Acrodermatitis enteropathica without hypozincemia: Therapeutic effect of a pancreatic enzyme preparation due to a zinc-binding ligand

The clinical course and intestinal absorption studies of a female infant who developed diarrhea after cessation of breast feeding, mood changes, and intermittently had mild perioral and perianal rashes are described. She showed a partial response to a pancreatic enzyme preparation which was attributed to its content of a zinc-binding ligand, picolinic acid. Complete recovery occurred on pharmacologic doses of zinc. Exacerbation occurred twice upon withdrawal of the oral zinc medication. The zinc concentrations of plasma and intestinal mucosa were normal.

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**Acrodermatitis enteropathica** is a hereditary syndrome, characterized by chronic diarrhea, failure to thrive, neuropsychiatric symptoms, and dermatitis over the peripheral aspects of the extremities and in oral, anal, and genital areas. Moynahan and Barnes first recognized that plasma zinc was low in this disorder and that recovery could be induced with zinc supplements. Human milk and diiodohydroxyquinolone, which have been used therapeutically, both seem to improve the bioavailability of zinc. Evans and Johnson (personal communication) have shown that human milk contains a zinc-binding ligand, picolinic acid, which facilitates zinc absorption. Thus, more zinc is available to breast-fed infants than to infants receiving cow milk, although the zinc content of the two does not differ significantly.

A yet unrecognized molecular defect is responsible for the hereditary zinc malabsorption in AE. In some cases this defect may involve a deficiency in the amount or function of zinc-binding ligand. The mechanism by which zinc deficiency affects the integrity of epidermis and intestinal mucosa, thus causing diarrhea and dermatitis, has remained obscure. The function of one or more metalloenzymes may be affected.

The study of metabolic diseases has frequently led, in the past, to the recognition of genetic heterogeneity. The same seems to hold true for AE, because a patient was described recently who had the clinical features of AE but no hypozincemia. The patient responded to pharmacologic doses of zinc, although the plasma zinc was elevated.

| Abbreviation used | AE: acrodermatitis |

We had the opportunity to study a similar patient who also required pharmacologic doses of zinc but had initially normal plasma zinc values. Marked improvement was observed following treatment with a pancreatic enzyme preparation, which later was shown to contain a zinc-binding ligand.

**Case Report**

This female patient was referred at 12 months of age because of chronic diarrhea which started at 4 months of age after she was weaned from the breast and given Enfamil. At 5 months of age the formula was changed to ProSobee. Nutramigen was then tried together with the following strained foods: fruits, squash, carrots, peas, noodles, rice, chicken, and lamb. Over a span of eight months stools were persistently loose or liquid, and ranged in frequency from 4 to 12/day. A gluten-free diet failed to cause
improvement. A rash of the buttocks, hands, and face, at 7 months of age, led to admission at another hospital where staphylococcal dermatitis was diagnosed. In addition the mother noted frequently well-demarcated, deep red lesions in the peri-anal area, as well as a fleeting, deep red discoloration of the cheeks. There were several ear infections in the course of the first year, treated by myringotomy and adenoidectomy at age 11 months. The parents sought consultation of a dermatologist who performed skin tests which were positive for milk, dust, pollen, and wheat. The history was otherwise noncontributory. The physical examination was normal, except for some skin fold redundancy due to recent weight loss, and a lesion suggestive of angular stomatitis. The facial skin was dry and rough, and the lips and cheeks were usually deep red. The height was 75.5 cm and weight 8.25 kg.

In the hospital homogenized cow milk was tried, but it caused an acute exacerbation with watery, explosive stools that showed reducing substances and a pH of 6. Stools returned to normal on CHO-free formula base (Syntex) with added fructose. While receiving a protein hydrolysate formula (Nutramigen) without supplements, stools were large and loose, but hydration could be maintained by forcing oral fluids.

Laboratory and gastrointestinal function tests. The results of WBC; urinalysis; BUN, electrolytes, Ca, P, alkaline phosphatase, serum carotene, serum vitamin A, and immunoglobulin levels; EEG; chest radiograph; and a barium study of the colon were normal. Stool examinations for occult blood, pathogenic organisms, ova, and parasites were negative and there were no free-fat globules. Except for a time following the challenge with homogenized milk, stools were repeatedly negative for reducing substances. Sweat chloride values were 32, 29, and 49 mEq/L. A VMA spot test was negative.

