

Autism and Schizophrenia: Intestinal Disorders

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We examined Dohan's hypothesis that schizophrenia is associated with the absorption of "exorphins" contained in gluten and casein. In addition, because of the work of Reichelt *et al.* (Reichelt, K.L., Saelid, G., Lindback, J. and Orbeck, H. (1986) *Biological Psychiatry* 21: 1279–1290) and Rodriguez *et al.* (Rodriguez, Trav, A.L., Barreiro Marin, P., Galvez, Borrero, I.M., del Olmo Romero-Nieva, F. and Diaz Alvarez, A. (1994) *Journal of Nervous and Mental Disease* Aug; 182(8): 478–479), we carried out similar studies on a group of children with autism. In both syndromes we found similar patterns of peptide containing peaks (Ninhydrin positive) after molecular screening with Sephadex G-15.

Immunoglobulin assay of IgA and IgG against gliadin and casein in serum was done. High titer IgG antibodies to gliadin were found in 87% of autistic and 86% of schizophrenic patients and high titer IgG antibodies to bovine casein were found in 90% of autistic and in 93% of schizophrenic patients. High titer IgA antibodies to gluten or casein were found in 30% of children with autism while in schizophrenic patients 86% had elevated IgA antibodies to gluten and 67% to casein; some normal children and adults have these antibodies but only in trace amounts. When schizophrenic patients were treated with dialysis or a gluten–casein free diet, or both (Cade, R., Wagemaker, H., Privette, R.M., Fregly, M., Rogers, J. and Orlando, J. (1990) *Psychiatry: A World Perspective* 1: 494–500) peptiduria and Brief Psychiatric Rating Scores fell while abnormal behavior diminished.

A gluten–casein free diet was accompanied by improvement in 81% of autistic children within 3 months in most of the behavior categories. Our data provide support for the proposal that many patients with schizophrenia or autism suffer due to absorption of exorphins formed in the intestine from incomplete digestion of gluten and casein.

Keywords: Gluten, Casein, Schizophrenia, Autism, Gliadorphin, β -Casomorphin, Diet

AUTISM AND SCHIZOPHRENIA

Dohan (1966), following a study of the dietary habits of societies in New Guinea and other South Pacific Islands reported that schizophrenia was very rare and mild among people who did not use wheat, barley, oats and rye. Conversely, he found that schizophrenia was common and severe in societies around the world when gluten containing grains made up a large part of the diet. As a result of his studies, Dohan raised the hypothesis that an overload of peptides derived from gluten or casein cause schizophrenia. Our studies were

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designed to answer questions that Dohan's hypothesis and its extension to autism (Reichelt *et al.*, 1981; Rodriguez *et al.*, 1994) raise. These are:

- (1) Is there an unusually high concentration of peptides in blood and urine of schizophrenic and autistic patients? (Reichelt *et al.*, 1981; Cade *et al.*, 1990).
- (2) If they enter the blood, can they penetrate the blood brain barrier? (Sun and Cade, 1999a).
- (3) What structures do they enter? (Sun and Cade, 1999a).
- (4) Could involvement of the brain structures the peptides enter cause the symptoms of autism and schizophrenia? (Sun and Cade, 1999b).
- (5) If the peptides are removed or greatly decreased in concentration (Cade *et al.*, 1990) are the disease symptoms and signs diminished or cured? (Knivsberg *et al.*, 1995).
- (6) If a normal animal is given one of the peptides will it produce symptoms similar to those of autism or schizophrenia? (Sun and Cade, 1999b).

While several of these problems have been approached by different laboratories (Reichelt, 1996; Reichelt *et al.*, 1981; 1996; Shattock and Lowdon, 1991; Shattock *et al.*, 1990; Knivsberg *et al.*, 1990; 1995; Whitely *et al.*, 1997) with a variety of methods, no one laboratory has attempted to join all of these questions together.

MATERIALS AND METHODS

Subjects and Treatments

A total of 270 human patients with either autism or schizophrenia were referred by their parents or physician and enrolled in these studies: 83 schizophrenic men (ages 17–49) and 37 schizophrenic women (ages 18–46 years), 128 boys with autism (ages 3.5–16 years) and 22 autistic girls (ages 3.5–14 years). When reviewed by our psychiatrist (H.W.) all met DSM III criteria for their disease (APA, DSM III, 1987). All schizophrenic patients had chronic disease documented

by psychiatric hospital records. None had experienced a remission, significant improvement, or a response to drug therapy for at least 3 years prior to enrolling in our study. All autistic patients had been ill for at least one year prior to entry in the study. None, as assessed by their parents, teachers or physicians, had shown any sustained improvement or response to therapy they received. These patients were all interviewed by our psychiatrist (H.W.) and thought to meet DSM III criteria. Control values for all laboratory tests, schizophrenics and autistic children, were obtained from roughly sex and age matched normal volunteers who had no detectable disease.

All patients, both schizophrenic and autistic, were referred by a responsible adult, usually a parent, or by their physician. Schizophrenic patients, in addition to a psychiatric interview, were evaluated with the MMPI (Cade *et al.*, 1990) and Brief Psychiatric Rating Scale (Hedlund and Vieweg, 1980). Severity of 18 psychiatric symptoms were rated by three experienced observers; two psychiatrists and one physician assistant independently from 1 (normal) through 7 (very severe) and averages and means were then calculated. Variations between the highest and lowest ratings of the three evaluators on an individual subject examination averaged 8% and were never greater than 13%. In the schizophrenic group the BPRS was given at every dialysis-clinic visit throughout the study and the MMPI at intervals of 4–6 months. Observations of patients on dialysis and a gluten–casein diet were not blind as we found it impossible to blind the patients and patients invariably told the observers.

Autistic children were admitted to the study if they had been diagnosed as having autism or Asperger's syndrome (Asperger, 1961) by their pediatrician or psychiatrist, and, if in the opinion of our study psychiatrist (H.W.), they met DSM III criteria for the disorder. During the study autistic children were assessed by their parents, usually, and physicians, independently, and in many instances, by their teachers (on a scale of 0–4) for the presence and severity of the diagnostic

manifestations of autism listed in Table IV. The parents and physicians assessments were then averaged. Variability of individual observer scores was less than 10%. This may have been so because much of the physician's evaluation was obviously affected by the parents historical observations done at home or teachers done at school. It was repeated after one month of treatment and then every 3 months for one year. Both schizophrenic subjects and autistic patients had all medications discontinued before dietary or dialysis treatment was begun and were, as well as we could control, kept off all medications while on the gluten-casein free diet. Supervised discontinuation of medications and treatments that had been proven to be ineffective was not considered hazardous. The Human Experimental Control Board of the hospital approved of the protocol and all parents gave informed consent.

A total of 57 patients with schizophrenia agreed to undergo hemodialysis for 6–8 h, weekly, depending on their body weight, for 1–14 years using a Fresenius Polysulfone membrane 0.75 m². Five of these patients were treated with dialysis plus a gluten-casein free diet, and seven patients from another patient pool were treated with diet alone. Autistic children, with the exception of one 13-year-old girl who was dialyzed and recovered in 7 weeks, were all treated with a gluten-casein free diet.

The gluten-casein free diet used by both schizophrenic and autistic patients is a synthesis of *The Milk-Free Kitchen* by Kidder (1988) and *The Gluten-Free Gourmet* by Hagman (1990). Diets before the study were frequently quite strange depending on the child's preferences. Most had a consuming desire for products containing milk or gluten and many experienced what appeared to be opium withdrawal when the diet was started. When their child entered the study parents usually made a serious effort to encourage a balanced diet devoid of gluten and casein. Some children added new foods to their intake readily. With most, however, 2–3 months were required to achieve a reasonably well balanced diet.

