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Transmissible gastroenteritis in piglets: A model of infantile viral diarrhea

Piglets infected with transmissible gastroenteritis virus, compared to matched-fed litter mates, had massive diarrhea characterized by increased quantities and concentrations of sodium, potassium, and chloride. Determinations of Na-K-ATPase in mucosal homogenates from small and large intestine revealed decreased activity of this enzyme in the upper small bowel. Our data indicate that a defect in active sodium transport in this region may be an important factor in the pathogenesis of the diarrhea. Further studies using this model should help to define the mechanisms producing diarrhea in acute infantile gastroenteritis.

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In most cases of acute infantile gastroenteritis occurring in temperate climates, no specific etiologic agent is isolated. Usually, the clinical course of the disease suggests an acute infection, presumably viral, although to date extensive virologic studies from our institution and elsewhere have been unrewarding. Nevertheless, we have begun a series of experiments to explore the mechanisms that cause diarrhea in a specific enteric viral infection in swine, transmissible gastroenteritis. This report will deal with our initial observations which characterize the clinical response and the diarrhea in this disease. Also, we will describe data on the activities of certain intestinal mucosal enzymes, in particular, sodium-potassium-dependent adenosine triphosphatase (Na-K-ATPase). This information may provide a clue to the site and nature of the primary defect of intestinal function and may lead to a greater understanding of a potentially fatal disease in infants.

Transmissible gastroenteritis is caused by a Corona virus. Symptoms of the disease are vomiting, diarrhea, and dehydration within 24 hours of infection. In piglets affected at or near birth, the mortality rate is close to 100 per cent; in older piglets there is severe diarrhea which subsides within two weeks. The virus infects the proximal small bowel where a mucosal lesion may occur, particularly in animals less than 14 days of age.

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MATERIALS AND METHODS

Pigs. Piglets from eight litters of York breed were used in this study. They were left with the sow until age 17 to 20 days when they were placed in holding pens and fed canned whole evaporated cow's milk (Farmer's Wife Brand, Cow & Gate Ltd., Brockville, Ontario, Canada).

Virus. The Purdue strain of transmissible gastroenteritis virus was used. It had been pig passaged six times, and the inoculum was prepared as described.7

Experimental plan. We used a total of 49 piglets. Of these, 31 were inoculated orally with transmissible gastroenteritis virus at 23 to 26 days of age. Control piglets were matched-fed litter mates (each fed the mean volume of the intake of the infected pigs in their own litter). Piglets were randomly assigned to one of three experimental groups: acute, convalescent, or recovered. Piglets were killed by electrocution as follows: acute, 42 hours post inoculation; convalescent, 1 week post inoculation; and recovered, 2 weeks post inoculation. During the 24 hour period before the pigs were killed we collected stool and urine from male pigs in each litter using metabolic cages constructed of Plexiglas, which we could adjust to the size of the piglet. We sampled blood from the anterior vena cava immediately prior to killing the pigs. Piglets did not receive intravenous therapy. Of the total number of pigs inoculated, five died and two failed to develop symptoms of the disease.

Preparation of tissue for histologic study. Tissues for light microscopy were stapled onto paraffin blocks for identification, fixed in Suza's fixative for 24 hours, and then transferred to 95 per cent alcohol and processed routinely for staining with hematoxylin and eosin. Each section was examined by one investigator who did not know whether it was from a control or infected pig. Villous height and crypt depth were measured with a standard micrometer eyepiece, previously calibrated, and epithelial cell damage and round cell infiltration of the lamina propria were rated.

