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STUDIES ON TRYPTOPHAN CATABOLISM IN ACRODERMATITIS ENTEROPATHICA

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Abstract. In 5 cases of acrodermatitis enteropathica, intestinal biopsies and biochemical tests of intestinal absorption were performed, and tryptophan catabolism in the kynurenine and xanthurenic acid pathway was studied. Intestinal biopsy demonstrated subtotal villous atrophy (in untreated children) and extensive lymphocytic inflammatory infiltrates. Impaired absorption of xylose and monosaccharides returned to normal during treatment. Absorption of amino acids, including tryptophan, was normal. Oral loading with *l*-tryptophan and some of the biochemical analyses were done during treatment with oxyquinoline drugs and during intervals in the administration of the drug. Urinary levels of kynurenine increased markedly after oral loading with *l*-tryptophan, especially during treatment. Excretion of xanthurenic acid was normal. The hypothesis of a toxic complex of polypeptides and kynurenine, and that of primary disorders in the tryptophan catabolism in acrodermatitis enteropathica is proposed. The mode of action of the oxyquinoline drugs is discussed.

Acrodermatitis enteropathica (AE) is a rare disease, described for the first time in 1942 by Danbolt & Closs (6). The disease usually starts in the first year of life with skin lesions and disorders of the alimentary tract. The erythematous, desquamating and bullous lesions are characteristically distributed around the natural orifices of the body. In the course of the disease, loss of hair (including the eyebrows and eyelashes) leads to complete alopecia. Persistent diarrhea is the cause of malnutrition, resulting in extreme debilitation and even death in untreated cases.

The pathogenesis of the disease is not known. The following theories have been proposed: transition from breast to artificial feeding, since the first clinical symptoms usually appear at this time (3, 7, 20); disorders in the intestinal absorption of tryptophan (6); formation of toxic peptides from undigested proteins as a result of deficiency of leucine-aminopeptidase (14); disorders of tryptophan metabolism in the kynurenine and xanthurenic acid pathway (1, 11, 15); and disorders of lipid absorption and metabolism (2, 4, 16, 18, 27).

However, none of these hypotheses explains satisfactorily all the aspects of the disease.

It was the purpose of the clinical observations and biochemical and histologic analysis to elucidate the pathogenesis of AE and the relation between the cutaneous and intestinal lesions, and to evaluate intestinal absorption in the disease.

Our study was concerned with: the catabolism of tryptophan, especially via kynurenine and indoles pathways; histopathologic changes in the intestinal mucosa; and intestinal absorption of carbohydrates and tryptophan.

Observations were made in the fully developed stage of the disease as a result of withdrawal of drug, and after prolonged administration of oxyquinoline derivatives in a state of remission of the skin lesions and intestinal disorders.

MATERIAL

The study material consisted of 5 patients with AE, all of whom were observed in the early stage of the disease before the institution of treatment. The characteristic skin lesions were typically localized. From the onset of the disease, all the children had intestinal disorders with persistent diarrhea leading to marasmus, and disorders of physical and psychomotor development. Treatment consisted mainly in the administration of iodochlorooxyquinoline, which was well tolerated and in all cases brought about remission of the skin lesions and gastrointestinal symptoms. Some of the clinical features of the cases and method of treatment are shown in Table I.

The period of observation of the children ranged from 3½ to 14 years. The diagnosis of AE was established between the ages of 2–14 months.

METHODS

A. *Intestinal biopsy*

The histologic changes in the intestinal mucosa were studied by intestinal biopsy in 4 children by the use of a Crosby-type aspiration tube. The biopsies were performed prior to treatment.

Table I. *Clinical features of 5 cases of acrodermatitis enteropathica*

Case	Age at onset of illness (months)	Breast feeding (months)	Diarrhea	State of nutrition	Therapy: Drug	Therapy: Dosage (g/24 hr)	Therapy: Period after which therapeutic effect was obtained[a]
1. B. S.	6	Unknown	+ + + +	General emaciation	Entero-Vioform Diiodoquin	1.50	2 years
2. E. S.	9	8	+ + +	Extreme emaciation	Entero-Vioform	0.75	5 months
3. A. G.	2	2	+ +	Slight emaciation	Entero-Vioform	0.75	1 month
4. L. S.	13	3	+ +	Moderate emaciation	Entero-Vioform Diiodoquin	0.37	3 weeks
5. G. G.	3	2	+ +	Moderate	Entero-Vioform	1.50	1 month

[a] Regression of the skin involvement and intestinal disorders.

