopler, E., Cueva, J.E. and Hallin, A. (1996) "Treatment of autistic disorder", *J. Am. Acad. Child Adolesc. Psychiatr.* **35**, 134–143.

Chapman, R.W.G., Laidlow, J.M., Colin-Jones, D., Eade, O.E. and Smith, C.L. (1978) "Increased prevalence of epilepsy in coeliac disease", *Br. Med. J.* **22 July**, 250–251.

Chen, L., Hamaguchi, K. and Ogawa, M. (1980) "pCPA reduces both monoaminergic afferents and non-monoaminergic synapses in the cerebral cortex", *Neurosci. Res.* **19**, 111–115.

Courchesne, E., Townsend, J. and Saitoh, O. (1994) "The brain in infantile autism: posterior fossa structures are abnormal", *Neurology* **44**, 214–223.

D'Eufemia, P., Celli, M., Finnochiaro, R., Pacifico, L., Viozzi, L., Zaccagnini, M., Cardi, E. and Giardini, O. (1996) "Abnormal intestinal permeability in children with autism", *Acta Paediatr.* **85**, 1076–1079.

Deykin, E.Y. and MacMahon, N. (1979) "The incidence of seizures among children with autistic symptoms", *Am. J. Psychiatr.* **136**, 1310–1312.

Egaas, B., Courchesne, E. and Saitoh (1995) "Reduced size of corpus callosum in autism", *Arch. Neurol.* **52**, 794–801.

Ermisch, A., Brust, P., Kretzchmar, R. and Buhle, H-J. (1983) "On the blood–brain barrier to peptides (3H) beta-casomorphin-5 uptake by eighteen brain regions *in vivo*", *J. Neurochem.* **41**, 1229–1233.

Feinberg, I. (1982) "Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence?", *J. Psychiatr. Res.* **17**, 319–334.

Frith, U. (1988) "Autism: possible clues to the underlying pathology", In: Wing, L., ed, Aspects of Autism: Biological Research (Gaskell, The National Autistic Society, London), pp. 19–30.

Furlano, R.I., Anthony, A., Day, R., Brown, A., McGarvey, L., Tomson, M.A., Davies, S.E., Berelowitz, M., Forbes, A., Wakefield, A.J., Walker-Smith, J.A. and Murch, S.H. (2001) "Colonic CD-8 and γ–δ-cell infiltration with epithelial damage in children with autism", *J. Pediatr.* **136**, 366–372.

Gardner, M.L.G. (1994) "Absorption of intact proteins and peptides", In: Johnson, L.R., ed, Physiology of the Gastrointestinal tract, 3rd Ed. (Raven Press, New York), pp. 1795–1820.

Geissler, A., Andus, T., Roth, M., Kullmann, F., Caesar, I., Held, P., Gross, V., Feuerbach, S. and Schömerich, F. (1995) "Focal white-matter lesions in brain of pateints with inflammatory bowel disease", *Lancet* **345**, 897–989.

Gillberg, I.C. and Gillberg, C. (1996) "Autism in immigrants: a population-based study from Swedish rural and urban areas", *J. Intellect. Disabil. Res.* **40**, 24–31.

Gillberg, C., Terenius, L. and Lønnerholm, G. (1985) "Endorphin activity in childhood psychosis: spinal fluid in 24 cases", *Arch. Gen. Psychiatr.* **42**, 780–783.

Gobbi, G., Bouquet, F., Gicco, L., Lambertini, A., Tassinari, C.A., Ventura, L. and Zaniboni, M.G. (1992) "Coeliac disease, epilepsy, and cerebral calcifications", *Lancet* **340**, 439–443.

Green, L-A., Fein, D., Modahl, C., Feinstein, C., Waterhouse, L. and Morris, M. (2001) "Oxytocin and autistic disorder: alterations in peptide forms", *Biol. Psychiatr.* **50**, 609–613.

Gschanes, A. and Windisch, M. (1999) "Early postnatal treatment of peptide preparations influences spatial navigation of young and adult rats", *Behav. Brain Res.* **100**, 161–166.

Hadjivassiliou, M., Grünewald, R.A., Chattopadhyay, A.K., Davies-Jones, G.A.B., Gibson, A., Jarratt, J.A., *et al.* (1998) "Clinical, radiological, neurophysiological, and neuropathological characteristics of gluten ataxia", *Lancet* **352**, 1582–1585.