Laboratory Methods

Blood was obtained initially for presence and quantification of IgA (Reichelt and Landmark, 1995) and IgG antibodies to gluten and to bovine casein (Scott *et al.*, 1985). A 24 h urine, chilled and acidified during collection with 6 N HCl, was then adjusted to pH 4 with NaOH and precipitated by addition of 10% ethyl alcohol saturated with benzoic acid. The pH was again adjusted to pH 4 and left in a cold room, sealed, for 24 h. The supernatant was then decanted. The precipitate was washed with ethanol and again centrifuged and decanted. The wash decant, rewash procedure was repeated 2 or 3 times until the OD at UV280 with a 1 cm light path was 0.3. An aliquot was then passed through a 100 cm × 1.8 cm column of Sephadex G-15 with 0.6 M NH₄HCO₃ as the solvent, flow rate was 1.8 ml/min and temperature was 17°F. The sample size applied to the column contained between 75 and 750 mg creatinine depending on the age and size of the subject (Hole *et al.*, 1979; Trygstad *et al.*, 1980). Small children excrete small amounts of urine and creatinine, so in some instances the amount of creatinine was quite small. The peak areas in Sephadex separations were recorded at UV280 and 230 and are expressed as area (cm²) under the peak (recorded at UV280) divided by mg of creatinine in the sample. Aliquots from peaks eluting from Sephadex filtration at 2.4 and 4.02 times the void volume were collected, acidified with acetic acid, then neutralized with KOH and tested by the Ninhydrin reaction. The remaining sample was then lyophilized and the residue stored at -71°C. A liquid aliquot of each sample was also stored at -71°C.

Each HPLC sample contained 250 μmol of creatinine. Patterns were developed using a Vydac 18 reverse phase column (0.250 × 4.6 nm) 1 ml/min. A Biorad column heater (No. 1250426) was used so that chromatography occurred at 35°C. Samples were scanned at 215, 230 and 280 nm and the area under the UV215 nm integrated at 1000 mV. The column was then cleaned by flowing mobile

phase A for 15–20 min. In many samples prints were made at 2 magnification (see Figure 2(c)) so that peaks quite large and quite small could be seen and examined with ease. The chromatographic procedure is based on the separation of peptides from amino acids and salts developed by Bohlen *et al.* (1980), and modified by Reichelt *et al.* (1998) and Shattock and Lowdon (1991). A separate single morning aliquot of chilled (Thymol preserved) urine was collected for direct HPLC. A 5 cm sample was filtered with costar spin and centrifugal cellulose acetate 0.22 μm filters at 3500 rpm for 20 min at 4°C. A urine aliquot containing 250 nmol of creatinine was applied to the column and the chromatogram developed as described above. Two pumps were used to perfuse the system. Pump A 0.1% TFA in water and pump B 0.1% TFA in 95/5 acetonitrile/H₂O. For the first 15 min only TFA in water was perfused, then pump B infused 0.1% TFA in acetonitrile (95%) H₂O (5%) and the gradient increased by 5% every 5 min until it was 60/40. After the separation of peptides, the system was cleaned by flowing TFA acetonitrile/H₂O 60% for 20 min; then back down to 1% over 5 min. All standards were obtained from Nova Biochem, Peninsular Biochem or from Sigma Biochem and stored at +4°F until used.

Statistical Evaluation

Statistical evaluation of data from both autistic children and schizophrenic adults was done by Kruskal–Wallis one-way analysis of variance on ranks (Shutte, 1977). Differences between values in pre-treatment, 1 month, 3 months, etc. were done by student Newman Keuls taking into account the non-parametric nature of the data.

RESULTS

Sephadex Separation

Figure 1(a)–(c), demonstrates the three patterns of peptide containing peaks which separate during

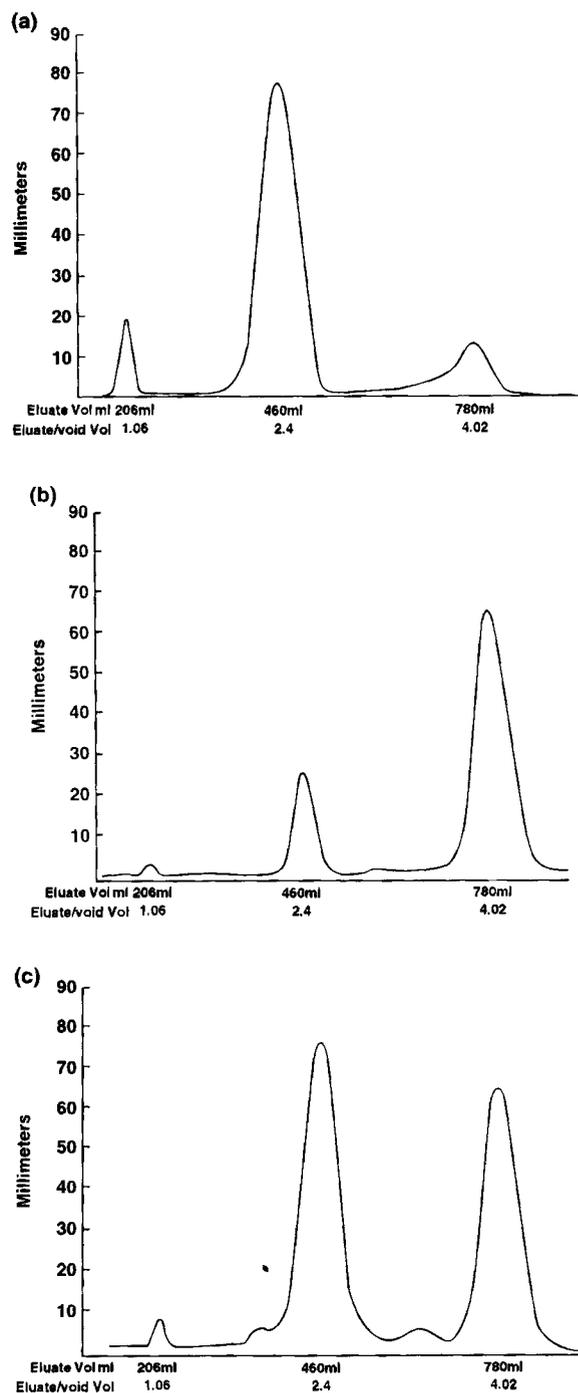


FIGURE 1 (a) Urinary peptide containing peak Pattern I from normal subjects following G-15 Sephadex separation, (b) Pattern II from normal subjects, (c) Pattern III from normal subjects. Normal values were estimated by using the highest and lowest values of peaks from urine of normal volunteers.

Sephadex filtration of urine from normal voluntary controls (76 adults, ages 16–71, 2/3 male) and 43 children (ages 3–11, 32 boys and 11 girls). Pattern I has a large peak which elutes at approximately $2.4 \times$ void volume (vv) and a small or non-existent peak at $4 \times$ vv. Pattern II has a small peak at $2.4 \times$ vv and a large peak at $4 \times$ vv. Pattern III has a large peak at both 2.4 and $4.0 \times$ vv. All peaks were strongly Ninhydrin positive, a finding also reported by Hole *et al.* (1979, 1988).

The same three patterns, but with larger peaks at 2.4 and $4.0 \times$ vv are also found in pre-treatment urine of both autistic children and patients with schizophrenia. The percentage occurrence of each pattern in the two patient groups and in normal controls is shown in Table I. Among pre-treatment patients with autism an abnormal Pattern I was found in 58%; Pattern II in 2% of pre-treatment samples, and in 6% of samples from children after 6 months of diet therapy, and Pattern III in 36% while 4 samples with a normal pre-treatment Pattern III were found. Among pre-treatment schizophrenic patients an abnormal Pattern I

was found in 30%, an abnormal Pattern II in 25%, and an abnormal Pattern III in 40%, while a normal Pattern III was found in 5%.