B. *Intestinal absorption*

Intestinal absorption was studied by the following methods:

(*a*) D-Xylose. After loading with 5 g D-xylose, its urinary excretion was determined by the method of Roe & Rice (19) in urine collected during 5 hours, before and during treatment.

(*b*) Glucose + galactose and fructose. A mixture of glucose and galactose was given in a dose 1.5 g/kg body weight and fructose in a dose 1.5 g/kg body weight, and blood glucose curves were determined (in 2 cases).

(*c*) Disaccharides. After loading with lactose, maltose or sucrose in doses of 1.5 g/kg body weight, blood glucose curves were determined (in 3 cases).

(*d*) Lactic acid. Lactic acid in stools was assayed by the method of Dische–Laszlo as modified by Tomaszewski (24), before and during treatment.

C. *Tryptophan metabolism*

Loading with *l*-tryptophan, 0.1 g/kg body weight, was done during a pause in drug administration and also during prolonged treatment. Tryptophan was given orally in fruit juice, and blood samples were obtained every 30 min for 3 hr, after which the children received normal meals. After loading, urine collected in two 6-hr and one 12-hr portions was refrigerated at −20°C prior to analysis.

Tryptophan (TRY) was assayed in the serum by the method described by Opieńska-Blauth (17); total indoles (T.I.) and indole-acetic acid (IAA) according to the method

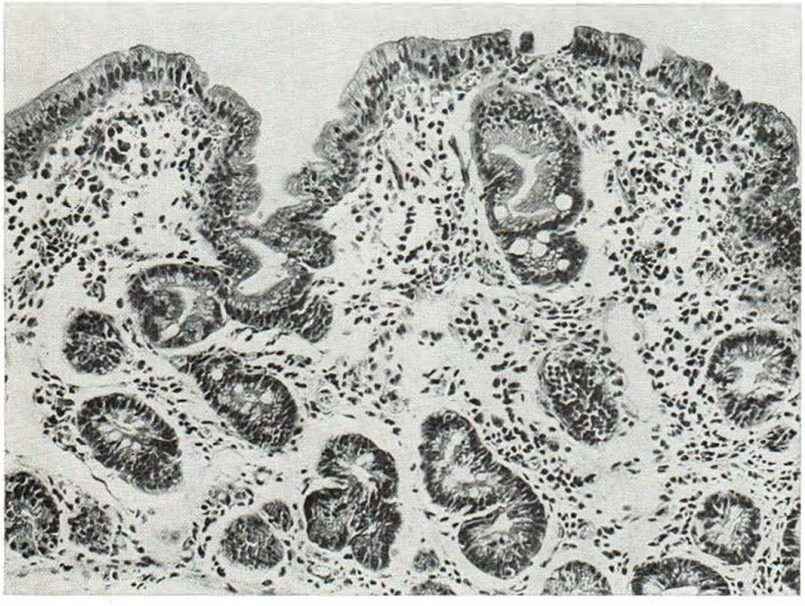

Fig. 1. Case 2. Jejunal biopsy. Subtotal villous atrophy and inflammatory infiltrates. H + E staining, ×400.

