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Serum Diamine Oxidase Activity in Acute Gastroenteritis in Children

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ABSTRACT. Serum diamine oxidase (DAO) activities were measured in 20 control patients and in 24 patients with gastroenteritis. Results (mean ± SE) were as follows: 1) control patients (n = 20), 36.2 ± 5.7 pmol h^{-1} ml^{-1}; 2) gastroenteritis (acute phase) (n = 11), 31 ± 4.9 pmol h^{-1} ml^{-1}; 3) gastroenteritis (healing phase) (n = 21), 18 ± 1.9 pmol h^{-1} ml^{-1}. Patients with gastroenteritis in the healing phase had significantly lower DAO values when compared to control patients ($p < 0.001$) and to gastroenteritis patients in the acute phase ($p < 0.05$). In eight patients where both acute and healing phase values could be measured, a significant decrease between acute and healing phase was found ($p < 0.001$). Patients with severe gastroenteritis tended to have lower DAO activity values than patients with moderate gastroenteritis ($p = 0.10$). Our results support the hypothesis that serum DAO activity is a marker of the total mass of functional enterocytes, the decrease of which during gastroenteritis is reflected in a decrease of serum DAO activity values. (*Pediatr Res* 19: 26–28, 1984)

Abbreviation

DAO, diamine oxidase

Diamine oxidase is an enzyme showing a high activity in small intestinal mucosal extracts of humans and other mammalian species (6). The enzyme is localized in the cytosol fraction of mature villous enterocytes (2). Mucosal DAO, through its implication in local polyamine and histamine catabolism, is thought to play a role in cell division, as well as a local protective role against histamine (2, 3). We have shown that a positive correlation exists between mucosal DAO and disaccharidase activities in children with a histologically normal duodenal mucosa (5). This supports the hypothesis that mucosal DAO activity reflects mucosal functional integrity. DAO activity is measurable not only in mucosal extracts but also in serum. Further correlation between serum and ileal activities has been shown to exist in the rat (6). The physiological function of circulating DAO is not known.

Acute gastroenteritis is accompanied by mucosal destruction and decreased mucosal disaccharidase activities (1). If serum DAO activity accurately reflects small intestinal functional integrity, as stated above, a decrease of serum activities would be expected in the course of acute gastroenteritis. The aim of the present study was to look at serum DAO activities during the acute and healing phases of gastroenteritis in children.

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PATIENTS AND METHODS

Patients. Control serum DAO activities were obtained in the following way. Serum DAO activities from 10 children (six girls and four boys; age range, 0.1–9.2 years) without gastrointestinal disease were measured. Informed consent was obtained in every case. The mean and standard deviation were found to be similar to those of 10 young healthy adults. These 20 values were consequently pooled and used as serum DAO control values (Fig. 1). Twenty-four children with acute gastroenteritis were studied (13 girls, 11 boys; age range, 0.1–4.5 years). The majority of our patients had a bacterial gastroenteritis (primarily *Salmonella* and *Escherichia coli*). Rotavirus was isolated in only a few cases. Serum DAO activities were measured in the acute and healing phase of the disease in eight of these children. In the others, they were estimated either in the acute or in the healing phase. No measurements were performed during periods of dehydration. The acute phase was arbitarily defined as a period during which the child had at least three fluid stools per day. The healing phase was defined as a period when stool frequency was less than three per day and stool consistency was more or less normal. Serum DAO activity in the acute phase of gastroenteritis was measured in 11 children. Serum DAO activity in the healing phase was measured in 21 children. Seven of the latter were considered on clinical grounds to have had a severe gastroenteritis while the 14 others were considered to have had moderate disease. Duration of gastroenteritis was the main criterion used to distinguish severe (more than 8 days) from moderate (less than 8 days) disease.