Enzyme assays. We designed an assay for Na-K-ATPase based on its biochemical characteristics in whole homogenates of intestinal mucosa from normal pigs. Thawed homogenates were rehomogenized with an equal volume of 0.1 per cent sodium deoxycholate in 2.5 mM of EDTA and 20 μl of this preparation was added to a total volume of 1.0 ml of either an incubation medium made up of 5 mM of MgATP (Sigma Chemical Co., St. Louis, Mo.), 120 mM of NaCl, and 20 mM of KCl in 50 mM of TRIS buffer, or a second medium containing in addition 0.2 mM of ouabain (Sigma Chemical Co., St. Louis, Mo.). The pH of both solutions was 7.2 at 37°C. We performed reactions in duplicate in both solutions at 37°C, stopping them after 15 minutes by plunging the tubes into an ice-water bath and adding 0.5 ml of cold 8 per cent perchloric acid. Tubes were kept on ice until inorganic phosphate was measured in a Technicon autoanalyzer by the method of Gomori.8 Since ouabain selectively inhibits the ATPase activity stimulated by sodium and potassium, the difference in inorganic phosphate produced without
and with ouabain represents Na-K-ATPase activity and was expressed as micromoles of p_i produced per hour, per milligram of protein as determined by the method of Lowry and associates\textsuperscript{10} modified by Eggstein and Kreutz\textsuperscript{11} using bovine albumin as standard. Mg-ATPase was determined from p_i liberated from the medium containing ouabain and was expressed similarly.

Sucrase and lactase were measured by the method of Dahlqvist\textsuperscript{12} at a substrate concentration of 0.11 mM, and alkaline phosphatase by the method of Kelly and Hamilton.\textsuperscript{13} Disaccharidase units were micromoles of glucose produced per minute; alkaline phosphatase units were millimoles of p-nitrophenol produced per hour. Statistical analyses were based on distribution of enzyme activity following a lognormal skew. All data were converted to logarithms before analyses. The mean was plotted as the antilogarithm and scatter expressed as the antilogarithm of one standard error above and below the mean.

**Stool determinations.** Stools were homogenized in a Sorvall “OmniMixer” (Ivan Sorvall, Inc., Norwalk, Conn.), using a minimum volume of deionized water.

To determine chloride a 1 Gm. aliquot was mixed in a total volume of 4 ml. of chilled 10 per cent nitric acid. After incubating the mixture for 10 minutes at room temperature, the supernate from a 10 minute low-speed centrifugation was analyzed.\textsuperscript{14} Recover ranged from 89 to 92 per cent.

For sodium and potassium determinations stool homogenates were wet ashed by incubating 1 Gm. of homogenate in 10 ml. of concentrated nitric acid overnight in a 100 ml. evaporating dish; the contents were then boiled gently with several aliquots of concentrated nitric acid and finally with 10 ml. of deionized water, always with a small volume (about 4 ml.) of liquid remaining in the dish. This mixture and rinsings from the dish were made up to a volume of 10 ml. in a volumetric flask. A flame spectrophotometer was used to determine sodium and potassium on diluted aliquots of the supernate from a 10 minute centrifugation at low speed. Ninety-two to 104 per cent of potassium was recovered.

The pH was determined on fresh stool using pH paper. The stool was hydrolyzed by boiling for one minute with 1.0N HCl and neutralized; it was then assayed for reducing substances.\textsuperscript{15}

**Serum determinations.** Standard methods were used for the following determinations: pH,\textsuperscript{16} venous carbon dioxide tension,\textsuperscript{17} magnesium,\textsuperscript{18} calcium,\textsuperscript{19} chloride,\textsuperscript{14} and total proteins.\textsuperscript{20} Serum sodium and potassium were determined using a flame spectrophotometer, protein by electrophoresis on cellulose acetate, and osmolality by freezing-point depression.

**RESULTS**

**Acute phase.** Eight infected pigs were studied during the acute phase of the disease; they were compared with 10 matched-fed litter mates. Mean weight of all pigs at the start of the experiment was 4.78 \pm 0.86 Kg. After 42 hours the infected pigs had lost 20 per cent of their body weight; weight of control pigs remained unchanged (Table I). All infected pigs had loose watery stools 24 hours post inoculation. Stools collected 18 to 42 hours post inoculation from five male infected pigs and six male control pigs were analyzed (Table II). Stool weight and electrolytes in transmissible gastroenteritis-infected pigs increased tremendously over values for control pigs. The concentrations of the electrolytes expressed as per gram of stool were also significantly increased. There was no difference in fat excretion. Fresh stool had a pH between 7.0 and 8.0. Hydrolyzed

| Time after injection | Control* | Infected | \( p \) |
|----------------------|----------|----------|------|
| 42 hr.               | 100.0 \pm 2.0 | 80.3 \pm 2.1 | < 0.01 |
| 1 wk.                | 104.2 \pm 1.2 | 93.5 \pm 5.5 | N.S.   |
| 2 wk.                | 113.3 \pm 7.7 | 118.8 \pm 8.7 | N.S.   |

The values represent the mean \pm 1 S.E.M.