Table II shows the area (cm^2/mg creatinine) under the peaks eluting at 2.4 and $4.0 \times$ vv for normal controls, pre-treatment children with autism and pre-treatment patients with schizophrenia. Table III shows the titer of IgA and IgG antibodies to gluten and casein (Scott *et al.*, 1985). Among autistic children elevated IgA antibody (titer) to gluten or casein was found in 30% (Reichelt and Landmark, 1995) and averaged 2.1 against gluten and 1.9 against casein on a 0–6 scale. Elevated IgG antibodies to gluten, expressed as Rast scores, were elevated in 87% and to casein in 90%. Among schizophrenic patients, IgA antibodies to gluten were elevated in 86% and against casein in 67%. IgG antibodies to gluten were elevated in 86% and to casein in 93%. Both normal control children and adults had IgA titers to gluten of 0–trace and IgG titers to gluten and casein of < 120 –1600. It seems plausible from the report of Pratesi *et al.* (1998) that IGA damage to cerebral vessels could be part of the cause for chronic symptoms.

TABLE I Frequency (%) of Sephadex column patterns of peptide containing peaks from urine of autistic and schizophrenic patients and normal volunteers

Pattern type	I	II	III
Autism	58	2	36
Normal volunteers	30	25	40
Schizophrenia	30	25	40

All peaks were collected and tested for amino acid and peptides by the ninhydrin reaction; 5 of schizophrenic and 4 of autistic patients had Pattern III peptiduria with both peaks in a normal range.

HPLC Separations

Figure 2(a)–(f) show the initial pre-treatment HPLC separation of peptides from the urine of a 7-year-old boy with autism. An aliquot of urine was kept at $+4^\circ\text{C}$ until processed for HPLC study. Figure 2(b) shows the HPLC of urine from the same child after one year on a gluten–casein free diet. It is apparent that the amount of peptides, which elute between 30 and 70 min, is much less in

TABLE II Size of peptide peaks (cm^2/mg creatinine) following Sephadex separation from normal, roughly age matched controls, schizophrenic patients and autistic children

Eluate vol/void vol	Pattern I		Pattern II		Pattern III	
	2.40	4.02	2.40	4.02	2.40	4.02
Normal	0.10–0.18	0–0.05	0–0.1	0.1–0.20	0.1–0.18	0.1–0.18
Autism	0.24–13.0	0–0.05	—	—	0.25–17.0	0.25–8.0
Schizophrenia	0.30–17.5	0–0.05	0–0.1	0.22–14.0	0.13–21.0	0.26–16.1

TABLE III Frequency (%) and concentration of plasma IgA and IgG antibodies to gluten and casein in autistic and schizophrenic patients and normal volunteers

	IgA				IgG			
	Gluten		Casein		Gluten		Casein	
	Inc	Conc	Inc	Conc	Inc	Conc	Inc	Conc
Autism	15%	2.1	14%	1.9	87%	5240	90%	6720
Schizophrenia	86%	2.9	67%	2.97	86%	3024	93%	3373
Normal children volunteers	3%	0-Tr	6%	0-Tr	1%	0-1600	7%	0-1600
Normal adult volunteers	34%	0-Tr	51%	0-Tr	34%	1240	26%	0-820

IgA concentrations were dilution titration and IgG is expressed on Rast score. Normal anti-IgA was 0, anti-IgG as Rast units was normal, 0-1600.

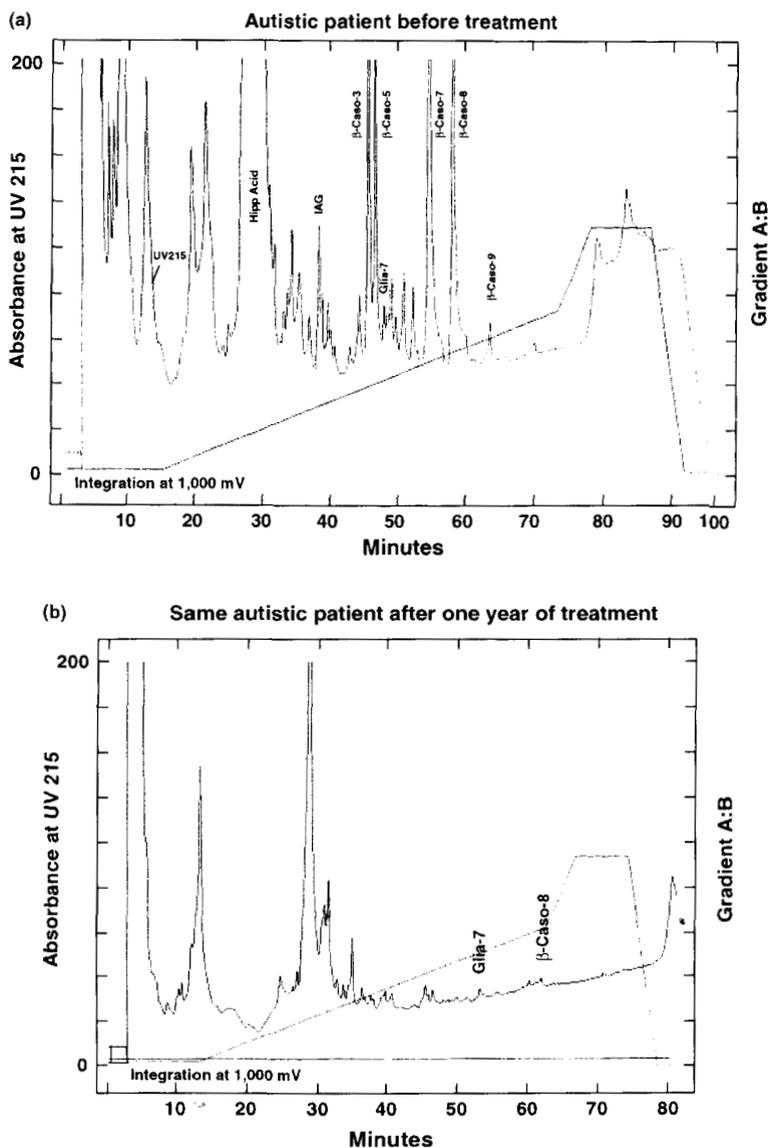


FIGURE 2(a) and (b)