Serum DAO activity measurement. The activity of DAO (EC 1.4.3.6) was estimated by a method close to the radiometric technique described by Okuyama and Kobayashi (8). In this method, [^{3}H]putrescine is used as substrate. The reaction product (Δ^1-pyrroline) is extracted in toluene and its radioactivity is estimated by using a scintillation spectrometer (Packard, 3225).

Details were as follows: 1 ml blood was taken and allowed to clot at room temperature for 45 min. After centrifugation, serum was kept at −20° C. Determinations were performed within 1 month. No decrease of activity was recorded.

[^{3}H]Putrescine was purchased from New England Nuclear Co. It had a specific radioactivity of 40.5 Ci $mmol^{-1}$. Before utilization, it was mixed with unlabeled putrescine in order to reach a specific radioactivity of approximately 1 Ci $mmol^{-1}$. To determine the DAO activity, 50 μl of serum was mixed with 50 μl of putrescine (160 pmol, yielding approximately 280,000 cpm). The mixture was incubated at 37° C for variable periods (0, 10, 20, 30, and 60 min). Activities were shown to be proportional to time and quantity of enzyme. Activities were thereafter measured

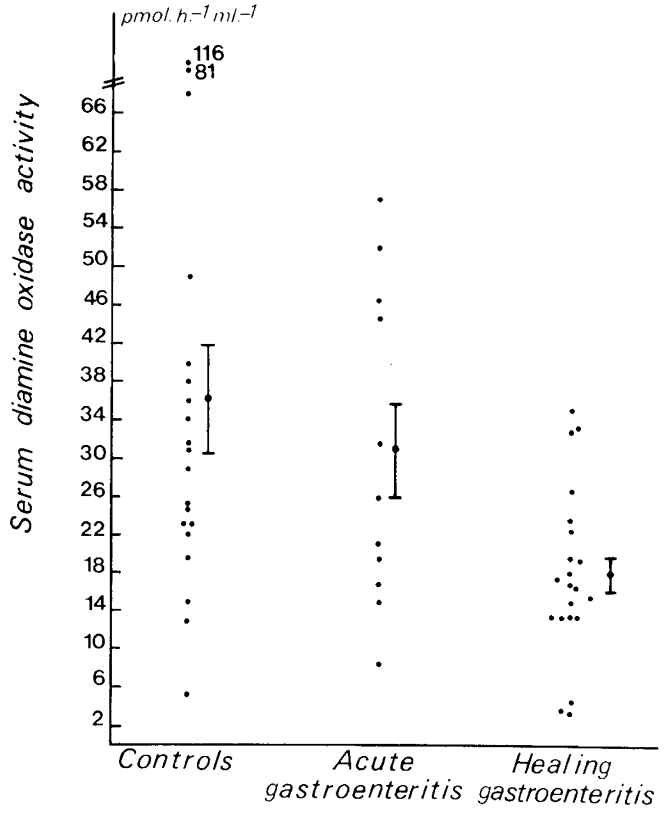

Fig. 1. Serum diamine oxidase activity in control patients and during the acute and healing phase of gastroenteritis.

routinely at 60 min with occasional checks of the time-activity relationship. The reaction was stopped by the addition of 10 μl of aminoguanidine (10^{-4} M). Fifty μl of saturated bicarbonate solution was then added to each tube in order to improve the toluene extractability (8). The toluene extraction was performed twice by using 500 μl of the organic solvent and by allowing the water phase to freeze. Eight hundred μl of the mixed toluene phases were poured into counting vials containing 6 ml of Lumagel. Fifty μl of substrate solution added in Lumagel served as standard. Each determination of DAO activity was performed in duplicate or in triplicate. Results are expressed in pmol putrescine deaminated h^{-1} ml^{-1} serum.

Statistical methods. Mean and standard error were calculated as usual. Because of skewness in distribution, nonparametric tests were used for comparison among groups. The two-sample Wilcoxon test was used to evaluate serum DAO activity differences between the following groups: controls *versus* all acute phase values; controls *versus* all healing phase values; all acute phase values *versus* all healing phase values; and healing phase values (severe disease) *versus* healing phase values (moderate disease). The Wilcoxon rank sum test for paired samples was used to compare the eight paired acute and healing phase values.

