

# Correction of Enhanced $\text{Na}^+\text{-H}^+$ Exchange of Rat Small Intestinal Brush-Border Membranes in Streptozotocin-induced Diabetes by Insulin or 1,25-Dihydroxycholecalciferol

Pradeep K. Dudeja, Ramesh K. Wali, Alan Klitzke, Michael D. Sitrin, and Thomas A. Brasitus  
Department of Medicine, University of Chicago, Chicago, Illinois 60637

## Abstract

Diabetes was induced in rats by administration of a single i.p. injection of streptozotocin (50 mg/kg body wt). After 7 d, diabetic rats were further treated with insulin or 1,25-dihydroxycholecalciferol [ $1,25(\text{OH})_2\text{D}_3$ ] for an additional 5–7 d. Control, diabetic, diabetic + insulin, and diabetic +  $1,25(\text{OH})_2\text{D}_3$  rats were then killed, their proximal small intestines were removed, and villus-tip epithelial cells were isolated and used to prepare brush-border membrane vesicles. Preparations from each of these groups were then analyzed and compared with respect to their amiloride-sensitive, electroneutral  $\text{Na}^+\text{-H}^+$  exchange activity, using  $^{22}\text{Na}$  uptake as well as acridine orange techniques.

The results of these experiments demonstrated that (a)  $\text{H}^+$  gradient-dependent  $^{22}\text{Na}$  uptake as well as  $\text{Na}^+$  gradient-dependent transmembrane  $\text{H}^+$  fluxes were significantly increased in diabetic vesicles compared to their control counterparts, (b) kinetic studies demonstrated that this enhanced  $^{22}\text{Na}$  uptake in diabetes was a result of increased maximal velocity ( $V_{\max}$ ) of this exchanger with no change in apparent affinity ( $K_m$ ) for  $\text{Na}^+$ , (c) serum levels of  $1,25(\text{OH})_2\text{D}_3$  were significantly lower in diabetic animals compared with their control counterparts; and (d) insulin or  $1,25(\text{OH})_2\text{D}_3$  treatment restored the  $V_{\max}$  alterations to control values, without any significant changes in  $K_m$ , concomitant with significantly increasing the serum levels of  $1,25(\text{OH})_2\text{D}_3$  in diabetic animals. These results indicate that  $\text{Na}^+\text{-H}^+$  activity is significantly increased in proximal small intestinal luminal membranes of streptozotocin-induced diabetic rats. Moreover, alterations in the serum levels of  $1,25(\text{OH})_2\text{D}_3$  may, at least in part, explain this enhanced antiporter activity and its correction by insulin. (*J. Clin. Invest.* 1991; 87:1755–1762.) Key words: antiporter • diabetes mellitus • sodium-hydrogen ion exchange • streptozotocin • vitamin D

## Introduction

Chemically induced diabetes mellitus has been shown to be associated with a number of structural abnormalities in the mammalian small intestine, such as hypertrophy (1), as well as several functional alterations, including enhancement of the uptake of hexoses (2), various amino acids (3), bile salts (4), as

well as sodium (5, 6). Insulin therapy, moreover, has been reported to correct many of these intestinal alterations associated with diabetes, including sodium absorption (5, 6). Although it has been postulated that this increased sodium absorption observed in diabetes may be an adaptive response to excess loss of urinary sodium as a result of osmotic diuresis produced by glycosuria (6), to date, the mechanism(s) underlying this intestinal phenomenon in this pathological state remain(s) enigmatic.

In this regard, recent studies in renal proximal tubular brush-border membrane vesicles isolated from streptozotocin-induced diabetic rats have demonstrated an increase in electroneutral amiloride-sensitive,  $\text{Na}^+\text{-H}^+$  exchange activity in these preparations compared to their control counterparts, which was partially corrected by insulin administration (7, 8). Since this ubiquitous transporter(s) has been implicated in a number of diverse physiological processes, including cellular proliferation (reviewed in reference 9), these authors suggested that this enhanced  $\text{Na}^+\text{-H}^+$  exchange might underlie known complications of the diabetic state such as renal hypertrophy (7, 8).