Following an oral dose of xylose (0.5 gm/kg) blood levels rose to 42 mg/dl. An oral load of 5% lactose solution (2 gm/kg) caused a rise of blood glucose from 63 to 77, 83, 79, and 81 mg/dl at 15, 30, 60, and 90 minutes, respectively. An equal load of 5% sucrose solution caused a rise from 68 to 102, 91, 77, and 88 mg/dl at 30, 60, 90, and 120 minutes, respectively. Duodenal intubation for pancreatic enzyme analysis was unsuccessful.

A pancreatic enzyme preparation (Viokase) was started on a trial basis; first three teaspoons (6.75 gm/day), and later four (9.06 gm/day). The patient went home on this medication, but, due to a misunderstanding, continued to receive Nutramigen and low-protein solid foods. During the ensuing six weeks diarrhea was much improved; she gained 1,400 gm, and remained free of rashes. When the protein intake was subsequently increased by giving homogenized milk and meats, stools again became looser. The marked improvement on Viokase could not be attributed to its enzyme content. Consequently other properties of Viokase were investigated.

Zinc analyses, measured by atomic absorption were performed in the laboratory of Dr. A. Prasad. The Viokase powder used by the patient and Viokase tablets purchased in a local pharmacy contained 125.5 and 121.3 µg/gm of zinc, respectively. The plasma zinc level was 120 µg/dl at 13 months of age; at 20 and 23 months of age, after zinc therapy had been discontinued for three and seven weeks, it was 116 and 96 µg/dl, respectively (normal, 112 ± 13.6); RBC zinc values were 26.9 and 31.4 µg/gm Hgb at 20 and 23 months of age, respectively (normal, 39.8 ± 4).

Response to zinc therapy and withdrawal. At 15 months of age zinc sulfate therapy was started, at three tablets per day, equivalent to 45 mg elemental zinc. Stool consistency improved within several days and the parents reported that they saw, for the first time, formed stools. The stools remained normal when the pancreatic enzyme preparation was subsequently discontinued, and when homogenized milk and protein-rich table foods were added. There were even episodes of constipation.

At 19 months of age, while receiving zinc sulfate, the plasma zinc level was 176 µg/dl and RBC zinc value 32.1 µg/gm Hgb. Zinc therapy was then discontinued. One week later diarrhea recurred. After two weeks of constant severe diarrhea the mother insisted on reinstitution of zinc therapy, which caused improvement within a few days. She commented spontaneously on the mood change associated with the changes in stool consistency. Later, she increased the dose to 6 mg elemental zinc per day. At age 21 months, zinc was again withdrawn and diarrhea recurred, this time after two weeks. The mother then changed the diet to the protein hydrolysate formula and low-protein solids, because this had been best tolerated. Nevertheless, stools remained abnormal throughout seven weeks, causing dehydration on one occasion and admission for intravenous fluid therapy. At that time a coin-shaped, deep red, sharply demarcated lesion developed at the angle of the mouth. After seven weeks on a zinc-free regimen, an intestinal biopsy was obtained and duodenal juice was aspirated. The aspirate had a normal trypsin and amylase content, and the intestinal biopsy showed only mild nonspecific inflammatory changes. Electronmicroscopy was not done, because only a limited amount of tissue could be obtained, and it was felt that tissue analysis for zinc was more important. This was performed through the courtesy of Dr. K. M. Hambidge, who found 74 µg/gm dry weight. Two control specimen contained 79 and 86 µg/gm. Dr. Hambidge did not consider the small difference significant.

Since reinstitution of oral zinc therapy at 23 months of age, the patient has been symptom free for one and one-half years. By 30 months of age, the weight had increased from the tenth to the seventy-fifth percentile; the weight was proportionate for height. Two attempts to decrease the zinc dosage were unsuccessful, as diarrhea occurred when the intake of elemental zinc was reduced to 15 mg twice a day, which is well in excess of the amount which the patient received while on Viokase. The patient's general health has been good, except for three minor motor seizures, well controlled by mephobarbital. The patient is developing normally.

