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Alterations in Jejunal Transport and (Na^+–K^+)-ATPase in an Experimental Model of Hypoxia in Rats (42228)

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Abstract. Hypoxia was induced by exposing rats to an atmosphere of 93% N_2, 7% O_2 for 4–48 hr. The animals became hypoxic as indicated by a decreased blood P_aO_2 (mean ± SEM: 48 ± 10 mm Hg). Hypoxia was accompanied by metabolic acidosis (pH 7.22 ± 0.02) and decreased serum bicarbonate levels (9.0 ± 4.0 meq/liter). Hypoxic rats also showed evidence of tissue hypoxia; liver tryptophan oxygenase levels were increased to 21 ± 2 nmole/min/mg protein. In the hypoxic animals there was decreased jejunal mucosal (Na^+–K^+)-ATPase activity and an inhibition of active intestinal transport of sodium, glucose, 3-*O*-methylglucose, galactose, tyrosine, phenylalanine, and glycine as determined by *in vivo* perfusion studies. Jejunal fructose transport, which has a large passive component, was unaffected by hypoxia. The electrolyte, carbohydrate, and amino acid transport alterations produced by hypoxia were seen in the absence of an effect on jejunal cell number, DNA synthesis, or cell turnover. There was also no evidence of histological or ultrastructural damage. Furthermore, studies with a luminal macromolecular tracer, horseradish peroxidase, indicated that the jejunal lumen-to-blood barrier to macromolecules was also unaltered in these hypoxic animals. *In vitro* local oxygenation of the jejunum, by bubbling of 95% O_2:5% CO_2, markedly improved sodium and glucose (but not 3-*O*-methylglucose) absorption in hypoxic rats and control rats. The (Na^+–K^+)-ATPase activity of the jejunal mucosa of hypoxic rats was significantly enhanced by the local bubbling of 95% O_2:5% CO_2. Overall, our data indicate that during relatively mild conditions of hypoxia there is an inhibition of jejunal (Na^+–K^+)-ATPase activity and related transport processes that is prevented by *in situ* oxygenation.

Hypoxia in newborn children, particularly in premature infants, is a severe condition often associated with carbohydrate intolerance and necrotizing enterocolitis (NEC) (1, 2), a complication with a high mortality rate (3, 4). Clinical findings suggest that a relationship may exist between hypoxia, malabsorption, and the damage to the small intestinal mucosa characteristic of NEC (2–6).

In experimental systems, severe changes in oxygen levels, occurring simultaneously with other alterations, adversely affect the structure and reduce the functional transport capacity of the small intestine; this occurs during anoxia (7–9) and under hyperbaric conditions (10). By contrast, fructose and sorbose absorption, which occur principally by passive processes, are unaffected (11). Several factors may underlie the injurious effects of severe hypoxia on the intestinal mucosa. For example, ischemia with reduced intestinal blood flow (9, 12–16), and serum hyperviscosity (17), may produce early changes in the intestinal microcirculation leading to a loss of organization of the mucosal cells (9, 17). Devascularized or clamped intestinal loops lead to ischemia, low intestinal oxygen levels, the destruction of enteric nerve plexi and ganglion cells as well as gross and histologic damage to the intestinal mucosa (18–21). Some of these structural changes can be reversed with local *in situ* oxygen administration (22).

In the present study, we have assessed the effects of mild hypoxia on the (Na^+–K^+)-ATPase activity and sodium, carbohydrate, and amino acid transport of the rat jejunal mucosa. The mechanisms by which hypoxia may alter (Na^+–K^+)-ATPase and related jejunal transport were also investigated. In this model, we have attempted to exclude some of the complications of severe hypoxia or anoxia, and the structural integrity of the intestinal mucosa was preserved.

Materials and Methods. *1. Experimental hypoxia.* Male Wistar rats (Charles River Labs., Crl: (WI) BR, Kingston, N.Y.) with a

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mean ± SEM weight of 62.8 ± 2.9 g were maintained on a standard Purina Lab chow diet with free access to water. They were then studied after exposure to an atmosphere poor in oxygen for various durations of time. The animals were kept in plastic cages within chambers of rigid molded acrylic (36 × 36 × 12 in.), custom made by Plas-Labs., Inc., Michigan. The experimental rats were exposed to 93% N_2:7% O_2 at normal sea level atmospheric pressure, obtained by mixing compressed nitrogen and air, for periods of 4, 24, and 48 hr. Controls were exposed to air (78% N_2) for identical periods. Six animals were used for each group for each exposure period. Gas proportions were maintained with Matheson gas regulators. The gas mixtures were passed through an air trap and a particle filter of hydrated silica gel and administered under positive pressure at a rate of 7 liters/min. An outflow port vented the gases to the atmosphere.

Rats had free access to food and water and could be handled through neoprene gloves without disturbing the internal milieu. In experimental rats the mean food intake was 1.69, 1.88, and 1.05 g on the first, second, and third days. In controls the mean food intake was 6.43, 7.88, and 8.58 g, respectively. Hypoxic rats lost an average of 7.6 g after 24 hr, and an additional 3.5 g after 48 hr. In contrast, control rats gained an average of 5.0 and 4.0 on each of the 2 days. All differences in body weight gain between hypoxic and control groups were significant at the 0.05 level using a nonpaired Student's *t* test.