*Control piglets are matched-fed litter mates.

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**Table I.** Mean final weight of piglets expressed as per cent of original weight before infection
Table II. Stool determinations on collections from male pigs 18 to 42 hours post inoculation

| Determinations | Control* | Infected | p  |
|----------------|----------|----------|----|
| Weight (Gm./24 hr.) | 36.3 ± 9.4 | 261.5 ± 74.4 | < 0.05 |
| Fat (% intake) | 3.9 ± 1.0 | 5.1 ± 1.5 | N.S. |
| Na (mEq./24 hr.) | 0.9 ± 0.6 | 22.0 ± 6.1 | < 0.01 |
| K (mEq./24 hr.) | 1.5 ± 0.5 | 14.2 ± 3.1 | < 0.01 |
| Cl (mEq./24 hr.) | 0.4 ± 0.3 | 19.0 ± 5.3 | < 0.01 |
| Na (mEq./Kg. stool) | 18.2 ± 7.5 | 82.0 ± 9.9 | < 0.01 |
| K (mEq./Kg. stool) | 28.0 ± 6.5 | 64.0 ± 11.8 | < 0.05 |
| Cl (mEq./Kg. stool) | 10.5 ± 5.3 | 66.0 ± 4.4 | < 0.01 |

The values represent the mean ± 1 S.E.M.
*Control pigs are matched-fed litter mates.

Stools contained no measurable reducing substances.

Except for a decrease in calcium, serum electrolytes were unchanged in the infected pigs. Serum sodium, potassium, chloride, magnesium, total protein, and protein patterns remained the same (Table III). However, pH fell from 7.36 to 7.30, and carbon dioxide tension and bicarbonate were significantly lowered in the infected group. Infected pigs did not differ from control pigs with respect to villous dimensions, epithelial cell structure, or round cell infiltration. There was no difference between groups with respect to weight of mucosa or mucosal protein content per 20 cm. segment. Results of enzyme determinations of mucosal homogenates from small and large intestine are shown in Figs. 1 and 2. The only difference in Na-K-ATPase activity was in the proximal jejunum where it was decreased in infected pigs. In this area Mg-ATPase, alkaline phosphatase, and lactase were also decreased. In the midjejunum, lactase, sucrase, and alkaline phosphatase were decreased. In the ileum, lactase and sucrase were decreased. In none of these enzymes was the activity changed in the proximal or distal colon.

**Table III. Serum determinations on blood obtained at 42 hours post inoculation**

| Serum          | Control* | Infected | P    |
|----------------|----------|----------|------|
| Na (mEq./L.)   | 147 ± 1  | 147 ± 2  | N.S. |
| K (mEq./L.)    | 6.6 ± 0.3| 5.9 ± 0.2| N.S. |
| Cl (mEq./L.)   | 104 ± 1  | 107 ± 2  | N.S. |
| Ca (mg.%)      | 11.0 ± 0.2| 10.3 ± 0.2| < 0.05|
| Mg (mg.%)      | 2.9 ± 0.1| 2.5 ± 0.2| N.S. |
| Osmolality     | 312 ± 4.0| 298.0 ± 11.0| N.S. |
| Protein (Gm.%) | 6.2 ± 0.2| 6.2 ± 0.2| N.S. |
| pH             | 7.36 ± 0.01| 7.30 ± 0.02| < 0.01|
| Pco₂ (mm. Hg)  | 39.0 ± 1.9| 32.7 ± 1.6| < 0.05|
| HCO₃ (mEq./L.) | 20.7 ± 0.5| 13.7 ± 0.8| < 0.01|

The values represent the mean ± 1 S.E.M.
*Control pigs are matched-fed litter mates.

Activities were determined at those intestinal sites where enzyme activities had been lowered in infected piglets in the acute phase, and except for decreased Na-K-ATPase activity in the proximal jejunum (Fig. 3) found they were not different from the same activities in control piglets. By chance, there were no male pigs in the infected group so stools could not be collected for analysis. The infected pigs weighed 97 per cent of their original weight which was not statistically different from the control pigs who weighed 104 per cent, suggesting that rehydration had taken place.