This carrier-mediated exchange process has also been shown to exist in the apical membranes of the mammalian small and large intestine and plays an important role in electroneutral sodium absorption in these organs (10–16). In the small intestine, this exchanger has been clearly documented in the apical membranes of the villus cells, however, its presence on the apical membranes of crypt cells is uncertain (17). Recent studies in both the large and small intestine have demonstrated that the activity of this antiporter is subject to hormonal regulation (18–21). For example, apical membranes isolated from colonocytes of rats treated with dexamethasone (18) exhibited increased  $\text{Na}^+\text{-H}^+$  exchange activity, whereas, estradiol treatment decreased this exchange process (19). Additionally, previous studies in our laboratory (20) and others (21) have demonstrated modulation of  $\text{Na}^+\text{-H}^+$  exchange by  $1,25\text{-dihydroxycholecalciferol}$  [ $1,25(\text{OH})_2\text{D}_3$ ] in CaCo-2 and chick small intestinal cells, respectively. These latter findings are particularly interesting in view of earlier studies (22), which documented a decrease in the serum levels of  $1,25(\text{OH})_2\text{D}_3$  in animals administered streptozotocin, secondary to decreased renal 1-hydroxylation. Insulin therapy, moreover, has been shown to correct these alterations in  $1,25(\text{OH})_2\text{D}_3$  in diabetes (22).

Based on the above observations, in the present experiments it was, therefore, of interest to determine whether  $\text{Na}^+\text{-H}^+$  exchange activity was altered in brush-border membrane vesicles prepared from the proximal intestinal villus cells of streptozotocin-induced diabetic rats as well as to explore the possible factor(s) responsible for such alterations, including the vitamin D status of these animals. The present results as well as a discussion of their physiological relevance in this experimen-

Address reprint requests to Dr. Brasitus, Section of Gastroenterology, Department of Medicine, University of Chicago, Box 400, 5841 S. Maryland Avenue, Chicago, IL 60637.

Received for publication 25 June 1990 and in revised form 11 October 1990.

*J. Clin. Invest.*

© The American Society for Clinical Investigation, Inc.

0021-9738/91/05/1755/08 \$2.00

Volume 87, May 1991, 1755–1762

tal model of diabetes mellitus serve as the basis for the present report.

## Methods

**Materials.** 1,25(OH)<sub>2</sub>D<sub>3</sub> was kindly provided by Dr. M. R. Uskokovic, Hoffman-LaRoche, Inc., Nutley, NJ. A 1,25(OH)<sub>2</sub>D<sub>3</sub> radioreceptor assay kit was acquired from Incstar Corp., Stillwater, MN. Streptozotocin (2-deoxy-2-[[[methyl-nitrosoamino]carbonyl]amino]-D-glucopyranose), *N*-methyl-D-glucamine, D-gluconic acid lactone, sodium gluconate, acridine orange, valinomycin, nigericin, and amiloride hydrochloride were from Sigma Chemical Co., St. Louis, MO. Stock solutions of *N*-methylglucamine gluconate (N-MGG)<sup>1</sup> were made by titrating 1 M *N*-methyl-D-glucamine with solid D-gluconic acid lactone. Iletin II purified pork insulin (500 U/ml) was a generous gift from Eli Lilly & Co., Indianapolis, IN. Mini-osmotic pumps (model 2001) were obtained from Alzet Corp., Palo Alto, CA. [<sup>3</sup>H]Thymidine (20 Ci/mmol) and <sup>22</sup>Na (24.1 Ci/mmol) were obtained from New England Nuclear, Boston, MA. All other materials were obtained from Sigma Chemical Co. or Fisher Scientific Co., Springfield, NJ, and were of the highest purity available.