The degree of blood oxygenation was assessed in samples drawn from the aorta of each rat at the time of sacrifice by determining P_aO_2 with a blood gas analyzer (Corning Model 168). Liver tryptophan oxygenase levels were assayed from liver homogenates prepared at the end of each experiment as described by others (23).

Following the period of exposure to different oxygen tensions, the rats were anesthetized inside the chamber with 1.2 g/kg/body wt of ip urethane. A midline abdominal incision was made to expose the bowel and one of the following two sets of studies described below was performed. In one set of experiments, the jejunum of hypoxic and control rats was assessed for the transport of Na, monosaccharides, and amino acids, and also assayed for ATPase activities of the mucosa as described in detail below. In the second series of studies, we examined the effects of *in vivo* and *in vitro* local oxygenation of the jejunum on the transport properties and ATPase activities of the hypoxic animals.

2. Intestinal enzymes. The jejunal mucosa was carefully scraped from a 20-cm segment with a glass slide, immediately frozen, and stored at −60°C for less than 1 week. For the assay, frozen mucosal specimens from experimental and control rats obtained on the same day were homogenized in 1:4 ice cold 0.25 *M* sucrose, 5 m*M* disodium EDTA, 2.4 m*M* sodium deoxycholate and 38 m*M* Tris–HCl buffer at pH 6.8. ATPase activity was assayed in the presence and absence of ouabain (1 m*M*) to discriminate total, Mg^{2+}, and (Na^+–K^+)-ATPases according to the method of Kramer *et al.* (24). The incubation medium provided as final concentrations 3 m*M* ATP, 3 m*M* Mg^{2+}, 100 m*M* Na^+, 20 m*M* K^+, and 100 m*M* imidazole–HCl buffer at pH 7.2. Lactase, maltase, and sucrase specific activities of the homogenates were determined by Dahlqvist's methods using the corresponding disaccharides at a final concentration of 28 m*M* in a 0.1 *M* maleate buffer at pH 6.0 (25). Protein was determined according to the Lowry method (26).

In another set of experiments, two similar 20-cm segments of jejunum were removed, the mucosa was scraped and homogenized as above and (Na^+–K^+)-ATPase activity assayed as described above at a series of pH values: 5.5, 6.0, 6.5, 6.8, 7.0, 7.35, and 8.0. The pH of the incubation medium was adjusted with NaOH or HCl.

3. Intestinal transport. All intestinal transport studies were done within the enclosed chambers. Surgical procedures and manipulation of the intestine were done by means of neoprene gloves. A 20-cm segment of jejunum distal to the ligament of Treitz was cannulated and perfused *in vivo* following a 60-min equilibration. Body rectal temperature was maintained at 37°C throughout the study. Perfusion was done at a rate of 0.18–0.20 ml/min with a Krebs–Henseleit–Ringer bicarbonate buffer containing 118.5 m*M* NaCl, 4.7 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, 24.8 m*M* $NaHCO_3$. One of the fol-

lowing was added to the buffer: 4 m*M* of either glucose, 3-*O*-methylglucose, galactose, or fructose; and 1 m*M* phenylalanine, tyrosine, glycine, or lysine. Trace amounts of ^{14}C and ^{3}H-labeled carbohydrates and amino acids sufficient to provide 10,000 cpm/ml were included in the perfusion solution. Eight, 15-min fractions were collected from each perfused animal. A more extensive description of the intestinal perfusion procedure has already been published (28). The concentrations of the solutes in each fraction were determined by liquid scintillation isotope dilution estimation (Beckman LS-230). The sodium concentration was determined in perfusates by flame photometry (IL Model 143). Polyethylene glycol, mol wt 3000–3700 (PEG), was used as a nonabsorbable marker for net water exchanges. The actual pumping rate and volume of each sample collected were used to calculate the PEG recovery following passage through the gut lumen (27). The net absorption of solutes was determined from the amount disappearing from the perfused solution. The amount of PEG recovered in perfusates did not differ statistically from 100% in both hypoxic and control rats.

4. Intestinal DNA synthesis and turnover. DNA synthesis and turnover were studied by determining the incorporation rate of [^{3}H]thymidine (sp act, 45 Ci/mmole, New England Nuclear, Boston, Mass.), following ip injection at 1 μCi/g body wt. Animals were anesthetized with ip nembutal, the gut excised, jejunal mucosa obtained as described above, and homogenized 1:5 with 2% citric acid. DNA was extracted according to Schneider (29). [^{3}H]Thymidine labeled DNA was counted by liquid scintillation 4, 24, and 48 hr after ip injection of the tritiated compound. Jejunal mucosa was obtained as described above and the total DNA, expressed as milligram per gram mucosa and as 10^{6} nuclei/g mucosa, was determined following extraction of the material (29).