Hadjivassiliou, M., Boscolo, S., Davies-Jones, G.A.B., Grünewald, R.A., Not, T., Sanders, D.S., Simpson, J.E., Tongiorgi, E., Williamson, C.A. and Woodroofe, N.M. (2002) "The humoral response in the pathogenesis of gluten ataxia", *Neurology* **58**, 1221–1226.

Harper, D.N., Nisbet, R.H. and Siegert, R.J. (1997) "Dietary gluten and learning to attend to redundant stimuli in rats", *Biol. Psychiatr.* **42**, 1060–1066.

Hart, P.E., Gould, S.R., MacSweeney, J.E., Clifton, A. and Schon, F. (1998) "Brain white matter lesions in inflammatory bowel disease", *Lancet* **351**, 1558.

Hashimoto, T., Tayama, M., Masahito, M., Sakorama, N., Yoshimoto, T. and Murakawa, K. (1992) "Reduced brain stem size in children with autism", *Brain Dev.* **14**, 94–97.

Hauser, K.F., McLaughlin, P.J. and Zagon, I.S. (1989) "Endogenous opioid systems and the regulation of dendritic growth and spine formation", *J. Comp. Neurobiol.* **281**, 13–22.

Hole, K., Bergslien, A.A., Jørgensen, H., Berge, O-G., Reichelt, K.L. and Trygstad, O.E. (1979) "A peptide containing fraction from schizophrenia which stimulates opiate receptors and inhbitis dopamine uptake", *Neuroscience* **4**, 1139–1147.

Horvath, K., Papadimitriou, J.C., Rabsztyn, A., Drachenberg, C. and Tildon, J.T. (1999) "Gastrointestinal abnormalities in children with autistic disorder", *J. Pediatr.* **135**, 559–563.

Husby, S., Jensenius, J.C. and Cant, A.J. (1984) "Passage of un-degraded dietary antigen into the blood of healthy adults", *Scand. J. Immunol.* **22**, 83–92.

Israngkun, P., Newman, H.A., Patel, S.T., Duruibe, V.A. and Abou-Issa, H. (1986) "Potential biochemical markers for infantile autism", *Neurochem. Pathol.* **5**, 51–70.

Kahn, A., Rheffat, E., Blum, D., Casimir, G., Duchateau, J., Mozin, J. and Jost, R. (1987) "Difficulty in initiating sleep and maintaining sleep associated with cow's milk allergy in infants", *Sleep* **10**, 116–121.

Keller, J. (1997) "Impact of autism-related peptides and 5-HT system manipulations on cortical development and plasticity", *Ist. Annual report for the EU project, BMH4-CT96-0730*, pp. 1–10.

Kilshaw, P.J. and Cant, A.J. (1984) "The passage of maternal dietary protein into human breast milk", *Int. Arch. Allergy Appl. Immunol.* **75**, 8–15.

Kinney, H.C., Burger, P.C., Hurwitz, B.J., Humans, J.C. and Grant, J.P. (1982) "Degeneration of the central nervous system associated with celiac disease", *J. Neurol. Sci.* **53**, 9–22.

Knivsberg, A-M., Reichelt, K.L., Nødland, M. and Høien, T. (1995) "Autistic syndromes and diet: a follow up study", *Scand. J. Edu. Res.* **39**, 223–236.

Knivsberg, A-M., Reichelt, K.L., Høien, T. and Nødland, M. (2002) "A randomised, controlled study of dietary intervention in Autistic syndromes", *Nutr. Neurosci.* **5**, 251–261.

LaBella, F.S., Geiger, J.D. and Glavin, G.B. (1985) "Administrated peptides inhibit the degradation of endogenous peptides,