RESULTS

Serum DAO activities in controls and in patients with gastroenteritis are shown in Figure 1. Mean and SEM were as follows: control patients (n = 20), 36.2 ± 5.7 pmol h^{-1} ml^{-1}; gastroenteritis patients in acute phase (n = 11), 31 ± 4.9 pmol h^{-1} ml^{-1}; gastroenteritis patients in healing phase (n = 21), 18 ± 1.9 pmol h^{-1} ml^{-1}. The significance of differences between groups was as follows: controls *versus* acute phase gastroenteritis, not significant; controls *versus* healing phase gastroenteritis, $p < 0.001$; acute phase gastroenteritis *versus* healing phase gastroenteritis, $p < 0.05$.

Healing phase gastroenteritis patients (n = 21) were further subdivided into severe (n = 7) and moderate disease (n = 14). Results in these subgroups are shown in Figure 2. Mean ± SE values were as follows: severe disease (n = 7), 11.8 ± 2.8 pmol h^{-1} ml^{-1}; moderate disease (n = 14), 21 ± 2.1 pmol h^{-1} ml^{-1}. The difference did not reach statistical significance ($p = 0.10$).

In eight patients where serum DAO activities were measured during both the acute and healing phases of the disease, a significant ($p < 0.001$) decrease of DAO activity between acute and healing phase was noted (Fig. 3). Two patients with low serum DAO activity values (13.5 and 3.9) could be retested 2 months after healing. DAO activity values reverted to normal (35 and 45, respectively).

DISCUSSION

The study of gastroenteritis in childhood is difficult because of the poor accessibility of the small intestine for histological studies. Peroral small intestinal biopsies have been performed in only

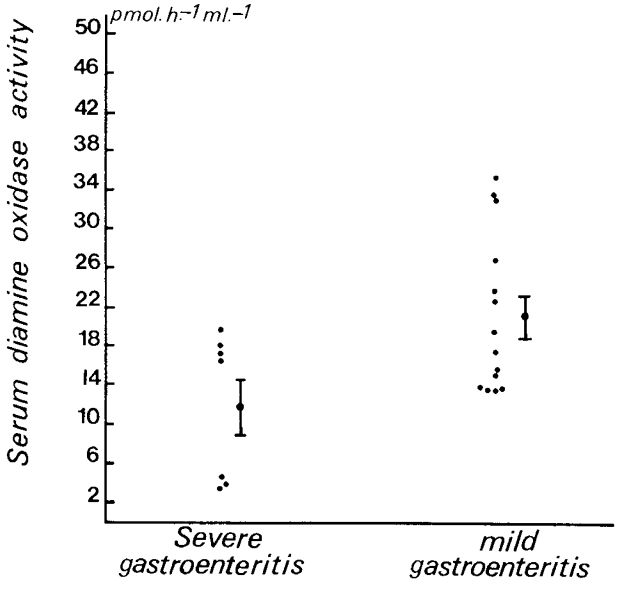

Fig. 2. Comparison of serum diamine oxidase activities during the healing phase of gastroenteritis according to severity of gastroenteritis.