**Induction of experimental diabetes and administration of insulin and 1,25(OH)<sub>2</sub>D<sub>3</sub>.** Male Lewis rats (250–300 g body wt; Charles River Breeding Laboratories, Inc., Wilmington, MA) were divided into two groups. Diabetes mellitus was induced in one of the groups by the single intraperitoneal injection of streptozotocin (50 mg/kg body wt in 0.9% NaCl), while the control group received the vehicle alone. The diabetic rats were further subdivided into insulin-treated, 1,25(OH)<sub>2</sub>D<sub>3</sub>-treated, or nontreated groups. Insulin-treated diabetic rats received 4–6 U per day of Iletin II pork insulin by subcutaneously implanted osmotic mini-pumps for 5–7 d beginning 7 d after streptozotocin administration (23). 1,25(OH)<sub>2</sub>D<sub>3</sub>-treated diabetic rats were injected subcutaneously with calcitriol (dissolved in 5% ethanol in propanediol) at a daily dose of 10 ng/100 g body wt for 5–7 d, beginning 7 days after streptozotocin administration. Previous studies from our laboratory have shown that this dose was physiologic as it did not overcorrect Ca and P absorption in vitamin D-deprived rats (24). The streptozotocin-treated rats were periodically analyzed for glycosuria and were eliminated from the study if they did not show at least 1% glycosuria at all times. Tail vein blood was used to monitor glucose levels using the Glucoscan 2000 (Lifescan Inc., Mountain View, CA). Only those insulin-treated rats in which the blood glucose levels stabilized at ~140±20 (mg/dl) for at least 3 d before sacrifice were used in the present experiments. Serum 1,25(OH)<sub>2</sub>D<sub>3</sub> levels were assayed by a nonequilibrium competitive protein-binding assay based on a thymus receptor, using a kit and procedure supplied by Incstar Corp.

**Isolation of villus enterocytes.** Control, diabetic, insulin-treated diabetic, and 1,25(OH)<sub>2</sub>D<sub>3</sub>-treated diabetic rats were fasted for 16 h with water ad lib. before sacrifice. Animals were killed by rapid cervical dislocation, the proximal half of their small intestines was quickly removed, their wet weights were determined, and nine fractions of enterocytes along the crypt-villus axis were sequentially harvested using a technique which combines chelation of divalent cations with mild mechanical dissociation as previously described (25, 26). For each preparation two or three animals in each group were used. Fractions 1–3, representing villus-tip cells were pooled for these experiments. Preparations from each of the different experimental groups were comparable with respect to sucrase specific activity and [<sup>3</sup>H]thymidine incorporation into DNA, indicating similar populations of mature enterocytes, as previously described (26).

**Brush-border membrane preparation.** Brush-border membranes were prepared from villus-tip cells of all four groups by using 10 mM MgCl<sub>2</sub>, instead of 10 mM CaCl<sub>2</sub>, as the precipitating agent as previously described (27). Purity and comparability of the preparation

as well as contamination by microsomal, mitochondrial, and basolateral membranes were assessed by using appropriate marker enzymes (27). All brush-border membrane preparations were equally purified 15–20-fold compared with the original homogenates, using sucrase and alkaline phosphatase (*p*-nitrophenylphosphatase) as marker enzymes, and showed minimal contamination by other membranes as previously described (27). Protein was measured by the method of Lowry et al. (28), with bovine serum albumin as standard. All enzymatic activities were measured under conditions that were linear with respect to time and protein (27).

**<sup>22</sup>Na uptake experiments.** Brush-border membrane vesicles from each group were prepared and the final pellet was resuspended and washed twice as previously described (29). The first buffer consisted of 300 mM mannitol, 5 mM Tris-Hepes (pH 7.5). In experiments, in which the intravesicular pH was 7.5, a second washing with 144 mM KCl, 5 mM 2-(*N*-morpholino) ethane sulfonic acid (MES), 13 mM Tris, and 13 mM Hepes (pH 7.5) was used and the final pellet was suspended in the same buffer. In studies in which the intravesicular medium was pH 5.5, the second washing solution contained 150 mM KCl, 25 mM MES, and 4.6 mM Tris (pH 5.5).