5. Structure and ultrastructure of intestinal mucosa. Jejunal segments were fixed in 2.5% glutaraldehyde buffered with 0.1 *M* cacodylate at pH 7.3 and prepared for light and electron microscope study, as described elsewhere (5, 34). Jejunal fragments were oriented during embedding in flat molds so that sections cut through all layers of the intestine, from serosal to mucosal surface. Epon "thick" sections were cut on a Porter–Blum MT2-B ultramicrotome, stained with toluidine blue, and examined by light microscopy. Thin sections were cut and examined on a JEOL JEM-100 electron microscope. Histologic analysis of Epon thick sections was carried out in coded "blind" experiments during which the investigators were unaware of the physiological treatment to which the material was subjected. A total of six hypoxic and six control rats were studied histologically. Twelve to 24 villi per animal were analyzed. Villous height was measured in a blind fashion using a micrometer inserted in the eyepiece of a standard Nikon light microscope. The scores were then assessed for statistical significance with a χ^2 test and the code was then broken. None of the results were accepted as valid unless repeatable in three consecutive experiments.

To assess the effect of low oxygen tension on the barrier integrity of the jejunal mucosa, in an additional set of morphological studies, the jejunum of experimental and control rats was perfused for 60 min with an isotonic Krebs–Henseleit–Ringer buffer to which we added 0.5 g% horseradish peroxidase (HRP) a 40,000 mol wt glycoprotein tracer as extensively detailed elsewhere (30, 31). After perfusion, a 1-cm fragment of jejunum in the center of the perfused segment was fixed in cold 0.1 *M* cacodylate buffered (pH 7.3) 2.5% glutaraldehyde for 60 min, and then rinsed several times and overnight in cold 0.1 *M* cacodylate buffered 7% sucrose. Slabs of intestine were then rinsed in cold 7.5% sucrose, frozen on the head of a freezing microtome, rinsed in buffer, and then incubated at room temperature for the localization of peroxidase activity (14). The intestinal tissue fragment was then postfixed in osmium tetroxide and prepared for electron microscopy by standard procedures (30, 31).

6. In situ oxygenation: ATPases and transport. While under exposure to hypoxic or control atmospheres for 4 hr as described above, gas mixtures were bubbled directly into the jejunum at a rate of 1 liter/min for the same period. The gas mixtures were 95% O_2:5% CO_2, 7% O_2:93% N_2. In a third group of both control and hypoxic animals, no gas was bubbled. In these *in situ* studies, the outflow port of the intestinal segment exposed to the gases

was 20 cm distal to the port of entry which was at the ligament of Treitz. Tissues were kept moist during the bubbling of gases, with pads soaked with isotonic saline. After *in situ* oxygenation, the jejunal mucosa was scraped and assayed for ATPases as described above.

The effects of *in situ* oxygenation on jejunal transport capacity was studied by perfusing Krebs–Henseleit–Ringer buffer containing either glucose, 3-*O*-methylglucose and 1 m*M* phenylalanine under conditions described in section 3, while continuously bubbling the solution with one of the above gases.

Results. *1. Hypoxia assessment.* Blood P_aO_2 levels were significantly less in the experimental group than in controls. This decrease in blood P_aO_2 was apparent at 4 hr and persisted throughout the entire 48-hr experimental period (Table I). After 48 hr of exposure to a low oxygen atmosphere, simultaneous with hypoxia, there was a partially compensated metabolic acidosis as indicated by decreased serum bicarbonate and a slightly depressed serum pH. In addition, there was evidence of tissue hypoxia as indicated by the elevated hepatic tryptophan oxygenase activity, a well recognized marker for hypoxia (23). The control rats appeared to be slightly hypercapnic; the calculated P_aCO_2 in the rats was approximately 53 mm Hg, but the controls were not hypoxic and had a normal blood pH. In all instances the P_aO_2 and tryptophan oxygenase levels of the rats did not change as a result of *in situ* oxygenation (Table I).

TABLE I. THE EFFECTS OF HYPOXIA ON HEPATIC ENZYMES AND BLOOD GASES

	Hypoxic	Control	*P*
P_aO_2 (mm Hg)	48 ± 10[a]	115 ± 7	<0.01
HCO_3 (meq/l)	9.0 ± 4.0	31.0 ± 0.4	<0.01
pH	7.22 ± 0.02	7.38 ± 0.02	<0.01
Tryptophan oxygenase (nmol/min/mg protein)	21 ± 2	11 ± 1	<0.01

Note. Data shown are derived from hypoxic rats exposed to 93% N_2:7% O_2 atmosphere for 48 hr. Control rats were exposed to compressed air with 78% N_2:21% O_2. The tryptophan oxygenase levels were determined in the liver, and blood gases were measured in blood obtained from the abdominal aorta. The P_aO_2 levels of hypoxic and control rats given *in situ* oxygen for 4 hr did not change from those seen above. The tryptophan oxygenase level in the 4 hr hypoxic rat was 8.2 ± 2.0 vs control 4.3 ± 1.5 nmol/min/mg) protein ($P < 0.05$), which is lower than that seen in the 48 hr hypoxic animals. *In situ* oxygenation did not change the hypoxic effect on tryptophan oxygenase (7.0 ± 0.5 in hypoxic rats and 4.2 ± 0.2 in controls $p < 0.05$).

