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# Inhibition of Sodium Intestinal Transport and Mucosal ($Na^+$-$K^+$)-ATPase in Experimental Fanconi Syndrome[1] (39068)

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The Debre–de Toni–Fanconi syndrome, in its full expression, is a proximal tubular defect consisting of hyperaminoaciduria, renal glycosuria, hyperphosphaturia and acidosis due to excessive bicarbonaturia (1). These manifestations are secondary to decreased renal reabsorption by the proximal tubule.

Substances that cause proximal tubular damage produce a condition comparable to the Fanconi syndrome, e.g. heavy metals (2, 3) or phosphorylated sugars (4, 5). Berliner *et al.* (6) and later Harrison and Harrison (7) produced a comparable condition in experimental animals by iv or ip injections of sodium maleate. In further studies in rats, Kramer and Gonick (8) showed an inhibition of renal cortical ($Na^+$-$K^+$)-ATPase and a reduction of endogenous kidney ATP in addition to renal glycosuria, phosphaturia and aminoaciduria. The transport defect induced by maleate, has served to demonstrate the correlation between the resorptive renal mechanisms of sodium, calcium, magnesium, and urate (9) and the reabsorption of bicarbonate (10, 11).

The genetically determined human disorders cystinuria, Hartnup disease and Lowe's syndrome (12) demonstrate amino acid reabsorption defects in both the proximal tubule and the jejunum. In order to investigate if a comparable damage to that of the kidney could be demonstrated in the intestinal mucosa we studied the intestinal transport *in vivo* of electrolytes, glucose and amino acids and ($Na^+$-$K^+$)-ATPase following injections of sodium maleate in rats. These experiments indicated that the induction of a Fanconi-like syndrome produced sharp changes in sodium and potassium transport and ($Na^+$-$K^+$)-ATPase without affecting the intestinal absorption of glucose and amino acids.

*Materials and methods.* Male Wistar rats weighing approximately 150 g were given ip doses of either 1.5 or 9.0 mmoles/kg of 0.15 *M* maleic acid solutions adjusted to pH 7.0 with NaOH (8). Control animals received a comparable volume of saline. Following a 30 min induction period, after which all experimental animals developed glycosuria and proteinuria, the rats were anesthetized with 1.2 g/kg of urethane, the abdominal cavity opened and a 20 cm jejunal segment was perfused for 2 hr with Krebs–Henseleit bicarbonate isotonic buffers, with 20 or 40 m*M* glucose and 1 m*M* of either L-phenylalanine, L-lysine, glycine or α-aminoisobutyric acid (α-AIB). Tracer amounts of $^{14}C$ or $^{3}H$-labeled glucose or amino acids were included, as well as 600 mg/100 ml of polyethylene glycol, m.w. 3000–3700 (PEG), as a nonabsorbable marker. Amino acids and glucose were purchased from Sigma Chem. Co., St. Louis, Mo.; salts and chemicals from Mallinckrodt Chem. Works, St. Louis, Mo.; PEG from J. T. Baker & Co., Phillipsburgh, N. J., and radiolabeled substances from New England Nuclear, Boston, Mass. Full details of the perfusion technique and the analytical procedures have been described previously (13, 14). In some experiments, the concentration of sodium in the buffers was decreased and the tonicity maintained by the addition of mannitol to the solutions. Jejunal mucosa was scraped from an area contiguous to the perfused segment and homogenized with 4 vol of ice-cold 0.15 *M* NaCl for the determination of ($Na^+$-$K^+$)-ATPase (15).

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TABLE I. INTESTINAL TRANSPORT OF SODIUM AND INTESTINAL MUCOSA ($Na^+$-$K^+$)-ATPase IN MALEATE-TREATED RATS.

| Treatment | Sodium[a] | ($Na^+$-$K^+$)-ATPase[b] |
|---|---|---|
| 1.5 mmoles/kg sodium maleate | 94.0 ± 53.6[c] (34)[d, e] | 218.5 ± 75.0[c] (6)[d, f] |
| 9.0 mmoles/kg sodium maleate | 51.7 ± 22.3 (10)[e] | 185.7 ± 76.1 (6)[f] |
| Saline | 165.8 ± 39.5 (18) | 317.2 ± 9.9 (12) |

[a] Values expressed in μequiv/min/cm.
[b] Enzyme activity expressed in nmoles *P* hydrolyzed/min/mg of protein.
[c] Mean ± SEM.
[d] Number of determinations.
[e] $P < .001$.
[f] $P < .05$.