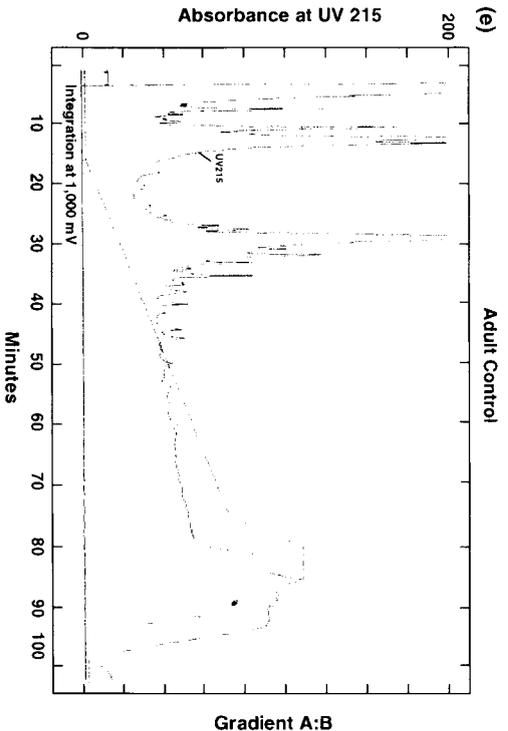
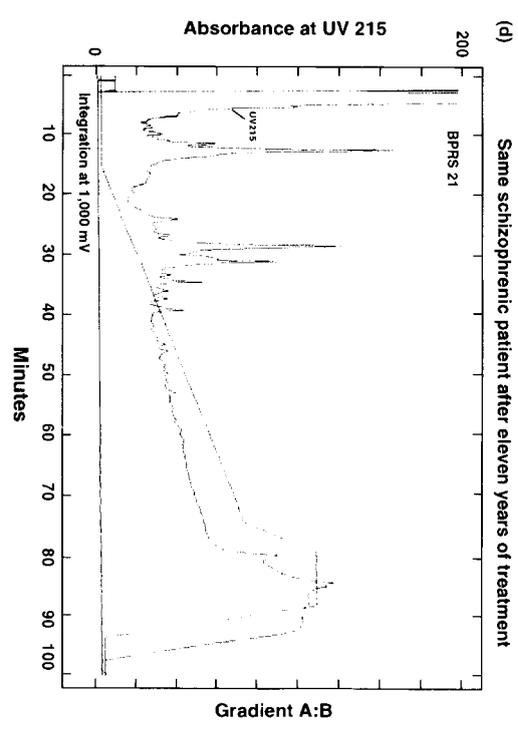
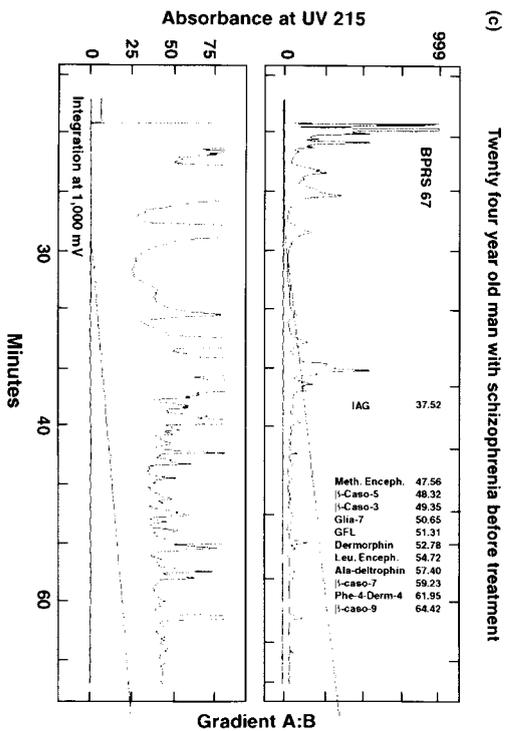


FIGURE 2(c)-(e)

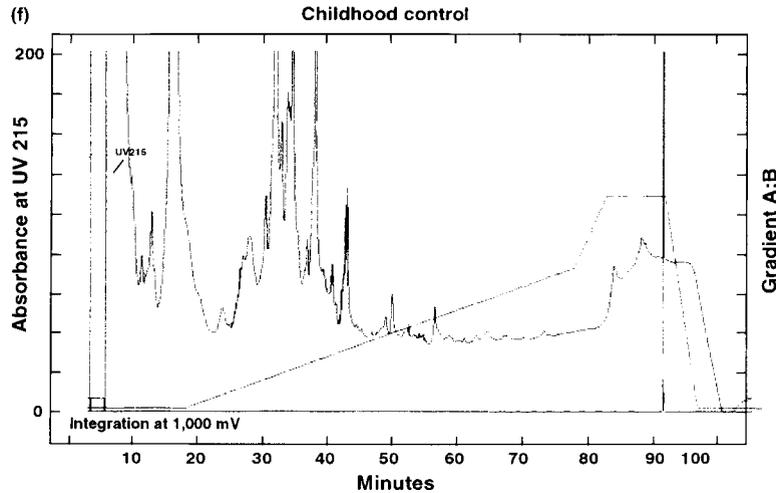


FIGURE 2(f)

FIGURE 2 Shows the result of HPLC separation of peptides from urine. All samples were stored at +4°F until processed, usually the day they were received. After preparation, a sample containing 250 μ mol of creatinine was put on the column; (a) shows the HPLC tracing of peptides in the urine of an 8 year boy before diet treatment and (b) shows the tracing from the same child after 1 year on the diet; (c) shows the pre-treatment tracing of a young man who was schizophrenic and (d) shows a tracing of the same man after 11 years of treatment; (c) shows an enlargement so that small peaks are easier to see and evaluate. The BPRS scores on (c) and (d) show the values at the time these urines were collected. Both tracings were made at UV215 and integrated at 1000 mV. Figure (e) shows the tracing from urine from a normal man and (f) shows the tracing from a 7-year-old normal boy.

the treatment sample than in the pre-treatment sample and, in particular, the peaks of casomorphin, gliadorphin and IAG were much smaller. Indolyl-3-acryloylglycine (IAG) is thought to be a product of Tryptophan degradation in the gut. It has been found in large amounts in multiple neurologic disorders. Most recently in Autism and Schizophrenia by Shattock *et al.* (1990) and Shattock and Lowdon (1991). It is thought to increase intestinal permeability. Figure 2(c) shows the HPLC of a young man with schizophrenia for 5 years before treatment began. It is apparent that the eluate peaks between 30 and 70 min, where peptides elute, are quite large. Figure 2(d) shows the HPLC of the same man after 11 years of treatment. He was treated with dialysis alone for 8 years and improved significantly but continued to have relapses. Dialysis was continued and a casein-gluten free diet was then added and he improved again but relapsed each time he broke his diet. We then added Creon^(R)10, a mixture of

pancreatic enzymes supplied by Solvay Pharmaceutical containing (Lipase 10,000 USP units, Amylase 30,000 USP units and Protease 30,000 USP units) one tablet with each meal and also if he mistakenly ate something containing gluten or casein between meals. He has now been on triple treatment for 18 months; his BPRS which is shown on HPLC tracings c and d are the values obtained at the time that urine was collected. His BPRS scores are now consistently normal as are his Sephadex peaks and HPLC. In addition, he has a full time job and supports himself. Figure 2(e) shows the HPLC tracing from a normal young man and Figure 2(f) that of a normal 8-year-old boy.

Figure 3A shows a comparison of progress of patients treated with dialysis alone and dialysis plus diet. The mean weekly BPRS scores were done by the same three observers mentioned previously in Subjects and Treatments for 5 Pattern I schizophrenic patients whose symptoms