[a] Means ± SEM, $n = 6$ rats/group.

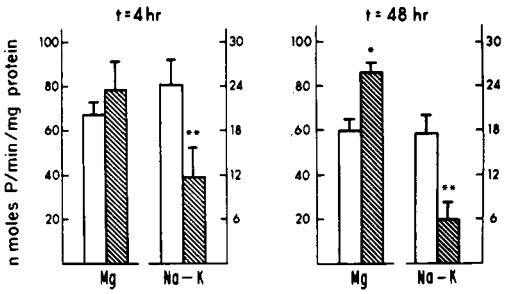

FIG. 1. (Mg^{2+})-ATPase (Mg), and (Na^+–K^+)-activated ATPase (Na–K) in rat jejunal mucosa after 4 and 48 hr of experimental hypoxia. (Na^+–K^+)-ATPase levels were depressed after 4 and 48 hr of hypoxia (▨) as compared with nonhypoxic controls (□). (Mg^{2+})-ATPase activity was higher in hypoxic rats after 48 hr. $n = 6$ rats/group. The bars indicate means ± SEM; $^{**}P < 0.01$.

2. Jejunal mucosal ATPase activity in vivo. Hypoxia of 4-hr duration significantly decreased jejunal mucosal (Na^+–K^+)-ATPase activity. After 48 hr of continuous hypoxia, jejunal (Na^+–K^+)-ATPase activity remained markedly depressed. In contrast, after 4 hr (Mg^{2+})-ATPase was not affected and the enzymatic activity of (Mg^{2+})-ATPase was indistinguishable in hypoxic and controls (Fig. 1). The difference in (Mg^{2+})-ATPase between hypoxic and control animals at 48 hr was significant. However, this appeared to be due to a small decrease in mean control values and a small increment in mean hypoxic values.

When the jejunum was oxygenated locally *in vivo* with either 7% O_2:93% N_2 or with 95% O_2:5% CO_2 the (Na^+–K^+)-ATPase was markedly increased in the hypoxic rats, while there was no effect in controls (Fig. 2). (Mg^{2+})-ATPase was unaffected by *in situ* oxygenation in both hypoxic and control animals.

3. Effect of pH on (Na^+–K^+)-ATPase of the mucosa in vitro. Jejunal (Na^+–K^+)-ATPase activity was sensitive to variation in pH (Fig. 3). The pH optimum for maximal activity of (Na^+–K^+)-ATPase was close to normal physiological conditions. Marked shifts to acidosis or alkalosis were associated with marked inhibition of the enzyme: A drop of mucosal pH to 6.8 induced about a 50% decrease in the

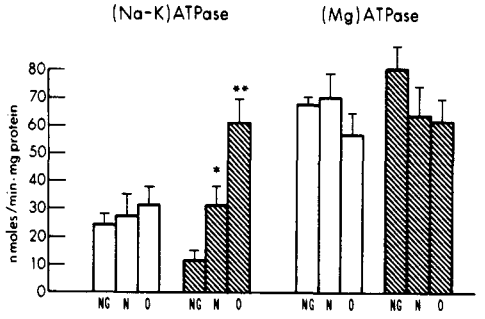

FIG. 2. Effect of *in situ* oxygenation on rat jejunal ATPase of hypoxic (▨) and control (□) rats. NG, no bubbling of any gas; N, bubbling of the hypoxic chamber gas mixture containing 7% oxygen and 93% nitrogen; O, bubbling with 95% oxygen, 5% carbon dioxide. (Na^+-K^+)-ATPase of jejunal mucosa assayed after 4 hr of hypoxia as in Fig. 1. Note that oxygenation (0) had no effect on the (Na^+-K^+)-ATPase or (Mg^+)ATPase activity in controls. In hypoxia rats the (Na^+-K^+)-ATPase of the (0) group was greater than the NG ($P < 0.01$) and N ($P < .05$) animals. N and NG (Na^+-K^+)-ATPase levels were not statistically different. *In situ* oxygenation had no effect, on the (Mg^{2+})-ATPase of hypoxic animals. The bars indicate means ± SEM. $n = 6$ rats/group; $^{**}P < 0.01$; $P < .05$.

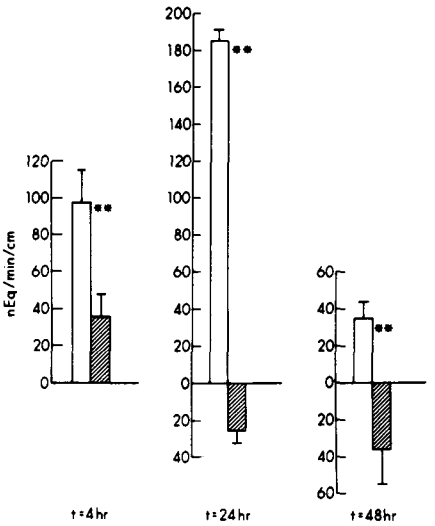

FIG. 4. Jejunal sodium transport in rats maintained for either 4, 24, or 48 hr under hypoxic (▨) or normal (□) conditions. Note the continual alterations in sodium fluxes in hypoxic rats, from malabsorption after 4 hr to secretion after 48 hr of exposure. Means ± SEM, $n = 6$ rats/group, $^{**}P < 0.01$.

hydrolytic activity of the enzyme as compared with optimum conditions. Maximum activity was observed at pH 7.35.