the dilemma of distinguishing direct from indirect effects", *Peptides* **6**, 645–660.
LeBoyer, M., Bouvard, M.P., Lensing, P., Launay, J-M., Tabuteau, F., Arnaud, P., Waller, D., Plumet, M-H., Recasens, C., Kerdelhue, B., Duags, M. and Panksepp, J. (1990) "Opioid excess hyothesis of Autism", *Brain Dysfunct.* **3**, 285–298.
LeBoyer, M., Bouard, M.P., Racasens, C., Philippe, A., Guillod-Bataille, M., Bondeaux, D., Tabateau, F., Dugas, M., Panksepp, J. and Launoy, J-M. (1994) "Difference between plasma N-and C-terminally directed beta-endorphin immunoreactivity in infantile autism", *Am. J. Psychiatr.* **151**, 1797–1801.
Lindstrøm, L.H., Nyberg, F., Terenius, L., Bauer, K., Besev, G., Gunne, L.M., Lyrenaas, S., Willdeck-Lund, G. and Lundberg, B. (1984) "CSF and plasma beta-casomorphin-like opioid peptides in post-partum psychosis", *Am. J. Psychiatr.* **141**, 1059–1066.
Lucarelli, S., Frediani, T., Zingoni, A.M., Ferruzzi, F., Giardini, O., Quintieri, F., Barbato, M., D'Eufemia, P. and Cardi, E. (1995) "Food allergy and infantile autism", *Panminerva Med.* **37**, 137–141.
Lucassen, P.L.B., Assendelft, W.J.J., Gubbels, J.W., vanEijk, J.T.M., vanGeldrop, W.J. and Knuistingh Neven, A. (1998) "Effectiveness of treatments for infantile colic: a systematic review", *Br. Med. J.* **316**, 1563–1569.
Mahe, S., Tome, D., Dumontier, A.M. and Desjeux, J.F. (1989) "Absorption of intact morphiceptin by diisopropylfluorophosphate-treated rabbit ileum", *Peptides* **10**, 45–52.
Mednick, S.A., Schulsinger, F., Bell, B., Venables, P.H. and Christiansen, K.O. (1974) Genetics, environment and psychopathology (North Holland Press, Amsterdam).
Migliore-Samour, D. and Jollet, P. (1988) "Casein, a prohormone with immunostimulating role in the newborns?", *Experientia* **44**, 88–93.
Nelson, K.B., Grether, J.K., Croen, L.A., Dambrosia, J.M., Dickens, B.F., Jelliffe, L.L., Hansen, R.L. and Phillips, T.M. (2001) "Neuroeptides and neurotophins in neonatal blood of children with autism and mental retardation", *Ann. Neurol.* **49**, 597–606.
Nyberg, F., Liberman, R., Lindstøm, L.H., Lyrenaes, S., Koch, G. and Terenius, L. (1989) "Immunoreactive beta-casomorphin-8 in cerebrospinal fluid from pregnant and lactating women: correlation with plasma level", *J. Clin. Endocrinol. Metab.* **68**, 283–289.
Panksepp, J., Normansell, L., Sivily, S., Rossi, J. and Zolovick, A.J. (1978) "Casomorphins reduce separation distress in chickens", *Peptides* **5**, 829–831.
Paul, K-D., Henker, J., Todt, A. and Eysold, R. (1985) "EEG-Befunde in Zoeliaki- kranken Kindern in Abhængigkeit von der Ernæhrung", *Z. Klin. Med.* **40**, 439–443.
Pedersen, O.S., Ying, L. and Reichelt, K.L. (1999) "Serotonin uptake stimulating peptide found in plasma of normal individuals and in some autistic urines", *Pept. Res.* **53**, 641–646.
Persico, A.M., Baldi, A., Reichelt, K-L., Gonzalez, A., Keller, F., (1998) "Serotonin uptake-stimulating peptides extracted from the urines of autistic patients: potential significance for the pathogenesis of autistic disorders". *American Neuroscience Meeting*, Los Angeles. abs. No 2.
Persico, A.M., Militerni, R., Brvaccio, C., Schneider, C., Melmed, R., Trillo, S., Montecchi, F., Palermo, M.T., Pascucci, T., puglisi-Allegra, S., Reichelt, K.L., Conciatori, M., Baldi, A. and Keller, F. (2000) "Adenosine deaminase alleles and autistic disorders: case–control and family-based association studies", *Am. J. Med. Genet.* **96**, 784–790.
Persico, A.M., D'Agruma, L., Maiorano, N., Totaro, A., Militerni, R., Bravaccio, C., Wassink, T.H., Schneider, C., Melmed, R., Trilo, S., Montecchi, F., Palermo, M., Pascucci, T., Puglisi-Allegra, S., Reichelt, K.L., Conciatori, M., Marino, R., Quattrocchi, C.C., Baldi, A., Zelante, L., Gasparini, P. and Keller, F. (2001) "Reelin gene alleles and haplotypes as a factor pre-disposing to autistic disorder", *Mol. Psychiatr.* **6**, 150–159.
Piven, J., Arndt, S., Bailey, J. and Andreasen, N. (1996) "Regional Brain enlargement in autism: a magnetic resonance imaging study", *J. Am. Acad. Child Adolesc. Psychiatr.* **35**, 530–536.
Pratesi, R., Gandolfi, L., Friedman, H., Farage, L., DeCastro, C.A.M. and Catassi, C. (1998) "Serum IgA antibodies from patients with coeliac disease react strongly with human brain blood-vessel structures", *Scand. J. Gastroenterol.* **33**, 817–821.