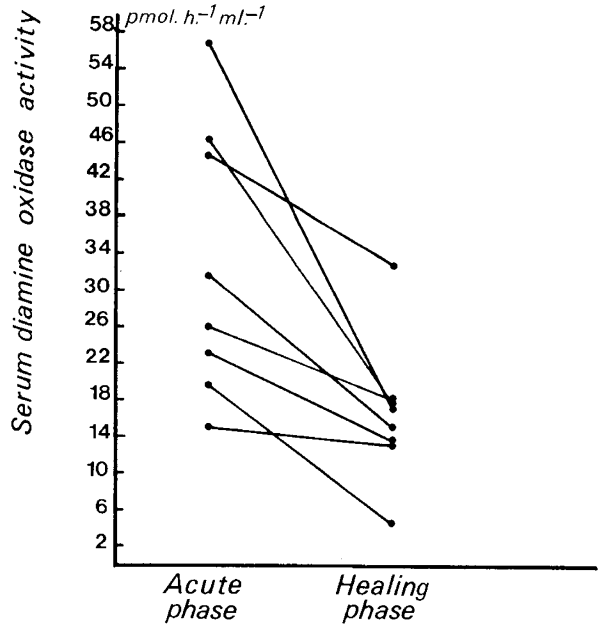

Fig. 3. Serum diamine oxidase activity in eight patients during the acute and healing phases of gastroenteritis.

very few studies. These studies have shown that a variable degree of mucosal damage with a decrease of brush border disaccharidase activities frequently accompanies childhood gastroenteritis (1). It has been shown in newborn lambs infected with rotavirus that there persists, for 4 or more days after complete clinical recovery, a state of accelerated cell turnover with functionally immature cells clothing the villi (4).

The immature intestine of neonatal rat has been shown to have a low DAO content reflected in a low serum DAO activity (6). These data aid in understanding our own results. The low DAO activity values in the healing phase of gastroenteritis probably reflect the loss of functional enterocytes replaced in the healing phase by "immature" cells. The normal values found in the acute phase of gastroenteritis probably indicate that time is needed for mucosal damage to occur. Our results further show that patients in the healing phase after severe gastroenteritis tend to have lower serum DAO activity values than patients recovering from moderate gastroenteritis. This finding supports the hypothesis that serum DAO activity might be dependent on the total mass of functional enterocytes (6), since severe gastroenteritis is most probably accompanied by a heavier loss of functional enterocytes than gastroenteritis of moderate degree. In the rat, serum DAO activity has been shown to accurately quantitate the length of acute intestinal mucosal injury (7).

The low serum DAO activity values found in healing gastroenteritis are sometimes lower than those found in acute celiac disease (unpublished data). This would logically mean that loss of functional enterocytes can be more important in gastroenteritis than in acute celiac disease. Literature data tend to support this by showing that a complete destruction of the small gut mucosa along all its length can occur in acute gastroenteritis, whereas in celiac disease the mucosal damage is mainly localized to the proximal intestine (9). Although isolated serum DAO activity measurements are difficult to interpret because of the wide variation in normal values, we believe serial serum DAO activity measurements may be useful in evaluating the severity of small intestinal functional loss in childhood gastroenteritis. Further serial serum DAO activity measurements may turn out to be a most useful tool in the follow-up of childhood enteropathies.

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Incorporation of [^{14}C]Glucose into α-1,4 Bonds of Glycogen by Leukocytes and Fibroblasts of Patients with Type III Glycogen Storage Disease

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ABSTRACT. In two patients assay of α-1,6-amyloglucosidase activity by incorporation of [^{14}C]glucose into glycogen revealed normal activity in leukocytes, erythrocytes, and fibroblasts, whereas no activity was detected in liver and muscle. No activity in any tissue was found when enzyme activity was assayed by following the release of glucose from a phosphorylase limit dextrin. Labeling of glycogen by incubation with crude tissue homogenates according to the protocol used for the [^{14}C]glucose method and subsequent degradation of the outer portion of the polysaccharide molecule with β-amylase showed that with tissues from normal controls more than 90% of the label of the glycogen was retained in the limit dextrin. When fibroblasts or leukocytes of the patients served as enzyme source up to 80% of the label was released after incubation with β-amylase or phosphorylase *a*. Addition of Tris to the assay inhibited enzyme activity in fibroblast homogenates of the patients and of controls to the same extent and had no effect on the distribution of the label between supernatant and limit dextrin after β-amylolysis of the labeled glycogen. A pH curve performed with fibroblast preparations from the patients and a normal control did not reveal

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