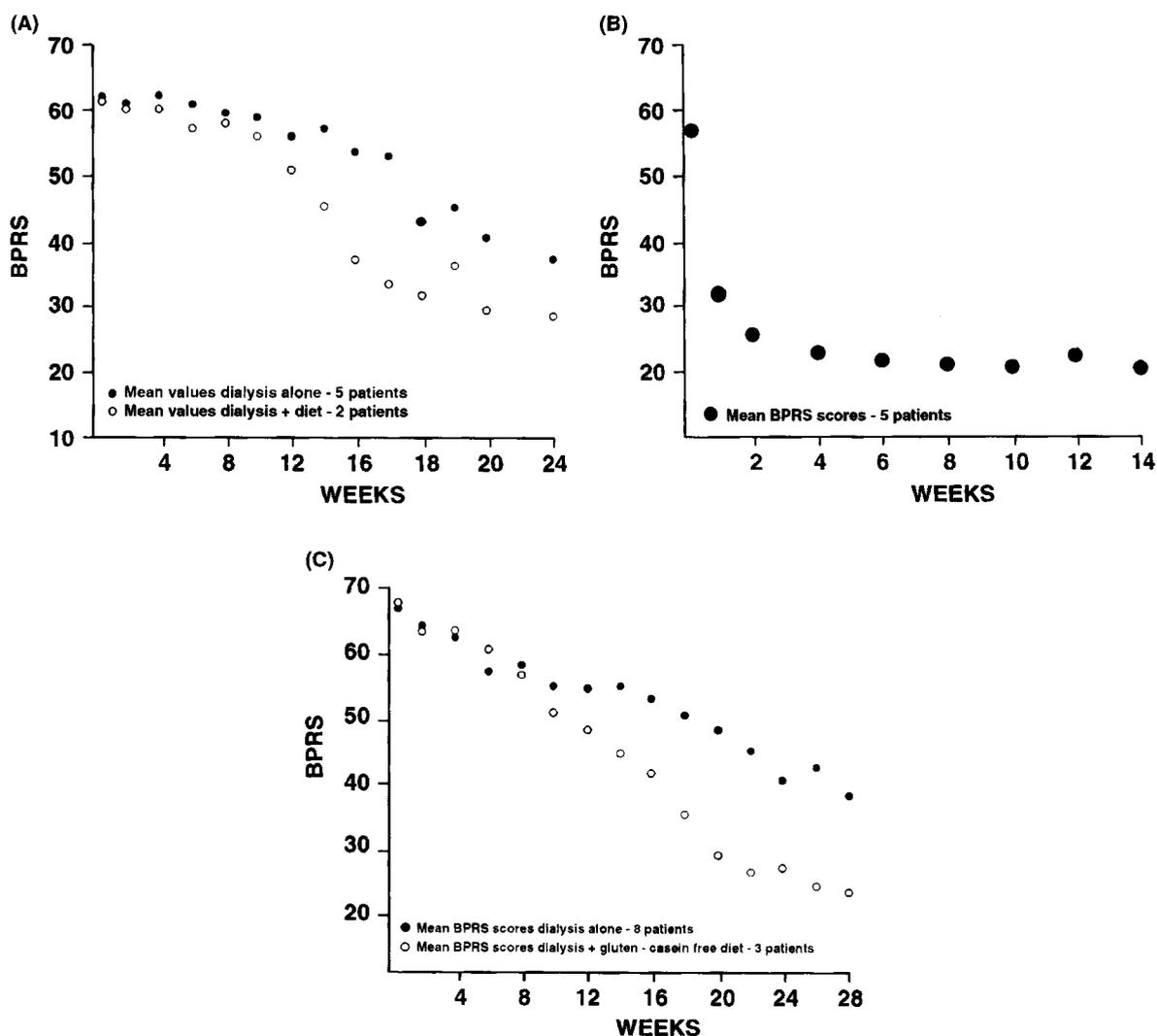


FIGURE 3 (A) Mean BPRS scores by the three observers previously mentioned in Subjects and Treatments of schizophrenic patients with Pattern I peptiduria during hemodialysis alone ● (5 patients) and during hemodialysis plus a diet devoid of gluten and casein ○ (2 patients), (B) mean BPRS scores during dialysis of 5 patients who had a Pattern II peptiduria, (C) mean BPRS scores of patients with a Pattern III peptiduria during hemodialysis alone ● (8 patients) and during hemodialysis plus a gluten-casein free diet ○ (3 patients). All patients shown in (A)–(C) improved significantly or became completely normal.

improved significantly or disappeared completely while on dialysis and an additional two patients who were treated with dialysis and diet simultaneously. While improvement was apparent from the beginning, it is also apparent the improvement occurred more quickly and completely in the two patients on combined therapy.

Figure 3B shows BPRS scores from 5 patients with Pattern II peptiduria. It is apparent that rapidity of improvement was much greater in patients with Pattern II and recovery more complete (Jorgensen and Cappelen, 1992; Reichelt, 1996) than among either Pattern I or in Pattern III patients whose data is shown in Figure 3C. From a molecular standpoint the particles in peak II after

Sephadex separation are much smaller than those in peak I and are therefore more easily removed with dialysis. In Pattern III also, recovery was more rapid and complete when diet and dialysis were used together. Thus, of 57 patients who received dialysis or dialysis plus diet, 23 (40%) patients, those shown in Figure 3A–C, all demonstrated major improvement or became completely normal. Eighteen patients dropped out during the first 3–6 months and 10 additional patients dropped out after 6 months even though they had experienced significant improvement.

In Figure 4 we have plotted BPRS values for 7 schizophrenic patients treated with a gluten–casein free diet alone. Each of these patients had a Pattern III peptiduria. Patients 1 and 6 showed significant improvement after only 2 months on the diet. After 4 months on diet, all seven patients had improved and there was a statistically significant improvement $p < 0.04$ for the group as a whole comparing control with experimental values. Among five patients who continued

the diet with only occasional minor digressions, improvement was continuous and four of them reached normal BPRS scores, obtained jobs and, according to their families and peers, behave in normal fashion. Two patients (patients 1 and 6) improved for several months but, with repeated dietary digressions, relapsed. Patient seven had multiple dietary digressions between the sixth and tenth months and during that time suffered a recrudescence of abnormal behavior and ideation. Evaluation of the entire group by Kruskal–Wallis Anova showed a $p < 0.17$. When only the 5 who stayed on the diet were considered, the p for Anova was < 0.001 . Comparing pre-treatment control values with experimental values, showed no significant difference at 2 months; after 4 months the p was < 0.07 , at 6 months p was < 0.03 and remained less than 0.05 through 16 months when only the 5 compliant patients were considered. The p value was greater than 0.05 at the 16th month, however, when all seven subjects were considered. Sephadex filtration and HPLC

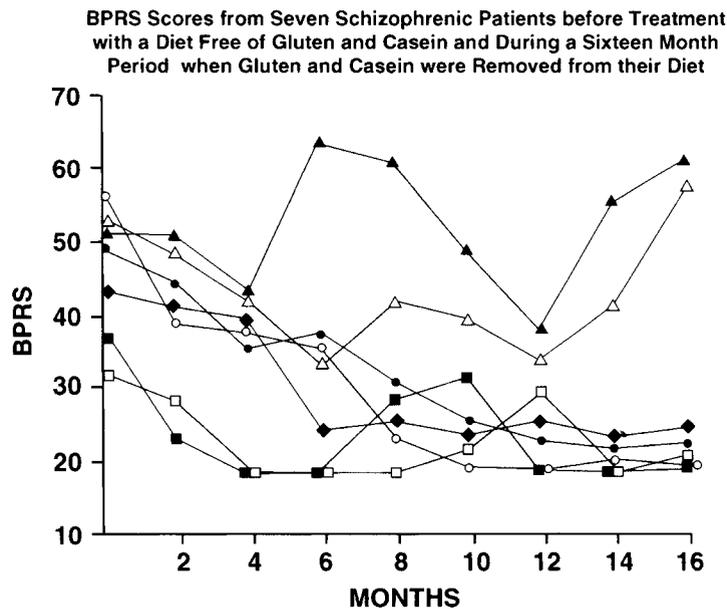


FIGURE 4 BPRS scores of 7 schizophrenic patients with Pattern III peptiduria done by the same 3 observers mentioned in Subjects and Treatments. Independent scores were summed and then averaged. Both observer and patient were blind when Creon^R was given but we were unable to blind the gluten–casein free diet or dialysis from the patients who inevitably told the observers what they were on; patient 1 ▲; 6 △; 7 ■.