4. Sodium, carbohydrate, and amino acid transport. The hypoxia induced reduction in (Na^+-K^+)-ATPase was paralleled by changes in jejunal sodium transport (Fig. 4). After 4 hr of hypoxia net sodium absorption was markedly decreased; after 24 hr of hypoxia sodium uptake was abolished and by 48 hr of hypoxia net sodium secretion occurred.

Hypoxia also impaired the jejunal transport capacity for glucose, 3-*O*-methylglucose, a nonmetabolizable analog of glucose, and galactose, when each was perfused at 4 m*M* concentrations (Fig. 5). However, protracted hypoxia had no effect on the transport of fructose, a monosaccharide which is absorbed by mediated, as well as by nonmediated mechanisms.

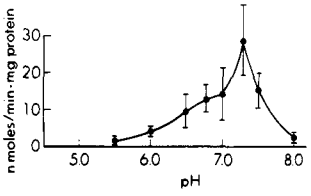

FIG. 3. Effect of pH changes *in vitro* on rat jejunal (Na^+-K^+)-ATPase specific activity. The pH optimum is at 7.35. Shifts in pH toward acidosis or alkalosis markedly inhibit enzyme activity. The points of the graph represent means ± SEM. $n = 6$ rats.

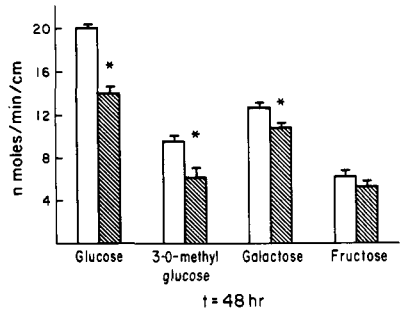

FIG. 5. The effect of 48 hr of hypoxia on carbohydrate transport in perfused rat jejunal segments. Hypoxic animals show a decreased absorption of glucose, 3-*O*-methylglucose and galactose. Fructose transport, which occurs largely by passive mechanisms, was unaffected by 48 hr of hypoxia. All carbohydrates were perfused at 4 m*M* concentration. (▨), Hypoxic; (□), control. Means ± SEM, $n = 6$ rats/group, $^*P < 0.05$.

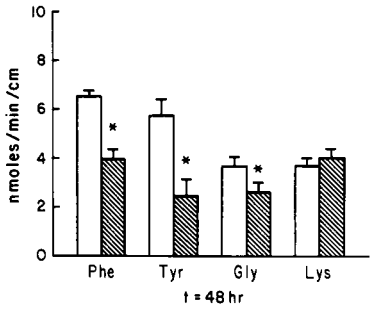

FIG. 6. Amino acid transport in rat jejunal mucosa following 48 hr of hypoxia. The transport of neutral amino acids, phenylalanine (Phe), and tyrosine (Tyr), and of glycine (Gly), were all decreased in the hypoxic (▨) animals as compared with controls (□). The transport of lysine (Lys), a basic amino acid was unaffected. All amino acids were perfused at a 1 m*M* concentration. Means ± SEM, $n = 6$ rats, $^*P < 0.05$.

Exposure to hypoxic conditions for 48 hr also led to a decrease in the jejunal absorption capacity for several neutral, actively transported amino acids including phenylalanine, tyrosine, and glycine (Fig. 6). The transport of a dibasic amino acid, lysine, was, however, unaffected under identical experimental conditions.

Perfusion of the jejunum with an oxygenated buffer solution improved sodium transport in hypoxic as well as control rats (Fig. 7). The uptake of glucose by the jejunum was also enhanced with the oxygenated buffer, but only in the hypoxic animals. However, the transport of 3-*O*-methylglucose in hypoxic rats was not enhanced by *in situ* oxygenation. It may be that the increased glucose removal from the intestinal lumen during perfusion with the oxygenated solution is at least in part due to a greater glucose utilization by the mucosa, rather than an enhanced absorption.

5. Histology and ultrastructure. The alterations in jejunal transport and ATPase were seen without marked alterations in the histology or fine structure of the mucosal epithelium. Mitochondria of epithelial cells from hypoxic rats were normal in appearance and indistinguishable from controls. The microvillar brush border was also unremarkable in hypoxic rats (Fig. 8). Although a number of platelets were seen in the capillaries of the lamina propria of hypoxic rats, no evidence of platelet aggregates or thrombi was apparent as has been reported under more severe conditions (7, 9). After 48 hr of hypoxia, there were no differences apparent in villus height between hypoxic and control animals.

In hypoxic animals as well as controls, cytochemical localization by light and electron microscopy showed that HRP was restricted to the microvillar brush border and was not seen penetrating between the epithelial cells.