**Recovered phase.** Six recovered pigs were compared to three control pigs two weeks post inoculation. No significant differences in intestinal enzyme activities or in serum pH, electrolytes, and protein existed between the two groups. Stool weights, fat, and electrolytes obtained on three male piglets and three control piglets were similar.

**DISCUSSION**

Transmissible gastroenteritis proved to be a useful model for the study of acute diarrhea. Oral administration of transmissible gastroenteritis virus produced a consistent self-limited disease in piglets characterized by anorexia, vomiting, diarrhea, and dehydration, similar in its clinical presentation to infantile gastroenteritis. Our preliminary data have provided important information for
future investigations of this condition.

Water moves in and out of the intestine passively in response to osmotic pressure gradients. Diarrhea can be produced when osmotically active material is present within the gut lumen in excessive amounts, stimulating water movement into the lumen. The massive fecal output of infected pigs contained large quantities of sodium, potassium, and chloride. The concentration of these ions in stool was also increased. Specifically, the high sodium concentration suggested that there was either a defect in intestinal absorption of sodium or an increased secretion of this ion into the gut lumen. In simple osmotic diarrhea, such as would be produced by water moving in response to mannitol in the lumen, the stools contain much less sodium in proportion to water.

There is now considerable evidence relating active transport of sodium to Na-K-ATPase in many organs. In dog kidney, Nechay and Nelson found a direct correlation between distribution of this enzyme and sodium reabsorption in cortex, medulla, and papilla. In normal pigs we found that the activity of the Na-K-ATPase was significantly greater in the proximal jejunum than in other regions of the bowel, implying that, contrary to current concepts, this region does absorb sodium by an active transport process. In the acute stage of the disease, the activity of the enzyme was depressed in the proximal jejunum only, suggesting that sodium absorption was defective at that site. If this suggestion is valid, the distal bowel must have been unable to compensate for the increased load of sodium, since losses in stool were excessive. The same pattern of Na-K-ATPase activity was present in animals one week post inoculation, but the activity had returned to control values within another week (Fig. 3).

In relating activity of Na-K-ATPase to
Fig. 3. Proximal intestinal Na-K-ATPase (mean ± S.E.M.) and fecal electrolytes in infected pigs compared with control pigs. Na-K-ATPase activity was lowered at 42 hours and 1 week post inoculation. Fecal electrolytes were increased at 42 hours. Two weeks post inoculation both values had returned to control levels.

sodium transport, the possibility is recognized that the asymmetrical properties of the cells have been destroyed by homogenization. However, the assays were performed under optimum conditions for the enzyme so that, since both control and diseased tissue were processed exactly alike, our results should have quantitative relevance. To date there have been few other quantitative determinations of intestinal Na-K-ATPase activity in the normal and diseased state. Activity of this enzyme in biopsies from the proximal jejunum is lower in the acute than in the convalescent phase of cholera, a disease associated with tremendous losses of sodium and water in stool. Recent studies of this disease in human subjects and in animals have shown significantly increased activity of adenyl cyclase in intestinal mucosa, linking the mechanism of diarrhea to cyclic adenosine monophosphate and chloride secretion. Obviously, it is important to assess this enzyme system in piglets with transmissible gastroenteritis.

Although activity of lactase in the small intestine decreased and piglets were ingesting small quantities of dietary lactose, their stools were not acidic and contained no sugar, indicating that disaccharidase deficiency was unlikely to be the primary cause of the diarrhea.

In the tissues examined by light microscopy, the groups did not differ with respect to villous measurements. No villous atrophy described in younger pigs was found. However, our enzyme data indicate that the epithelial cells were functionally damaged, probably a direct effect of the virus which replicates in the epithelial cells of the upper small bowel. The animals were matched fed; therefore, the decreases in enzyme activity were not due to poor nutrition of the infected pigs. Nor are the decreases likely to be due to gross derangement of ionic concentration of extracellular fluid, since during the acute phase values for serum sodium, potassium, and chloride were alike in infected and control animals. We have no explanation for the hypocalcemia in infected pigs.

To further define the mechanisms of diarrhea, we intend to proceed with studies on transmissible gastroenteritis. Such studies should lead to a better understanding of the perplexing problem of acute infectious diarrhea of infancy.

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