TABLE IV Scores before diet treatment and while ingesting a gluten-casein free diet for nine characteristic findings which frequently occur in autistic children; 4+ very severe disturbance, 3+ severe, 2+ moderate, 1+ minimal, 0 normal

70 patients	Social isolation	Eye contact	Mutism	Learning skills	Hyperactivity	Stereotypical activity	Hygiene	Panic attacks	Self mutilation
Pre-diet	4.0	4.0	4.0	3.0	3.0	4.0	2.0	2.0	0.8
1 month	3.0	3.0	2.0	3.0	2.0	2.0	2.0	0.00	0.5
*p	< 0.001	< 0.001	< 0.002	< 0.001	< 0.006	< 0.006	< 0.04	< 0.06	< 0.17
3 months	2.0	2.0	1.0	2.0	2.0	2.0	2.0	0.00	0.3
*p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.05	< 0.02
6 months	2.0	2.0	1.0	1.0	1.0	1.0	1.0	0.00	0.3
*p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.02
12 months	1.0	1.0	1.0	1.0	1.0	1.0	2.0	0.00	0.4
*p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
+p KWAV	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.007	< 0.001	< 0.05

n – number of patients; *p difference between pre-treatment values and experimental observations, +p for Kruskal-Wallis Anova for the characteristic under consideration.

of the two non-compliant subjects showed continued high excretion of multiple caseinate exorphins. In addition, family members informed us that exacerbation of their symptoms occurred when they stopped the gluten-casein free diet. While we cannot be sure, these patients and their families all denied the use of analeptics during the study.

We have followed 70 autistic children ingesting a gluten-casein free diet for 1-8 years. Of these, 81% improved significantly by the third month. Among 13 children (19%) who did not improve, five, from whom we obtained repeat urines, continue to have high urinary peptides as estimated by Sephadex separation and by HPLC. They continued to excrete excessive bovine β -casomorphins and gliadorphins which strongly suggests they were not following the prescribed diet.

Table IV shows the behavioral abnormalities that were evaluated in the autistic patients who were treated with a gluten-casein free diet. The rank values for each characteristic before diet therapy was begun are shown at the top of the table. Assessments made at 1, 3, 6 and 12 months following institution of diet therapy are shown below. The p value shown at each re-evaluation refers to a comparison between the pre-treatment values and the experimental value obtained at that re-evaluation period. The p value shown at

the bottom of each column of data refers to the analysis of variance for the characteristic shown. It is apparent that significant improvement occurred in most characteristics after one month of diet therapy and in all characteristics after 3 months of treatment and that overall improvement continued for the first year. While every effort was made to control the diets of our patients this was not always possible in children who lived at home in the care of their parents.

DISCUSSION

Dohan (1966), first called attention to the relationship between a diet high in cereal content and the occurrence and severity of schizophrenia. Dohan (1983), Asperger (1961), and Marchi *et al.* (1974), also pointed out the high incidence of emotional disorders, including schizophrenic and autistic-like behavior in children with celiac disease, an illness known to be due to a sensitivity to wheat and other gluten containing grains.

Our studies, as has the work of Shattock *et al.* (1990), Shattock and Lowdon (1991), Reichelt *et al.* (1986, 1990) demonstrate that hyperpeptiduria occurs in most autistic patients (Reichelt *et al.*, 1981; Hole *et al.*, 1979), found a peptide from patients with schizophrenia which induced

bizarre behavior in rats (Table I, Figure 2). We also found, as did Shattock *et al.* (1990), Shattock and Lowdon (1991) and Reichelt *et al.* (1986); bovine β -casomorphin fragments. Intact casein and gliadin (Gardner, 1994; Lucarellie *et al.*, 1993) who showed that the intestinal wall was excessively permeable to lactulose/dextran in the urine (Figure 2(a)–(f)) of both schizophrenic and autistic patients. While 72% of normal adults and 54% of childhood controls, from our data, probably have bovine β -casomorphins in their urine, the concentrations are so low they are difficult to identify and quantitate or to collect a pooled sample adequate for mass spectrometry to aid in identification. Compare Figure 2(a) and (b). The finding of low titer, β -casomorphins in the urine of normal controls would be expected from the report of Husby *et al.* (1985), who showed that trace amounts of bovine casomorphin could be found in the blood of normal nursing infant subjects and Stuart *et al.* (1984), who found bovine casomorphin in the blood of nursing mothers and of their infants and by Gardner (1994) who demonstrated that intact casein and gliadin are absorbed from the intestine.

The presence of large amounts of β -casomorphins and gliadorphins found in the urine of autistic children and adult patients with schizophrenia could be explained in several ways: 1) an increased permeability of the intestinal mucosa has been demonstrated by Gardner (1994) and D'Eufemia *et al.* (1996) who showed that 47% of children with autism had an increased lactulose/mannitol ratio which resulted from increased permeability of the tight junctions. A second mechanism could be inadequate or deficient enzymes for either intestinal or blood breakdown of gliadorphins or casomorphins which allows the exorphins in large amounts to gain access to the blood and circulate until taken up by the brain. A third mechanism, in 30% of children with autism and 96% of patients with schizophrenia, could be that the fixation of exorphins in the intestinal wall by IGA antibodies damages intracellular metabolic processes which causes increased

permeability of the intestinal wall. It has also been reported by Pratesi *et al.* (1998) that the IgA antibodies from patients with celiac disease react strongly with brain blood vessels. Another possible explanation for elevation of both endorphins and exorphins has been reported by Rui and Goldstein (1982), and Hui *et al.* (1983) who found that opioid and other peptides inhibit the metabolism of peptides. Gastro-intestinal disorders have been reported in autistic and schizophrenic patients by Coleman (1976) and by Goodwin *et al.* (1971). Also children with coeliac disease which is caused by a sensitivity to gluten have a much higher incidence of schizophrenia than occurs in the general population (Dohan, 1983). The demonstration of elevated IgA and IgG antibodies to gliadorphins and casomorphins can surely be viewed as a protective response to defend the body against attack by a noxious protein. For present purposes, the finding of IgG antibodies to gluten and casein is another indication that the foreign peptides reach the blood intact in large enough amounts to stimulate a vigorous IgG response and are therefore available for uptake by the brain. As mentioned above, the finding of IgA antibodies suggests a possible mechanism for the increased intestinal permeability and possibly for some of the symptoms in both autism and schizophrenia.

Dialysis Treatment of Patients with Schizophrenia

Approximately 40% of schizophrenic patients treated with dialysis alone for one year improved significantly or became well while weekly dialysis was given. All five patients treated with dialysis plus a gluten–casein free diet (see Table III) improved significantly or became well. Patterns I and III patients treated with dialysis and diet improved more quickly and completely than those treated with dialysis or diet as a single modality. Five of seven schizophrenic patients we treated with diet alone improved significantly or

became completely well. The rate of improvement in these patients, however, was much slower than the rate of improvement with combined therapy. This agrees well with Dohan's report (Dohan and Graberger, 1973) that hospitalized schizophrenic patients treated with a gluten-casein free diet, in addition to their usual medication, improved more rapidly and could be discharged earlier than when treated pharmacologically without diet. It is also of interest to note that the two schizophrenic patients (Figure 4) who did not become well on dietary therapy alone improved nicely when the diet was adhered to but relapsed when they resumed ingestion of gluten and casein containing foods.