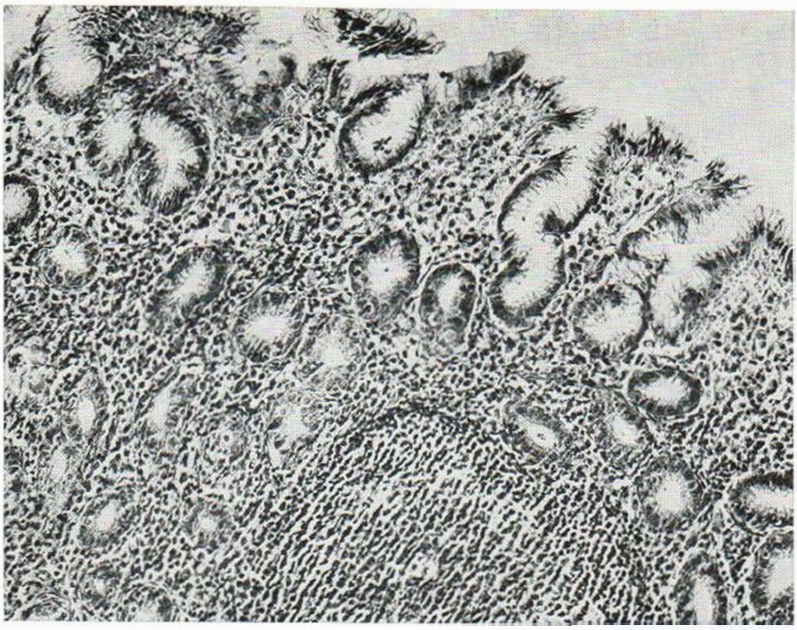

Fig. 2. Case 4. Jejunal biopsy. Leaf-like villi and heavy inflammatory infiltrates. H+E staining, × 300.

of Fischl & Rabiah (8), indican (IS) according to Meiklejohn (13), kynurenine (K) according to Thompsett (22), and xanthurenic acid (XA) according to Weller (26).

RESULTS

The changes in the mucous membrane of the small intestine before treatment are illustrated in Figs. 1 and 2 and Table II. Table II shows the results of the tests of intestinal absorption. Urinary levels of tryptophan metabolites before and after oral loading with *l*-tryptophan, and before and during treatment, are presented in Table III.

A. *Intestinal biopsy*

In all the cases examined during the period of emaciation and diarrhea, complete or partial atrophy

Table II. *Results of tests of intestinal absorption and intestinal biopsy in 5 cases of acrodermatitis enteropathica*

Case and age	Jejunal biopsy	Hb %	Test with D-xylose (normal 25%)		Fecal lactic acid (mg/ml) (normal traces)		Loading tests with		Serum total protein (g%)	Serum α-amino nitrogen mg% (normal 4.0–7.0)	
			A	B	A	B	Glucose galactose	Disaccharides		A	B
1. B. S. 2 y.	Subtotal villous atrophy	73	3	35	1.9	0.2	—	Flat curve	6.5	4.2	4.3
2. E. S. 1½ y.	Subtotal villous atrophy	78	13	40	4.5	1.5	Flat curve	Flat curve	6.9	5.8	4.9
3. A. G. 4 mo.	—	78	—	—	—	—	—	—	6.0	5.5	5.7
4. L. S. 14 y.	Subtotal villous atrophy	69	13	44	3.0	0.2	—	—	7.8	8.0	6.5
5 G. G. 9 mo.	Slight flattening of villi	65	13	54	0.2	Traces	Normal	Mimimal disturbances	—	—	—

— = not done, A = without treatment, B = during treatment

Table III. *Tryptophan metabolites in urine in 4 children with AE, treated and untreated, before and after loading with l-tryptophan (0.1 g/kg body weight)*

Case	T.I. (micromoles/kg/24 hr)	IAA	IS	XA	K	Maximal serum level of tryptophan after loading (mg%)
1. *B. S.*, ♀, *4 years old*						
Without treatment (30 days)						
Basal values	59.7	4.8	13.5	1.7	0.4	
After loading	157.4	8.1	10.9	0.6	19.0	24.1
Increase	97.7	3.3	0	0	18.6	
During treatment						
Basal values	93.2	3.7	7.8	2.7	4.2	
After loading	116.5	4.7	8.0	4.0	90.4	16.8
Increase	23.3	1.0	0.2	1.3	86.2	
2. *E. S.*, ♀, *3½ years old*						
Without treatment (25 days)						
Basal values	114.0	4.2	12.3	1.6	1.1	
After loading	221.0	9.8	12.1	0.8	8.4	16.7
Increase	107.0	5.6	0	0	7.3	
During treatment						
Basal values	57.0	2.7	8.7	2.5	3.9	
After loading	77.1	4.5	12.1	1.3	21.7	15.4
Increase	20.1	1.8	3.4	0	17.8	
3. *A. G.*, ♂, *3½ years old*						
Without treatment (28 days)						
Basal values	133.0	3.6	9.7	0.2	0.3	
After loading	167.7	5.2	6.6	0.6	4.4	11.6
Increase	34.7	1.6	0	0.4	4.1	
During treatment						
Basal values	71.2	2.3	7.9	0.6	1.8	
After loading	91.2	3.4	6.9	1.2	13.7	9.1
Increase	20.0	1.1	0	0.6	11.9	
4. *L. S.*, ♀, *14 years old*						
Without treatment (28 days)						
Basal values	76.0	2.7	10.1	0.3	0.1	
After loading	70.7	2.6	7.5	1.0	4.5	19.1
Increase	0	0	0	0.7	4.4	
During treatment						
Basal values	93.2	2.6	4.7	0.9	0.04	
After loading	73.5	2.7	1.0	3.1	11.5	11.5
Increase	0	0.1	0	2.2	11.4	
Normal children (10) (1–10 years old)						
Basal values	45–110	1.5–2.5	5.0–9.0	0.3–0.5	0.3–0.4	
After loading	100–200	3.5–5.0	8.0–10.0	1.2–2.5	3.0–8.0	9.5–16.0[a]
Increase	55–110	2.0–2.5	0–1.0	0.7–3.0	2.7–8.5	