IDENTIFICATION AND QUANTITATION OF THE ZINC-BINDING LIGAND

Method. One tablet of Viokase (325 mg) was ground into a fine powder and suspended in 10 ml deionized water. The suspension was centrifuged at 100,000 × g, and the supernatant was subjected to ultrafiltration under nitrogen at 60 psi in an Amicon Ultrafiltration Cell equipped with an Amicon UM-2 membrane. The ultrafil-
Table. Thin-layer chromatography of the purified zinc-binding ligand and various pyridine carboxylic acids

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<thead>
<tr>
<th>Sample</th>
<th>R&lt;sub&gt;t&lt;/sub&gt; values</th>
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<tbody>
<tr>
<td></td>
<td>Butanol:acetic acid:H&lt;sub&gt;2&lt;/sub&gt;O 4:1:2</td>
</tr>
<tr>
<td>Quinolinic acid*</td>
<td>0.16</td>
</tr>
<tr>
<td>Nicotinic acid*</td>
<td>0.69</td>
</tr>
<tr>
<td>Picolinic acid*</td>
<td>0.40</td>
</tr>
<tr>
<td>Picolinic acid + Zn†</td>
<td>0.40</td>
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<tr>
<td>ZnBL‡</td>
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*Sigma Chemical Co.
†Equimolar ratio of picolinic acid adjusted to pH 7.0 with LiOH and Zn(NO<sub>3</sub>)<sub>2</sub>
‡Zinc-binding ligand recovered from Sephadex G-15.

trate was applied to a 0.9 x 13 cm column, packed with Dowex 50 in the hydrogen ion form. The sample was eluted with deionized water and the eluted fractions were monitored with an LKB Uvicord II, set at 254 nm. The ultraviolet-detectable fractions, eluted from Dowex 50 with the water rinse, were pooled and applied directly to a 0.9 x 5 cm column of Dowex 1 in the formate form. The column was then washed with 80 ml deionized water, followed by 80 ml 0.05M formic acid, and the pooled fractions were freeze dried.

The freeze-dried fraction recovered from Dowex 1 was dissolved in 2 ml 0.153 mM Zn(NO<sub>3</sub>)<sub>2</sub>; the solution was adjusted to pH 7.4 with 0.1M LiOH, and applied to a 1.5 x 90 cm column packed with Sephadex G-15 that had been equilibrated with 0.153 mM Zn(NO<sub>3</sub>)<sub>2</sub>. The same solvent was subsequently used to elute the sample. Fractions of 0.8 ml were collected, diluted with 9.2 ml deionized water, and assayed for zinc content by atomic absorption spectrometry (Varian, Model 1250). Fractions which comprised a zinc peak were pooled and freeze dried for analysis by either thin-layer chromatography, or infrared spectroscopy. Ascending thin-layer chromatography was carried out on Gelman ITLC-SA sheets that had been activated at 100°C for 1.5 hour. After sample application, the sheets were equilibrated for 15 minutes in the chromatography tank; three different solvents were used for chromatography: butanol-acetic acid-water, 4:1:2; butanol saturated with 2.5M NH<sub>2</sub>OH; and 80% isopropanol. Organic substances were detected with a Mineralight UVSIL-25 ultraviolet lamp (Ultra-Violet Products, Inc., San Gabriel, CA). The infrared spectrum of the zinc-binding ligand was determined with a Perkin Elmer Model 467 grating spectrophotometer.

To determine the quantity of zinc-binding ligand in Viokase tablets and powder, the ligand was first purified as described. The freeze-dried zinc-containing fractions recovered from Sephadex G-15 were dissolved in 1.9 ml deionized water. Thereafter, 0.1 ml of 0.1 N HCl was added to the solution and the absorption at 265 nm was determined in a Beckman DB-GT spectrophotometer. The quantity of zinc-binding ligand (picolinic acid) was calculated from the molar extinction coefficient of picolinic acid in 5 mM HCl.