*Results.* The intestinal sodium absorption in maleate-treated rats was markedly impaired. However, there were no differences between animals injected with either 1.5 or 9.0 mmoles/kg of maleate (Table I). When the concentration of sodium in the perfused buffers was reduced from 140 mequiv/liter to 78 mequiv/liter, the absorption of this ion ceased, and a net efflux, that is, a blood-to-lumen secretion, occurred, at equal rates in animals given maleate or in controls given saline (Fig. 1). However, when sodium concentration in the buffers was further decreased to 26 mequiv/liter, the secretion of sodium intensified in control rats but was not further modified in maleate-treated ones.

Potassium secretion was lower in maleate-treated animals than in control rats in all three levels of sodium employed. However, the control rats exhibited a marked transfer of potassium from the circulation to the intestinal lumen during perfusions with solutions combining intermediate or low sodium levels. In contrast, experimental animals had a modest secretion of potassium when the medium had 78 mequiv/liter of sodium and demonstrated a net absorption of potassium when the sodium perfusate concentration was reduced to 26 mequiv/liter (Fig. 2).

The sodium transport alterations correlated with changes occurring in ($Na^+$-$K^+$)-ATPase activity of the intestinal mucosa. Experimental animals developed a marked inhibition of this enzyme with no differences to be found between the two dosages of maleate given to the rats (Table I). In contrast, the intestinal transport rates of either

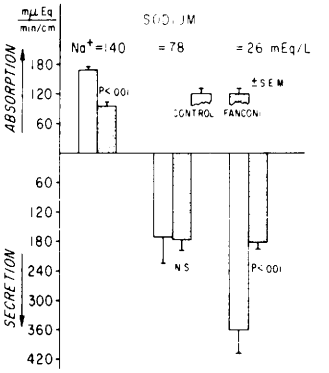


FIG. 1. Intestinal transport of sodium in rats injected with 9.0 mmoles/kg of maleate (Fanconi) and their controls. Measurements *in vivo* as described in the text were conducted when the concentration of sodium in the perfusing medium was either 140, 78 or 26 mequiv/liter. There was absorption of sodium at the highest concentration of this ion in the medium, but a reverse flux when sodium ionic strength was reduced. Tonicity was maintained with mannitol. Six rats were used in each group.

L-lysine, glycine, L-phenylalanine or α-AIB, when perfused separately at 1 m*M* concentration, were identical in maleate-treated and in controls. Similarly, the absorption rates for glucose in buffers, either at 20 or 40 m*M* levels, was the same as in control animals (Table II).

*Discussion.* Our experiments indicate that the same effects on ($Na^+$-$K^+$)-ATPase can be observed in the intestinal mucosa than those reported for the kidney, after injections of maleate. It has been well established that ($Na^+$-$K^+$)-ATPase is the enzyme considered to represent the biochemical vehicle for the active transport of sodium and potas-

sium across cell membranes (16). Its inhibition by maleate has been accepted as the explanation for the renal derangements observed in rats (6–11).

The renal effects of maleate injections in rats are of short duration and reversible, since the most easily observed abnormalities, such as glycosuria and alkaline pH subside in a few hours. They are also dose-related, at least up to 9.0 mmoles/kg (6, 8, 9, 10, 17). In spite of the transient nature of this damage, the disruption of intestinal mucosa ($Na^+$-$K^+$)-ATPase was sufficient to impair the sodium pump operating across this membrane and substantially reduce the transport of this ion to the serosal side. When the sodium concentration of the perfusates was reduced to 78 and 26 mequiv/liter, the transport capacity of the maleate-treated rats mucosa for sodium and potassium continued to be different to that of the non-treated animals. The divergent behavior exhibited by potassium transport at high and low concentrations of sodium is in agreement with earlier observations in the kidney where the potassium/sodium ratio of fractional secretions exceeded unity, suggesting independent reabsorptive mechanisms for these two ions. This is in contrast to an interrelated functional identity of sodium reabsorption with that of calcium, magnesium and urate (9).