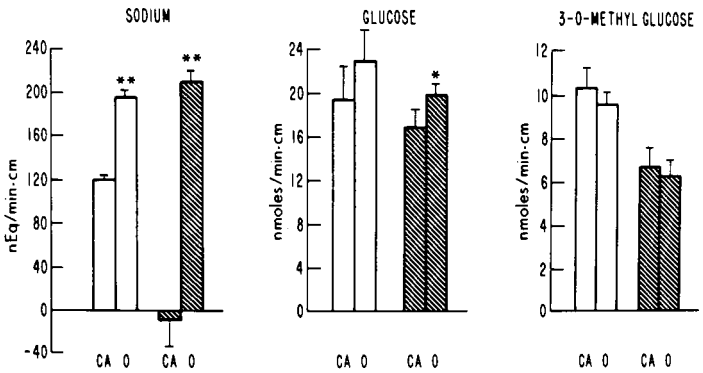

FIG. 7. Effects of *in vivo* local oxygenation on the jejunal transport of sodium, glucose, and 3-*O*-methylglucose in hypoxia. CA is chamber atmosphere; normal air in controls (□), 7% oxygen:93% nirogen in hypoxic (▨) animals. O is 95% oxygen;5% carbon dioxide. Gases were bubbled during perfusion carried out as in Figs. 4 and 5. Note that local oxygenation markedly enhanced jejunal sodium transport in hypoxic and control animals. Although glucose transport was also enhanced in the hypoxic group, there was no effect of oxygenation on the transport of 3-*O*-methylglucose. $n = 6$ rats/group, $^*P < 0.05$; $^{**}P < 0.01$. Means ± SEM.