T.I. = total indoles were determined using as a standard indoleacetic acid, IAA = indoleacetic acid, IS = indican, XA = xanthurenic acid, K = kynurenine.

[a] According to Yarbro (28).

of intestinal villi was noted. In contrast to coeliac disease, inflammatory infiltrates composed of lymphocytes and histiocytes were numerous.

B. *Intestinal absorption*

The 5-hr urinary excretion of xylose was markedly decreased in 4 cases prior to treatment, but returned to normal during treatment; absorption of glucose and galactose ("active" absorption) studied in 2 cases, was markedly impaired, whereas fructose was normally absorbed ("passive" absorption); contact digestion of disaccharides was markedly impaired in case 2, but only minimally so in cases 1 and 5. The latter 2 patients had no symptoms of malabsorp-

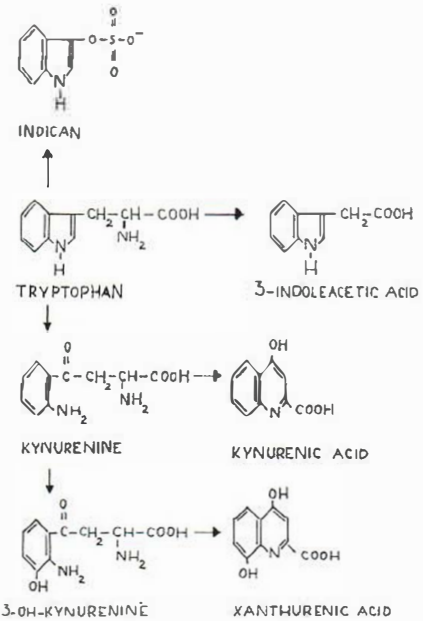

Fig. 3. Diagram (simplified) of tryptophan metabolism.

tion at the time of the examination; lactic acid levels in the stools were distinctly elevated in 3 cases (Nos. 1, 2 and 4), but returned to normal during treatment, while the intestinal symptoms subsided; serum levels of total protein and alpha-amino acid nitrogen were normal. The curves of tryptophan increase in the serum were also normal, and in 3 cases (Nos. 1, 2 and 4) the maximal serum levels after oral administration with tryptophan were even above normal.

C. *Tryptophan metabolism* (Fig. 3)

Total indoles. Urinary T.I. before and after loading with *l*-tryptophan were normal in children prior to as well as during treatment. However, the increase in T.I. after loading was much smaller in the treated children.

Indole-acetic acid. In 2 cases (Nos. 1 and 2), the basal values and increase of IAA in the urine were higher than normal after loading. The increase in IAA returned to normal during treatment.

Indican. The IS levels were normal before and after loading in both treated and untreated children.

Kynurenine and xanthurenic acid. In untreated children, after loading, the XA levels in the urine showed no increase (cases 1 and 2), or only very slight increase (cases 3 and 4). In the treated children, on the other hand, the increase in XA after loading was somewhat more pronounced, but within normal limits. Increase in K after loading in untreated children was normal, except for case 1, in which it was marked. In the treated children the rise in urinary K was much more considerable in all cases, and especially evident in cases 1 and 2.

DISCUSSION

All the children studied suffered from chronic diarrhea. Intestinal biopsy revealed important changes in the architectural structure of the villi, consisting in their subtotal atrophy, smoothing of the mucosa and inflammatory lymphocytic infiltrates, which neither Moynahan (14) nor Fry et al. (9) observed. By diminishing the absorbing surface of the intestine, these changes impair the absorption of sugars (xylose, glucose, galactose) and consequently increase the excretion of lactic acid in the stools and cause fermentative diarrhea.