Carpenter *et al.* (1983) reported there was no statistically significant improvement among 15 schizophrenic patients treated in a double blind crossover study in which they received real dialysis or sham dialysis during two 8 week periods. Examination of our present data and that of Wagemaker and Cade (1977) gives an explanation for his disparate results as do the reports of Reichelt (1996), and Jorgenson and Cappelen (1992) who report results very similar to ours. In Figure 3A-C we showed the BPRS scores for patients with Pattern I, II and III peptiduria while treated with dialysis. When Pattern II peptiduria was present, improvement was very rapid and complete; Pattern II peptiduria was found in 25% of our pre-treatment patients with schizophrenia. When Pattern I peptiduria was present (30% of patients) only questionable improvement had occurred after 14 weeks of dialysis. Patients with Pattern III peptiduria (Figure 3C) obviously improved from the first dialysis; statistically, significant improvement, however, was not demonstrable until the 10th week. These distinctions are important because, as they appear in Sephadex filtration the particles found in peak II are much smaller than those in peak I and can thus be removed by dialysis far more effectively. If only 1/4 of the patients in Carpenter's group had Pattern II peptiduria, the improvement they experienced would be obscured by the Patterns I

and III patients in the study who, from our data, would not be expected to improve until much later. Also, with the small sample of patients, it is possible that no Pattern II patients were included in his study group. Thus, Carpenter's study has two serious defects; the short period of dialysis and the small number of patients which almost assures lack of a representative distribution of the three peptide patterns.

In our studies of the effect of dialysis in Patterns I and III patients with schizophrenia we were unable to reduce the degree of hyperpeptidemia-peptiduria sufficiently or for long enough periods of time with dialysis as the sole modality (Cade *et al.*, 1990) in many patients with dialysis alone, while with dialysis plus a gluten-casein free diet the peptidemia and peptiduria decreased progressively and the patients improved as their peptiduria diminished (Cade *et al.*, 1990). We think that the frequent failure with dialysis alone is due to production and absorption of such large amounts of β -casomorphin and α -gliadorphin that dialysis was unable to reduce the concentration of exorphins sufficiently or continuously enough to have a clear beneficial effect.

While it was not a finding included in our study, five mothers volunteered that their autistic children had frequent grandmal seizures (Volkmar and Nelson, 1995; Olsson *et al.*, 1988) before beginning a gluten-casein free diet. Seizure frequency has decreased significantly in three and ceased in two. This, we think, suggests all children with unexplained epilepsy should be examined for exorphins (Pratesi *et al.*, 1998).

In a separate parallel study (Sun and Cade, 1999a) we injected β -casomorphin-7 IV, and a peak isolated from the urine and blood from autistic and schizophrenic subjects which we have tentatively identified on the basis of HPLC mobilities as bovine β -casomorphin-7, and shown that it is taken up, by a morphine receptor system, into the medial and lateral geniculate nuclei, the occipital and temporal cortices and multiple areas of the brain including: The nucleus accumbens, the hypothalamus, the entorhinal cortex, a

dopaminergic area, and the parietal cortex, several areas in the frontal and prefrontal lobes and one just anterior to the sylvian fissure which is the site of Broca's speech area. In addition, the caudate nucleus and the ventral tegmental area, another dopaminergic pathway, show very avid uptake of β -casomorphin-7. These findings show that β -casomorphin cannot only penetrate the blood brain barrier, but is quickly taken up by opioid receptors (Sun and Cade, 1999a) in areas of the brain where abnormal function could produce all of the symptoms which occur in patients with autism or schizophrenia. Specifically, hallucinations could occur because involvement of the geniculate nuclei could send false auditory and visual information to the temporal and occipital cortices or the involved cortices themselves could misinterpret the message. Known functions of the brain areas involved (Sun and Cade, 1999a) suggests that all the behavioral and associational abnormalities in autistic and schizophrenic patients could be explained by this involvement.

Our studies also show that reduction of the peptides in blood and urine by diet or by dialysis (Cade *et al.*, 1990) is accompanied by improvement in the BPRS and in the clinical state of the patient while lapses in the dietary regimen or the frequency of dialysis led to a relapse in their clinical state and BPRS. In another rat study (Sun and Cade, 1999b), we gave β -casomorphin to normal rats; fifty-seven seconds later the rats began running frantically, gnashing their teeth and foaming at the mouth. They then became hostile and defensive, attacking their normal cage mate if it came near. Pain sensitivity was greatly decreased, a finding occurring frequently in many patients with autism or schizophrenia. They also paid no attention when a bell was rung over their cage while normal rats invariably looked up for the source of the sound. This is of interest because most mothers of children with autism frequently think their child is deaf. Our behavior studies in rats given β -casomorphin have many parallels with the findings of Panksepp (1978) and Panksepp *et al.* (1980). Thus, all six questions

raised at the beginning of this study have affirmative answers. In addition, we think our data shows so much similarity between autism and schizophrenia that it is reasonable to wonder if they are due to the same basic defect. Treatment with a diet free of gluten and casein or dialysis plus diet therapy both decrease the degree of peptidemia in schizophrenia and in autism and this decrease is linearly related to improvement (Cade *et al.*, 1990) in BPRS scores and clinical behavior in schizophrenia (Cade *et al.*, 1990) and with clinical manifestations in autistic children (Cade *et al.*, 1990).

While exclusion of gluten and casein from the diet is an arduous task for parents, we believe that our results warrant the effort.

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References

- American Psychiatric Association (1987) *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edn., Washington, D.C.
- Asperger, H. (1961) Die Psychopathologie des coeliaki kranken Kindes. *Annual Paediatric* **197**: 346–351.
- Bohlen, P., Castillo, F., Ling, R. and Guillemin, R. (1980) Purification of peptides: An efficient procedure for the separation of peptides from amino acids and salts. *International Journal of Peptide Protein Research* **16**: 306–310.
- Cade, R., Wagemaker, H., Privette, R.M., Fregly, M., Rogers, J. and Orlando, J. (1990) The effect of dialysis and diet on schizophrenia. *Psychiatry: A World Perspective* **1**: 494–500.
- Carpenter Jr., W.T., Sadler, J.H., Light, P.D., Hanlon, T.E., Kurland, A.A., Penna, M.W., Reed, W.P., Wilkinson, E.H.