Reichelt, W.H. and Reichelt, K.L. (1997) "The possible role of peptides derived from food proteins in diseases of the nervous system", In: Gobbi, G., ed, Epilepsy and other Neurological Disorders in Coeliac Disease (John Libbey & Comp., London), pp. 225–235.
Reichelt, K.L., Saelid, G., Lindback, T. and Boler, J.B. (1986) "Childhood autism: A complex disorder", *Biol. Psychiat.* **21**, 1279–1290.
Reichelt, K.L., Ekrem, J. and Scott, H. (1990) "Gluten, milk proteins and autism: dietary intervention effects on behavior and peptide secretion", *J. Appl. Nutrit.* **42**, 1–11.
Reichelt, K.L., Knivsberg, A-M., Lind, G. and Nødland, M. (1991) "Probable etiology and possible treatemtn of childhood autism", *Brain Dysfunct.* **4**, 308–319.
Reichelt, K.L., Pedersen, O.S., Liu, Y., Knivsberg, A.M., and Nødland, M. (1997). "Possible role of peptides, exorphines and serotonin uptake stimulating peptides in autism". In: *Living and learning with Autism* (Edit: The Autism Res Unit. University of Sunderland), pp. 221–231.
Reichelt, W.H., E, J., Stensrud, M.B. and Reichelt, K.L. and (1998) "Peptide excretion in Celiac disease", *J. Pediatr. Gastroenterol. Nutrit.* **26**, 305–309.
Remme, J.F., Koetzner, S., Haugland, K., Reichelt, K.L., and Brønstad, G.O. (2001) "Analysis of neuroactive peptides with LC/MS in the urine of autistioc patients". *Proc. 49th Conf Mass Spectrometry and Allied Topics*, **Chicago, May 27–31**, pp. 2–3.
Rimland, B. (1988) "Comparative effects of treatment on child's behaviour", *Autism Res. Rev. Int.* **2**(suppl. 34B).
Ritvo, E.R., Ornitz, E.M., Eviatar, A., Markham, C.H., Brown, M. and Mason, A. (1969) "Decreased post-rotatory nystagmus in early infantile autism", *Neurology* **19**, 653–658.
Ritvo, E., Freeman, B.J., Scheibel, A.B., Duong, T., Robinson, H. and Guthrie, D. (1986) "Lower Purkinje cell counts in cerebella of four autistic subjects: initial findings of the UCLA-NSAC autopsy research report", *Am. J. Psychiatr.* **143**, 863–866.
Rogeness, G.A., Javors, M.A. and Pliszka, S.R. (1992) "Neurochemistry and child and adolescent psychiatry", *J. Am. Acad. Child Adolesc. Psychiatr.* **31**, 765–781.
Rosenblum, S.M., Ariock, J.R., Krug, D.A., Stubbs, E.G., Young, N.B. and Pelson, R.O. (1980) "Auditory brainstem evoked responses in autistic children", *J. Autism Dev. Disord.*, 215–225.
Scifo, R., Cioni, M., Batticane, N., Tirolo, C., Testa, N., Quattropani, M.C., Morale, M.C., Gallo, F. and Marchetti, B. (1996) "Opioid-immune interactions in autism: behavioural and immunological assessment during a double-blind treatment with naltrexone", *Ann. Ist. Super. Sanita* **32**, 351–359.
Shanahan, M.R., Venturini, A.J., Daiss, J.L. and Friedman, A.E. (2000) "Peptide diagnostic markers for human disorders", *Eur. Patent Appl.* **EP 0 969 015 A2 1-44**, 1–44.
Shattock, R. (1995). "Can dietary intervention be used successfully as a therapy for Autism?" In *Psychological Perspectives in Autism*. (Edit: The Autism Res Unit. University of Sunderland), pp. 203–206.
Shattock, P., Kennedy, A., Rowell, F. and Berney, T. (1990) "Role of neuropeptides in autism and their relationship with classical neurotransmitters", *Brain Dysfunct.* **3**, 328–345.
Siggins, G.R., Henriksen, S.J., Chavkin, C. and Gruol, D. (1986) "Opioid peptides and epileptogenesis in the limbic system: cellular mechanisms", *Adv. Neurol.*, 501–512.
Singh, V.K. (1995) "Neuropeptides as native immune modulators", *Prog. Drug Res.* **45**, 10–31.
Singh, V.K., Lin, S.X., Newell, E. and Nelson, C. (2002) "Abnormal measles–mumps–rubella antiboides and CNS autoimmunity in children with autism", *J. Biomed. Sci.* **9**, 359–364.
Stubbs, E.G., Crawford, M.L., Burger, D.R. and Vandenbark, A.A. (1977) "Depressed lymphocyte responsiveness in autistic children", *J. Autism Child. Schizophr.* **7**, 49–55.
Sun, Z. and Cade, J.R. (1999) "A peptide found in schizophrenia and autism causes behavioral changes in rats", *Autism* **3**, 85–95.
Sun, Z., Cade, R.J., Fregly, M.J. and Privette, R.M. (1999) "Beta-casomorphin induces Fos-like immunoreactivity in discrete brain regions relevant to schizophrenia and autism", *Autism* **3**, 67–83.
Tenconi, B., DiGiulio, A.M., Donadoni, M.L., Montegazza, P. and Gorio, A. (1991) "Perinatal exposure to opiates alters reactive