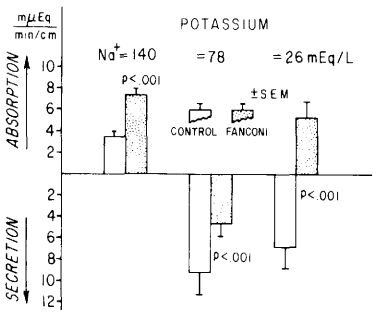


FIG. 2. Transport of potassium across the small intestinal mucosa under conditions described in Fig. 1. The potassium absorption was higher in the maleate-injected animals (Fanconi) than in those receiving saline when the medium had 140 or 26 mequiv/liter of sodium. When sodium was 78 mequiv/liter there was flux of potassium into the intestinal lumen in both groups of rats, but it was far less marked in the maleate-treated group.

The lack of any differences in the intestinal transport of glucose and amino acids, using selected representatives of diamino dicarboxylic, neutral, and the glycine-amino acid groups, has at least two possible explanations. The first is an insensitivity of the intestinal transport mechanisms per se to maleate. The second is that an impairment of sodium transport and of ($Na^+$-$K^+$)-ATPase is the sole effect of maleate and that no demonstrable action could be detected on the translocation of glucose and amino acids. A suggestion supporting the first of these hypotheses was offered by Rosenberg and Segal (16) who found that maleate can penetrate intestinal cells at a rate only 15% that observed in the kidney and that the renal aminoaciduria evident in animals treated with maleate is not due to a competitive type of inhibition with the transport

TABLE II. EFFECT OF 1.5 mmoles/kg OF MALEATE ON THE SMALL INTESTINAL ABSORPTION OF AMINO ACIDS AND GLUCOSE.

| Perfused metabolite | Treatment | |
|---|---|---|
| | 1.5 mmoles/kg of sodium maleate | Saline |
| L-lysine (1 m*M*) | 3.77 ± 0.14[a] (16)[b] | 3.08 ± 0.32 (15) |
| L-phenylalanine (1 m*M*) | 3.08 ± 0.11 (67) | 3.00 ± 0.10 (53) |
| Glycine (1 m*M*) | 4.32 ± 0.28 (16) | 4.06 ± 0.28 (15) |
| α-AIB (1 m*M*) | 3.80 ± 0.17 (32) | 3.47 ± 0.16 (30) |
| Glucose (20 m*M*) | 61.38 ± 3.56 (30) | 62.58 ± 2.38 (26) |
| Glucose (40 m*M*) | 109.78 ± 8.25 (30) | 109.13 ± 5.39 (27) |

[a] Mean ± SEM.

[b] Number of perfusate fractions analyzed. There were no significant differences between treated and control animals.

sites for amino acids, but to energy-yielding intracellular processes required to achieve active amino acid transport. Therefore, even although the intestinal mucosa may be less permeable to maleate, injections of 1.5 or 9.0 mmoles/kg are sufficient to inhibit ($Na^+$-$K^+$)-ATPase of the intestinal mucosa and to produce concomitant alterations in electrolyte transport. However, since sodium has been shown to be involved in glucose and amino acid transport (18, 19), the metabolic disruption produced is insufficient to alter glucose or amino acid intestinal transport *in vivo* during the period of maximum effects of maleate.

Moreover, Curran *et al.* (20) have shown that the entry of amino acids into the intestinal cells is not an energy-dependent process. Our failure to find an effect of maleate on the intestinal absorption of glucose and amino acids is consistent with these observations, since our experiments indicate that only the translocation of electrolytes, mediated by ($Na^+$-$K^+$)-ATPase is altered.

*Summary.* The administration of 1.5 or 9.0 mmoles/kg ip of maleate to rats induced, in addition to renal alterations similar to those occurring in the Fanconi syndrome, a decline in the intestinal mucosa ($Na^+$-$K^+$)-ATPase with a simultaneous decrease in sodium intestinal transport and an increase in potassium absorption. Further differences in the behavior of the two electrolytes were observed when the concentration of sodium in the perfusates was altered. No changes occurred in amino acid or glucose transport in experimental animals.

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