- and Bartko, J.J. (1983) The therapeutic efficacy of hemodialysis in schizophrenia. *New England Journal of Medicine* **308**: 669–675.
- Coleman, M. (1976) Calcium studies and their relationship to coeliac disease in autistic patients. In: *The Autistic Syndromes*, Coleman, M. (Ed.), pp. 197–205. North Holland Publishing Corp., Amsterdam, The Netherlands.
- D'Eufemia, R., Celli, M., Finocchiaro, R., Pacifico, L., Viozzi, L., Zaccagnini, M., Cardi, E. and Giardini, O. (1996) Abnormal intestinal permeability in children with autism. *Acta Paediatrica* **85**: 1076–1079.
- Dohan, F.C. (1966) Cereals and Schizophrenia. Data and hypothesis. *Acta Psychiatrica Scandinavica* **42**: 125–152.
- Dohan, F.C. and Graberger, J.C. (1973) Relapsed schizophrenics: Earlier discharge from the hospital after cereal-free, milk-free diet. *American Journal of Psychiatry* **130**: 685–686.
- Dohan, F.C. (1983) More on coeliac disease as a model for schizophrenia. *Biological Psychiatry* **18**: 561–564.
- Gardner, M.L.G. (1994) Absorption of intact proteins and peptides. In: *Physiology of the Gastrointestinal Tract*, 3rd edn., L.R. Johnson (Ed.) pp. 1795–1820. Raven Press, New York, N.Y.
- Goodwin, M.S., Cowen, M.A. and Goodwin, T.C. (1971) Malabsorption and cerebral dysfunction: A multivariate and comparative study of autistic children. *Journal of Autism and Childhood Schizophrenia* **1**: 48–62.
- Hagman, B. (1990) *The Gluten-Free Gourmet. Living Well without Wheat*. Henry Holt & Co., New York, New York.
- Hedlund, J.L. and Vieweg, B.W. (1980) The Brief Psychiatric Rating Scale (BPRS): A comprehensive review. *Journal of Operational Psychiatry* **11**: 48–65.
- Hole, K., Bergslien, H. and Jorgensen, H.A. (1979) A Peptide containing fraction in the urine of schizophrenic patients which stimulates opiate receptors and inhibits dopamine uptake. *Neuroscience* **4**: 1883–1893.
- Hole, K., Lingjaerde, O., Morkrid, L., Boler, J.B., Saelid, G., Diderichsen, J., Ruud, E. and Reichelt, K.L. (1988) Attention deficit disorders: A study of peptide-containing urinary complexes. *Journal of Developmental Behavioral Pediatrics* **9**: 205–212.
- Hui, K.-S., Hui, M., Banay-Schwartzl, M., DeGusman, T., Ling, N. and Lajhta, A. (1983) Enkephalin-containing polypeptides are potent inhibitors of enkephalin degradation. *Peptides* **4**: 639–646.
- Husby, S., Jensenius, J.D. and Svehag, S.E. (1985) Passage of undegraded dietary antigen into the blood of healthy adults. Quantification, estimation of size distribution, and relation of uptake to levels of specific antibodies. *Scandinavian Journal of Immunology* **22**: 83–92.
- Jorgensen, H.D. and Cappelen, C. (1992) Naloxone-induced reduction of schizophrenic symptoms: A case report. *Acta Psychiatrica Scandinavica* **63**: 102.
- Kidder, B. (1988) *The Milk-Free Kitchen, Living Well without Dairy Products*. Henry Holt & Co., New York, New York.
- Knivsberg, A.M., Wiig, K., Lind, G., Nodland, M. and Reichelt, K.L. (1990) Dietary intervention in autistic syndromes. *Brain Dysfunction* **3**: 315–327.
- Knivsberg, A.M., Reichelt, K.L., Nodland, M. and Høien, T. (1995) Autistic syndromes and diet. A four year follow-up study of 15 subjects. *Scandinavian Journal of Educational Research* **39**: 223–236.
- Lucarellie, S., Fredimma, T., Zingam, A., Ferizzi, Fl., Giardini, O., Quinterica, F., Barbato, M., D'Eufimia, P. and Corti, R. (1993) Food allergies and infantile autism. *Pan Minerva Medicine* **37**: 34–141.
- Marchi, A.C., Tuvo, F. and Mardio, S. (1974) Child autism and gluten intolerance. *Acta Paediatrica Scandinavica* **63**: 102.
- Oleson, I., Steffenberg, S. and Gillberg, C. (1988) Epilepsy in Autism and Autistic-like conditions. *Archives of Neurology* **48**: 666–668.
- Panksepp, J., Herma, B., Vilbeg, T., Bishop, P. and DeEsquinase, F.G. (1980) Endogenous opioids and social behavior. *Neuroscience Behavioral Review* **4**: 473–487.
- Panksepp, J. (1978) A neurochemical theory of autism. *Trends in Neurosciences* **2**: 174–177.
- Pratesi, R., Gandolfi, L., Friedman, H., Farage, L., de Castro, C.A. and Catassi, C. (1998) Serum IgA antibodies from patients with coeliac disease react strongly with human brain blood-vessel structures. *Scandinavian Journal of Gastroenterology* **33**(8): 817–821.
- Reichelt, K.L., Hole, K., Hamberger, A., Saelid, G., Edminson, P.D., Braestrup, C.B., Lingjaerde, O., Ledaal, P. and Orbeck, H. (1981) Biologically active peptide-containing fractions in schizophrenia and childhood autism. *Advances in Biochemical Psychopharmacology* **28**: 627–643.
- Reichelt, K.L., Saelid, G., Lindback, J. and Orbeck, H. (1986) Childhood autism: A complex disorder. *Biological Psychiatry* **21**: 1279–1290.
- Reichelt, K.L., Ekram, J. and Scott, H. (1990) Gluten, milk protein and autism: Dietary intervention effects on behavior and peptide secretion. *Journal of Applied Nutrition* **42**: 1–11.
- Reichelt, K.L. and Landmark, J. (1995) Specific IgA antibody increases in schizophrenia. *Journal of Biological Psychiatry* **37**: 410–413.
- Reichelt, K.L. (1996) Dialysis in schizophrenia: Successful only in a subtype? *Schizophrenia Research* **19**(2–3): 223–224.
- Reichelt, K.L., Seim, A.R. and Reichelt, W.H. (1996) Could schizophrenia be reasonably explained by Dohan's hypothesis on genetic interaction with a dietary peptide overload? *Progress Neuropharmacology and Biological Psychiatry* **10**: 1–32.
- Reichelt, W., Ek, J., Samsrud, M. and Reichelt, K.L. (1998) Peptide excretion in celiac disease. *Journal of Pediatric Gastroenterology and Nutrition* **26**: 305–309.
- Rodriguez, Trav, A.L., Barreiro Marin, P., Galvez, Borrero, I.M., del Olmo Romero-Nieva, F. and Diaz Alvarez, A. (1994) Association between autism and schizophrenia. *Journal of Nervous and Mental Disease*, August; **182**(8): 478–479.
- Rui, I. and Goldstein, A. (1982) Opioid and other peptides as inhibitors of leuorphan (Dyngorphin 13–29) converting activity. *Peptides* **7**: 87–90.
- Scott, H., Rognum, T.D., Midtvedt, T.J. and Brandtzaeg, P. (1985) Age related changes of serum antibodies to dietary and colonic antigens measured by enzyme-linked immunosorbent assay. *Acta Pathologica Microbiologica Scandinavica* (c) **93**: 65–70.
- Shattock, P., Kennedy, A., Rosell, F. and Berney, T. (1990) Role of neuropeptides in autism and their relationships with classical neurotransmitters. *Brain Dysfunction* **3**: 328–345.
- Shattock, P. and Lowdon, G. (1991) Proteins and peptidases. Part 2. Implications for the education and care of people with autism. *Brain Dysfunction* **4**: 323–334.
- Shutte, J.G. (1977) *Everything You Always Wanted to Know About Elementary Statistics but were Afraid to Ask*, Prentice-Hall, Inc. Englewood Cliffs, New Jersey.
- Stuart, C.A., Twiselton, R., Nicholas, M. and Hide, D.W. (1984) Passage of cow's milk protein in breast milk. *Clinical Allergy* **14**: 533–535.
- Sun, Z., Cade, J.R., Fregly, M.J. and Privette, R.M. (1999a) β -Casomorphin induces fos-like immunoreactivity in

- discrete brain regions relevant to schizophrenia and autism. *Autism* 3(1): 67–83.
- Sun, Z. and Cade, R. (1999b) A peptide found in schizophrenia and autism causes behavioral changes in rats. *Autism* 3(1): 85–95.
- Trygstad, O.E., Reichelt, K.L. and Foss, I. (1980) Patterns of peptides and protein associated peptide complexes in psychiatric disorders. *British Journal of Psychiatry* 136: 59–72.
- Volkmar, F.R. and Nelson, D.S. (1995) Seizure disorders in autism. *Journal of American Academy of Childhood and Adolescent Psychiatry* 29: 127–129.
- Wagemaker, H. and Cade, J.R. (1977) The use of hemodialysis in schizophrenia. *American Journal of Psychiatry* 134: 684–685.
- Whitely, P., Rodgers, J., Savory, D. and Shattock, P. (1997) Preliminary findings from the implementation of a gluten free diet in children with autism and associated spectrum disorders. In: *Living and Learning with Autism* Paul Shattock and Gillian Linfoot (Eds.), pp. 182–197. The Autism Research Unit, Sunderland Univ., UK.