Uptake of <sup>22</sup>Na was measured at 25°C by a Millipore filtration technique as previously described (29, 30). Unless otherwise stated, the incubation medium consisted of 144 mM KCl, 5 mM MES, 13 mM Hepes, 13 mM Tris, 1 mM NaCl (pH 7.5), and 5 μM valinomycin. Experiments were started by the addition of 80 μl of the incubation media containing (0.2–0.5 μCi of <sup>22</sup>Na) to 20 μl of the membrane suspension (~100 μg of protein). After designated time periods, the reaction was terminated by the addition of 5 ml of stop solution containing 150 mM LiCl, 16 mM Hepes, and 10 mM Tris (pH 7.5). The diluted samples were immediately filtered through a 0.45-μm Millipore filter using a Millipore manifold filtration assembly (Millipore Corp., Bedford, MA). Filters were further washed two times with 5 ml of cold stopping solution, dissolved in scintillation fluid (Filtercount, Packard Instrument Co., Inc., Downers Grove, IL) and the radioactivity was measured in a liquid scintillation counter (model 5800, Beckmann Instruments, Inc., Palo Alto, CA). Nonspecific binding of radioactivity to the filters was subtracted from the total counts of the sample. All incubations were performed in triplicate using membranes prepared the same day.

In studies in which the ionophore valinomycin was used, this agent (5 μM, final concentration) was added from an ethanolic stock solution resulting in a final ethanol concentration of 0.5% (vol/vol) in the incubation media during all measurements.

**Fluorescent dye studies.** The fluorescence quenching of acridine orange was employed to monitor formation as well as dissipation of pH gradients as previously described by our laboratory and others (11, 29, 31–33). The fluorescence of acridine orange was measured at 25°C with a spectrofluorometer (excitation 493 nm, emission 530 nm; model 650-40, Perkin-Elmer Corp., Norwalk, CT) equipped with a thermostated cuvette, stirring system, and adding port. Care was exercised to maintain constant pH and temperature, which previously have been demonstrated to influence the reproducibility of assay results (15, 31). Calibrations of the acridine orange fluorescence response with respect to membrane protein and various pH gradients were performed as described previously (15) to ensure that the dye response was not rate-limiting and could successfully be used to monitor transmembrane proton fluxes in small intestinal villus brush-border membrane vesicles prepared from both control and diabetic animals. In general, 25 μl of membrane vesicles (~500 μg of membrane protein) were added to 1,975 μl of incubation buffer containing 10 μM acridine orange. The initial fluorescence intensity of the incubation medium (without membranes) was adjusted to 800 arbitrary fluorescence units by altering the instrument attenuation and was kept constant throughout for various measurements. For proton influx studies, soon after the addition of vesicles loaded with Na<sup>+</sup> to incubation medium (pH 7.5 inside/7.5 outside), a time-based scan of fluorescence quenching was performed to assess the rate of transient intravesicular acidifications by drawing a tangent to the initial linear portion of the fluorescence signal

1. Abbreviations used in this paper: MES, 2-(*N*-morpholino) ethane sulfonic acid; N-MGG, *N*-methylglucamine gluconate.

(11, 15, 29, 34). For proton efflux studies, after the addition of vesicles to incubation medium (pH 5.5 inside/7.5 outside), as soon steady-state acridine orange fluorescence quenching developed, sufficient quantities of  $\text{Na}^+$ -gluconate stock were then added with constant stirring, resulting in reappearance of acridine orange fluorescence. The initial rate of fluorescence recovery was calculated as described above (15, 29). The detailed incubation conditions for each experiment are given in the table legends. After 300 s the pH gradient was dissipated by the addition of 150 mM  $\text{K}^+$  gluconate and 10  $\mu\text{g}$  of nigericin as previously described (15, 29). In studies, where ionophores were used, they were added from their ethanolic solutions as described above.

**Statistical analysis.** All experiments were performed using at least four or five freshly isolated independent membrane preparations. Results are expressed as mean $\pm$ SEM. Paired or unpaired Student's *t* tests and one way analysis of variance were used in statistical analysis as appropriate. A *P* value of  $< 0.05$  was considered statistically significant.