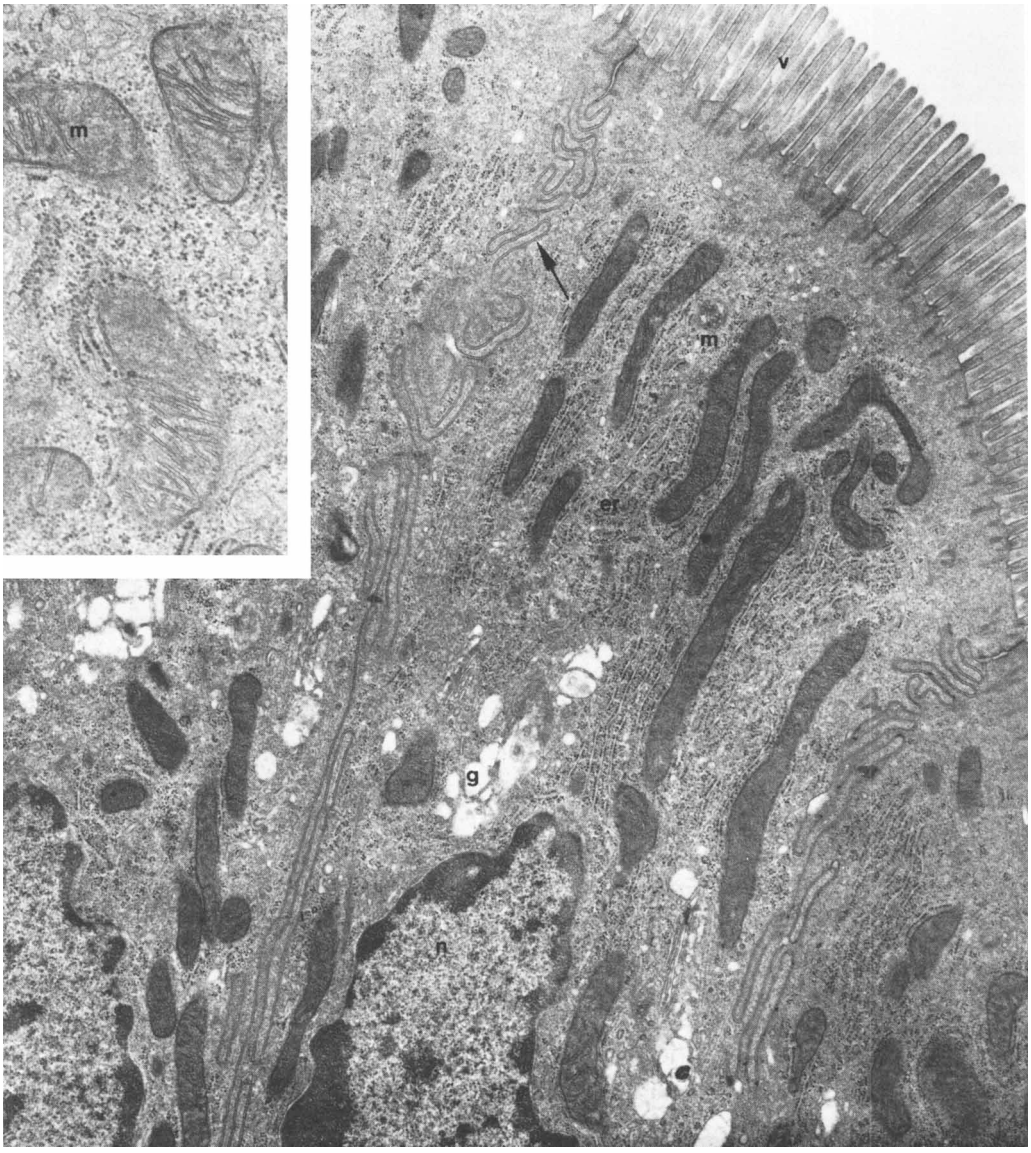

FIG. 8. Typical absorptive epithelial cells from the jejunum of a 48 hr hypoxic rat. In general, the organelles incuding mitochondria (m), microvilli (v), endoplasmic reticulum (er), Golgi apparatus (g), are unremarkable and normal in appearance. Intercellular space between adjacent cells is at arrow (×9840). The inset shows a higher magnification view of several mitochondrial swelling or structural abnormality is seen (×18,450).

Therefore, the integrity of the intestinal macromolecular barrier was apparently not affected by hypoxia.

6. *Intestinal disaccharidases.* Jejunal disaccharidase activities were also not affected by 48 hr of hypoxia; lactase, sucrase, maltase, and alkaline phosphatase were indistinguishable in hypoxic and control rats (Table II).

7. *DNA content and synthesis.* Mucosal DNA content and DNA synthesis were unaffected by hypoxia. DNA content in hypoxic and control rats was not statistically different

TABLE II. INTESTINAL DISACCHARIDASES, DNA CONTENT, AND [^{3}H]THYMIDINE INCORPORATION INTO MUCOSAL DNA OF HYPOXIC AND CONTROL RATS

	Hypoxic[a]	Controls[a]	P
Disaccharidases			
Lactase (IU/mg protein)	0.00250 ± 0.0004	0.00220 ± 0.0003	NS
Sucrase	0.0202 ± 0.0026	0.0167 ± 0.0017	NS
Maltase	0.5431 ± 0.0908	0.3467 ± 0.0686	NS
DNA mg/g mucosa	2.43 ± 0.14	2.30 ± 0.23	NS
Nuclei ($\times 10^{-6}$/g mucosa)	390 ± 28	382 ± 30	NS
[^{3}H]Thymidine incorporation (dpm/mg DNA $\times 10^{-3}$)			
4 hr	65 ± 7	53 ± 8	NS
24 hr	55 ± 4	54 ± 15	NS
48 hr	36 ± 6	26 ± 5	NS

Note. Hypoxia did not induce a change in mucosal disaccharides, mucosal DNA, nuclei, or incorporation of [^{3}H]thymidine into DNA.

[a] Means ± SEM, n = 6 rats/group.

(Table II). The calculated number of nuclei per gram of mucosa was also similar. The incorporation of [^{3}H]thymidine into mucosal DNA was also not significantly affected by hypoxia; the level of [^{3}H]thymidine containing DNA at 4, 24, and 48 hr was similar in hypoxic and control rats (Table II).

Discussion. In the present experimental model of hypoxia, induced by exposure to a low oxygen tension atmosphere, the animals developed not only a decreased P_aO_2, but also changes in serum bicarbonate levels with metabolic acidosis, all of which could play a potential role in the transport and enzyme alterations reported here.

Our data indicate that in this model of hypoxia there is rapid inhibition of intestinal active transport processes linked to the (Na^+–K^+)-ATPase; thus, the absorption of actively transported solutes such as sodium, glucose, and amino acids was decreased during hypoxia along with a depression in (Na^+–K^+)-ATPase activity, while the absorption of a largely passively transported monosaccharide, fructose, was not affected. These hypoxia-induced alterations in active solute absorption occur in the absence of marked histologic or ultrastructural damage to the intestinal epithelium, strongly suggesting that the transport alterations are not the result of an immediate cytotoxic effect of lowered P_aO_2 or other factors on mucosal integrity. Furthermore, good PEG recovery and the observation that the integrity of the mucosal barrier to macromolecular penetration was not altered by hypoxia lend support to the view that the changes in solute absorption concern transepithelial, membrane localized active transport mechanisms linked to the hydrolysis of ATP, and are probably not the result of a gross permeability change. It is of note that enterocyte mitochondria also show no signs of morphologic change induced by hypoxia.

The transport (Na^+–K^+)-ATPase alterations reported here also occurred in the absence of any artificial, surgically induced, ischemia and/or severe anoxia. The hypoxia levels in the present study were relatively mild and tolerated for extended periods. Nevertheless, it is clear that the pathophysiological link between hypoxia and reduction of active intestinal transport processes could be complex.

The decreases in active solute transport and jejunal Na^+–K^+-ATPase may either be due to acidosis (33) or to a direct effect of O_2 pressure on (Na^+–K^+)-ATPase activity (13), which has been shown under hyperbaric conditions. Alternatively, hypoxia may lead to a deficiency in energy-dependent protein synthesis, or to local low P_aO_2-induced ischemia (38).