**Results.** Evans and Johnson (personal communication) recently characterized the zinc-binding ligand in human milk, which was shown to be pyridine-2-carboxylic acid, commonly known as picolinic acid. Our studies, using thin-layer chromatography (Table) and infrared spectroscopy, proved that the zinc-binding ligand in Viokase tablets is also picolinic acid. The quantity of picolinic acid in Viokase was 416 µg (or 3.38 µmoles)/gm powder. Thus, the intake of the patient was approximately 466 mg with each meal, or 2,808 to 3,744 µg/day.

**DISCUSSION**

Food challenges and tolerance tests were not consistent with a specific malabsorption syndrome. The diarrhea following milk challenge and subsequent improvement on CHO-free formula base with added fructose suggested sensitivity to milk protein or lactose intolerance. However, on repeated trials, other hypoallergenic formulas were not beneficial. The absence of reducing substances in a majority of the watery stools, and the tolerance tests ruled out primary carbohydrate malabsorption.

The response to Viokase was dramatic. However, the patient was still on the protein hydrolysate formula and therefore not dependent on pancreatic enzyme. The occurrence of loose stools after institution of a high-protein diet confirmed our suspicion that the enzyme preparation had helped because of a factor other than its enzyme-content. The clinical picture was suggestive of AE, because the diarrhea, skin lesions, and mood changes, began after breast feeding was stopped. We considered the possibility that Viokase was beneficial because of its zinc content. However, the amount of zinc ingested in Viokase was only 1 mg/day, which is small if compared to the amount contained in the daily formula (approximately 4 mg/day). That zinc deficiency played an etiologic role, was then proven by the striking therapeutic effect of zinc sulfate. The effect of Viokase seemed to be due to a factor that improves the absorption of zinc available in the diet. The zinc dosage was apparently crucial because twice stools became abnormal on 30 mg elemental zinc per day, although they did not turn watery. This need for a higher zinc dose can explain the observation that on Viokase alone stool consistency was not entirely normal.
although much improved. It is possible that the effect of 30 mg elemental zinc was comparable to the effect of 5 mg elemental zinc, if given with a zinc-binding ligand. The apparent poor protein tolerance while the patient was receiving marginal zinc therapy remains unexplained. No efforts were made to confirm the maternal interpretation through objective tests.

All observations point strongly to a therapeutic effect of Viokase, which is due to its content of picolinic acid. Picolinic acid is known to be a strong, bidentate chelating ligand.5, 9 Paul et al9 as well as Clark and Williams10 have proven that picolinic acid-metal complexes are formed by coordination with the pyridine nitrogen and carbonyl oxygen on the adjacent carboxylic acid group. A low molecular weight zinc binding factor was demonstrated in rat intestinal lumen,11 as well as in the pancreas of rats and pancreatic secretions from a dog.12 Evens and Johnson showed that the low molecular weight zinc-binding ligand, contained in human milk, is picolinic acid. The ligand improves bioavailability by increasing zinc absorption. A substance with the same characteristics was demonstrated in Viokase because of our suspicion based on clinical observations. This substance appears to play a role as carrier in body fluids other than human milk and pancreatic secretions. Diodohydroxyquinolone, which is used like human milk in the treatment of AE, is structurally similar to picolinic acid.

The distribution and function of picolinic acid in different organ tissues and body fluids has not been assessed. Such investigations promise to clarify the genetic cause of AE and lead to the recognition of heterogeneity. We may be able to explain eventually why some patients with a clinical picture similar to AE do not have hypozincemia. It is possible that an abnormal ligand will be found that does not readily release zinc at the peripheral active tissue site.

The finding of abnormally low plasma zinc values in patients with cystic fibrosis who also have growth failure13 may be due to decreased zinc absorption, because, in the presence of significant pancreatic involvement, patients who are inadequately treated with Viokase have decreased amounts of ligand and will develop growth failure if fat and protein malabsorption are significant. Viokase is often given empirically by the practitioner and sometimes causes improvement of chronic diarrhea in cases that are not due to pancreatic insufficiency. It is possible that other cases of chronic diarrhea exist which are due to unrecognized zinc deficiency or zinc dependancy.

REFERENCES