## Results

**Profile of animal groups.** Body weights (initial and at sacrifice), intestinal wet weights at sacrifice, and blood glucose values of control, untreated diabetic, insulin-treated, and 1,25(OH) $_2$ D $_3$ -treated diabetic rats are included in Table I. As can be seen from this table, unlike control rats, untreated-diabetic rats failed to gain weight and actually lost body weight after induction of diabetes by streptozotocin. Insulin administration to diabetic rats for 5–7 d was found to partially correct this weight loss in these animals. Wet weights of the proximal small intestines of the diabetic animals at sacrifice, however, were found to be significantly greater than their control counterparts and this parameter was corrected by insulin treatment for 5–7 d. Blood glucose values for diabetic rats were also found to be significantly higher compared to values for control animals and insulin-treated animals. 1,25(OH) $_2$ D $_3$  treatment to diabetic rats, however, did not influence body weight loss, intestinal hypertrophy or hyperglycemia observed in untreated-diabetic rats (Table I).

**Conductance of protons and potassium.** Since assessment of  $\text{Na}^+$ - $\text{H}^+$  exchange activity involves measurements of  $\text{Na}^+$  gradient-dependent proton movement and/or proton gradient-dependent  $\text{Na}^+$  movement, it was important to examine proton conductance of control and diabetic brush-border mem-

brane vesicles in these studies. Acridine orange techniques, as previously described by our laboratory and others (11, 29), were used for these experiments. Vesicles from control and diabetic animals were loaded with 150 mM  $\text{K}^+$  gluconate, 10 mM Tris/Hepes, pH 7.5, and were diluted into a buffer containing 150 mM N-MGG, 10 mM Tris/Hepes, pH 7.5, containing 10  $\mu\text{M}$  acridine orange in the presence and absence of the potassium-selective ionophore, valinomycin. As shown in Table II in the absence of valinomycin the rate of intravesicular acidification, which represents intrinsic potassium conductance, remained unchanged in diabetic vesicles compared to their control counterparts. In the presence of valinomycin the rate of intravesicular acidification, representing intrinsic proton conductance, also remained unaltered (Table II) in diabetic compared to control vesicles. These findings, therefore, indicate that  $\text{K}^+$  and  $\text{H}^+$  conductances of small intestinal villus brush-border membrane vesicles remained unaltered in streptozotocin-induced diabetes, compared to their control counterparts.

**Time course of  $\text{H}^+$  gradient-dependent  $^{22}\text{Na}$  uptake.** Fig. 1 shows the effect of chemically-induced diabetes mellitus on the time course of pH gradient-dependent 1 mM  $^{22}\text{Na}$  uptake under voltage clamp conditions (presence of valinomycin with  $\text{K}^+$  on both sides of vesicles). In the presence of a pH gradient (pH 5.5 inside/7.5 outside),  $^{22}\text{Na}$  uptake in diabetic preparations was significantly enhanced at each time point (up to 3 min), compared to their control counterparts. At equilibrium (180 min), accumulation of  $^{22}\text{Na}$  was, however, similar in control and diabetic vesicles. As previously discussed (35, 36), the latter finding indicates that diabetes did not influence average intravesicular volume and, therefore, the increased  $^{22}\text{Na}$  uptake at early time points (up to 3 min) could not be ascribed to altered vesicular size in these experiments. Since similar aliquots of membrane proteins were used in the transport experiments, this also rules out changes in vesicular number as being responsible for increased  $^{22}\text{Na}$  uptake observed in these experiments. In the absence of a pH gradient (pH 7.5 inside/7.5 outside), there was no significant difference observed in  $^{22}\text{Na}$  uptake in vesicles from control and diabetic animals, indicating that  $^{22}\text{Na}$  permeability was also not influenced by the diabetic state. These findings are in agreement with previous experiments by Ghishan et al. (37), in which no differences were noted in  $^{22}\text{Na}$  permeability in jejunal brush-border membrane vesicles of control and streptozotocin-induced diabetic rats. 1

Table I. Profile of Animal Groups

Group	No. of animals	Initial body wt	Sacrifice body wt	Intestinal wt at sacrifice	Blood glucose
		g	g	g	mg/dl
Control	40	273 $\pm$ 6	338 $\pm$ 6	6.1 $\pm$ 0.4	118 $\pm$ 11
Diabetic	52	276 $\pm$ 5	250 $\pm$ 9*	9.3 $\pm$ 0.7*	430 $\pm$ 12*
Diabetic + insulin	44	252 $\pm$ 8*	298 $\pm$ 7*	6.5 $\pm$ 1.1	143 $\pm$ 22
Diabetic + 1,25(OH) $_2$ D $_3$	40	255 $\pm$ 10*	240 $\pm$ 9*	8.7 $\pm$ 0.8*	402 $\pm$ 18*

Values are means $\pm$ SEM of (*n*) number of separate animals in each group. It should be noted that the initial body weights for animals in the diabetic + insulin and diabetic + 1,25(OH) $_2$ D $_3$  groups were obtained 7 d after induction of diabetes by streptozotocin.