A potentially important factor in the alteration of (Na^+–K^+)-ATPase levels concerns the acidosis associated with hypoxia, as shown by the low blood pH and bicarbonate levels. In the present study we demonstrated that the intestinal (Na^+–K^+)-ATPase activity ranges between broad limits according to the treatment of the small intestine during the experiments (Figs. 1 and 2). We also showed that this enzyme is pH dependent; the optimal pH being in the physiological range with severe inhibition occurring at lower pH. A similar pH optimum, that is also related to ionic conditions, has been found in brain tissue (34). In our study, decreased sodium absorption could lead to suppression of coupled proton exchange, resulting in decreased intracellular pH (35). In this light we have earlier reported a model of maleate-induced experimental Fanconi syndrome characterized by acidosis due to bicarbonate loss in which there was also

inhibition of (Na^+-K^+)-ATPase activity, with a reduction of Na^+ transport (33).

Other investigators have shown that experimentally induced metabolic acidosis does not alter sodium transport of the jejunum, although other intestinal segments are affected (36). These studies did not evaluate (Na^+-K^+)-ATPase levels and they were acute, not extending beyond 4 hr, while the maximal sodium transport derangement reported in the present study occurred after 48 hr. We did not evaluate whether our hypoxic animals had lactic acid acidosis, which might be expected under conditions of hypoxia (37).

Others have found that O_2, under nonphysiological high pressure, can activate ATPase activities (10, 38). However, these reports concerned a combination of cation-activated total ATPases. As we have shown in the present study, altered O_2 pressures may affect (Na^+-K^+)-ATPase. In contrast there was no effect on (Mg^{2+})-ATPase.

Local oxygenation markedly enhanced both (Na^+-K^+)-ATPase activity as well as sodium transport in hypoxic rats. This occurred in the presence of systemic, metabolic acidosis and continuous hypoxia, suggesting that localized supply of O_2 may enhance the overall metabolic activity of jejunal cells. This contention is supported by the apparent increased utilization of glucose, reflected in its enhanced uptake during tissue oxygenation. The differing CO_2 levels in the bubbled gases appeared not to impair the stimulatory effect of O_2. The reason for enhanced (Na^+-K^+)-ATPase activity in the hypoxic rats is not clear. However, it could turn out that local oxygenation affects the turnover of the enzyme, its activation, or leads to an "unmasking" process that occurs to a greater extent in the hypoxic animal.

Although the notion that (Na^+-K^+)-ATPase activity was decreased because of a general inhibition of protein synthesis seems attractive, some data argue against this view. For example, (Mg^{2+})-ATPase activity was elevated even after 48 hr of hypoxia. Also, hypoxia had no effect on DNA synthesis, indicating that the enzymes involved in the nucleotide salvage pathway were intact, and disaccharidases were unaffected by hypoxia of 48 hr. Our data, however, do not rule out the possibility that (Na^+-K^+)-ATPase is an enzyme with comparatively very rapid turnover and hence may be affected to a greater extent than other catalytic proteins.

The possible effects of ischemia during hypoxia have been extensively reviewed (9, 14). An inadequate perfusion of the intestine due to experimental asphyxia with acidosis in ducks produced a 20–40% drop in intestinal regional blood flow (13). In piglets, there was a 35% reduction in local intestinal blood flow (9). In ultrastructural studies of asphyxiated animals there were changes in the microcirculation of the gut with damaged mucosal cells, platelet aggregates, thrombi, and blistering of the brush border (7). By contrast, none of these changes in vasculature or cell structure was found in the present study where hypoxia was milder. This notion is supported by the lack of obvious histological or ultrastructural damage and the integrity of the macromolecular barrier of the intestine as determined by horseradish peroxidase studies as well as our PEG recoveries and fructose transport. Under more severe conditions of cell damage, as seen with deconjugated bile salts or hyperosmosis, there is a loss of the integrity of the jejunal macromolecular barrier (30, 31).

Although the hypoxic rats failed to gain weight in the 48 hr of study, we do not believe the changes in transport and (Na^+-K^+)-ATPase are due to malnutrition of the animals. We previously demonstrated that sodium dependent intestinal transport of carbohydrates and amino acids is not decreased by even prolonged malnutrition in rats (28, 39). Furthermore, recent evidence from our own studies indicates that (Na^+-K^+)-ATPase is also not affected by protein-energy malnutrition in rats (40).

The intestinal effects of hypoxia in the present experimental model may bear some relation to clinical conditions that have been associated with pneumatosis intestinalis (2, 41), and necrotizing enterocolitis (NEC). Newborn preterm infants with hypoxia and acidosis due to perinatal distress, hyaline membrane disease and/or other conditions (2–6) are among those at risk for NEC. In these newborns a possible relationship between monosaccharide intolerance, carbohydrate malabsorption, and neonatal hypoxia has been described (1, 2, 42, 43). Under clinical conditions, a combination

of asphyxia, acidosis, ischemia, and shock may be seen. These alterations may be major contributing factors to intestinal injury and the development of NEC. The present observations suggest that one effect of hypoxia, inhibition of active carbohydrate transport, may increase the concentration of available fermentative substrates for intestinal microflora (41). Malabsorbed carbohydrate is a potential substrate for the generation of the gas under pressure needed for the development of pneumatosis intestinalis and NEC (2, 3).

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