The finding of abundant inflammatory infiltrations in the intestinal villi argues against atrophy of the villi as a result of marasmus. Most probable is a local toxic effect of the hypothetic complex of incompletely digested peptides with kynurenine. The normal levels of xylose in the urine and lactic acid in the stools in all treated children suggest that the inflammatory and atrophic lesions in the intestinal mucosa in AE are temporary and secondary, developing during the disease process.

The normal levels of alpha-amino acid nitrogen in the serum suggest unimpaired absorption of amino acids. Normal intestinal absorption of *l*-tryptophan is indicated by the normal rise in serum tryptophan after loading, normal level of urinary indican, and normal rise of total indoles. An increase in the urinary excretion of these metabolites is considered a sign of impaired intestinal absorption of tryptophan (8, 10, 21). The somewhat higher serum levels of tryptophan in two children after loading, before treatment, in comparison with healthy children, might be explained (according to Malczewski, 12) by damage of the liver impairing the ability of its cells to take up tryptophan and in consequence causing higher levels of this amino acid in the peripheral blood.

The contradictory results of tests of intestinal absorption reported in the literature (5, 6, 11, 15, 23, 25) are probably due to the variable state of the intestinal mucosa depending on the stage of the disease, clinical status of the patient, and treatment.

The intestinal changes in AE probably develop under the influence of unknown toxic factors acting

on the intestinal epithelium and simultaneously on the skin. Hence, Moynahan's suggestion (14) that the changes in the intestinal mucosa are primary and genetically determined is not acceptable. Both the intestinal and cutaneous lesions must be regarded as parallel and characteristic of the initial stage of the disease. The excessive increase in urinary kynurenine after loading during treatment, and the low levels of xanthurenic acid before and during treatment, can be explained by the fact that in AE, formation of xanthurenic acid is inhibited, either directly in the stage of transamination of 3-OH-kynurenine, or in the stage of hydroxylation of kynurenine.

Another possible cause of the hyperproduction of kynurenine, especially after treatment, could be the stimulation of tryptophan pyrrolase activity by oxyquinoline derivatives. However, in a control experiment in which a healthy child was given 1.5 g of Entero-Vioform daily for 12 days, no effect on the urinary excretion of kynurenine was observed.

Kynurenine is converted to 3-OH-kynurenine and xanthurenic acid in the liver. Hence, it must be assumed that the oxyquinoline derivatives act not only in the alimentary tract, but also influence tryptophan metabolism.

The increase in tryptophan metabolites (K and XA) observed in our patients during treatment, contradicts their toxic action on the skin and intestines, as suggested by Hansson (11).

The mode of action of the oxyquinoline derivatives is not clear. Hansson (11) proposed the hypothesis that as a result of marked chemical structural similarity of oxyquinoline to the tryptophan metabolites (mainly XA), these drugs act competitively, either by neutralizing the toxic metabolites of tryptophan, or by blocking their production.

Moynahan (14), on the other hand, suggested the hypothesis of a toxic action of products of incomplete digestion of proteins (owing to decreased leucine-aminopeptidase activity in the small intestine in AE). According to this hypothesis, the therapeutic effect of the oxyquinoline derivatives would consist in the binding of undigested peptides in indifferent complexes.

Our own studies suggest a hypothesis combining both of these theories. We assume that the toxic factor is not a polypeptide, but its complex with kynurenine. The action of the quinoline drugs would consist in their substitution in the place of kynurenine (preventing kynurenine from forming complexes with polypeptides), and thus giving rise to a different complex, devoid of toxicity. As a result, the urinary excretion of kynurenine from the body increases during treatment, as demonstrated in this study. In this respect, the disorders of tryptophan metabolism may be considered as primary and possibly genetically determined.

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ADDENDUM

After submitting our manuscript for publication we came across the paper by A. F. Robertson, et al. (J Pediatr *83:* 1012, 1973) in which they too suggested a decreased kynurenine hydroxylase activity in AE due to the dramatic increase of kynurenine, acetylkynurenine and kynurenic acid levels in the urine after l-tryptophan loading test + Diiodohydroxyquin, and normal levels of 3-hydroxykynurenine, xanthurenic acid and *N*-methylnicotinamide.