pruning and regeneration of serotoninergic neurones", *Brain Dysfunct.* **4**, 49–50.

Thibault, L., Coulon, J-F., Roberg, J., Craig, K.A., Meador-Woodruff, J.H., Goldman, R. and Green, J.F. (1988) "Changes in serum amino acids content and dopamine beta-hydroxylase activity and brain neuro-transmitter interaction in cats fed casein with and without gluten", *J. Clin. Biochem. Nutrit.* **4**, 209–221.

Torrente, F., Ashwood, P., Day, R., Machado, N., Furlano, R.I., Anthony, A., Davies, S.E., Wakefield, A.J., Thomson, M.A., Walker-Smith, J.A. and Murch, S.H. (2002) "Small intestinal eneropathy with epithelial IgG and complement deposition in children with regressive autism", *Mol. Psychiatr.* **7**, 375–382.

Troncone, R., Scarcella, A., Donatiello, A., Cannataro, P., Tarabusco, A. and Aurichio, S. (1987) "Passage of gliadin into human breast milk", *Acta Paediatr. Scand.* **76**, 453–456.

Wakefield, A.J., Anthony, A., Murch, S.H., Thomson, M., Montgomery, S.M., Davies, S., O'Leary, J.J., Berelowitz, M. and Walker-Smith, J.A. (2000) "Enterocolitis in children with developmental disorders", *Am. J. Gastroenterol.* **95**, 2285–2295.

Waring, R.H. and Ngong, J.M. (1993). Sulphate metabolism in allergy-induced autism: relevance to the disease etiology. In *Biological perspectives in Autism* (Edit: The Autism Res Unit. University of Sunderland), pp. 25–33.

Watanabe, Y., Kojima-Kumatsu, T., Iwaki-Egawa, A. and Fujimoto, Y. (1993) "Increased excretion of proline-containing peptides in dipeptidyl peptidase IV-deficient rats", *Res. Commun. Chem. Pathol. Pharmacol.* **81**, 323–350.

Whiteley, P., Rodgers, J., Savery, D. and Shattock, P. (1999) "A gluten-free diet as an intervention for autism and associated spectrum disorders: preliminary findings", *Autism* **3**, 45–65.

Zagon, I.S. and McLaughlin, P.J. (1987) "Endogenous opioid systems regulate cell proliferation in the developing rat brain", *Brain Res.* **412**, 68–72.