\* *P*  $< 0.05$  or less compared to control values.

Table II. Effect of Streptozotocin-induced Diabetes on  $\text{K}^+$ -Gradient-dependent Intravesicular Acidification ( $\text{H}^+$  Influx) as Assessed by Acridine-Orange Fluorescence Quenching\*

Conditions	$\text{K}^+$ -dependent $\text{H}^+$ influx	
	Control	Diabetic
fluorescence units/min $\cdot$ mg		
No addition	19.5 $\pm$ 7.6	23.5 $\pm$ 8.6
Valinomycin	100.3 $\pm$ 16.9	117.0 $\pm$ 20.2

Values are mean $\pm$ SEM of four or five independent membrane preparations. Brush-border membrane vesicles ( $\sim 500 \mu\text{g}$  of protein) were loaded with 150 mM  $\text{K}^+$ -gluconate, 10 mM Tris/Hepes (pH 7.5) and were diluted 1:80 into 150 mM N-MGG, 10 mM Tris/Hepes (pH 7.5), and 10  $\mu\text{M}$  acridine orange $\pm 5 \mu\text{M}$  valinomycin.

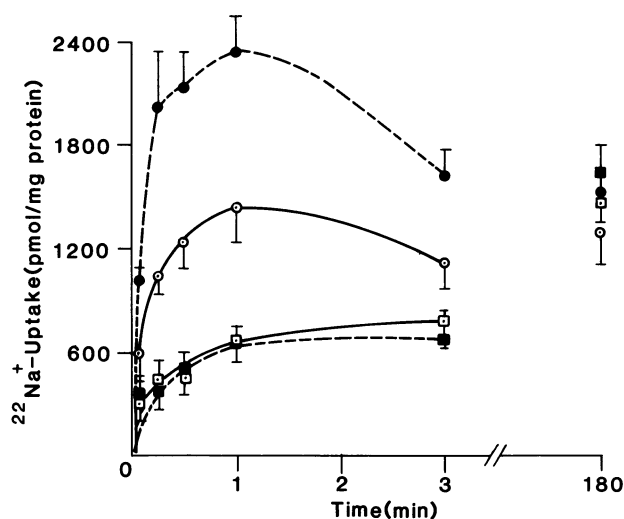


Figure 1. Time course of proton gradient-dependent 1 mM  $^{22}\text{Na}$  uptake in proximal small intestinal villus brush-border membrane vesicles of control and streptozotocin-diabetic rats.  $\text{Na}^+$  uptakes were measured at  $25^\circ\text{C}$  in membrane vesicles from control ( $\circ$ ) pH 5.5 in/7.5 out and ( $\square$ ) pH 7.5 in/7.5 out and diabetic ( $\bullet$ ) pH 5.5 in/7.5 out and ( $\blacksquare$ ) pH 7.5 in/7.5 out rats at various time periods indicated. Values represent mean  $\pm$  SEM of four or five independent membrane preparations.

mM amiloride significantly inhibited ( $\sim 85$ – $95\%$ , in 15 s)  $^{22}\text{Na}$  uptake in the presence of a pH gradient, in both the control ( $1,029 \pm 104$  to  $123.5 \pm 12.5$ ) and diabetic groups ( $2,020 \pm 346$  to  $151.3 \pm 20.6$ ). These findings, therefore, again indicate that  $^{22}\text{Na}$  uptake via the exchange mechanism was significantly increased, whereas  $^{22}\text{Na}$  uptake by ancillary pathways was not influenced in this experimental model.

It was also conceivable that the observed increase in  $^{22}\text{Na}$  uptake in brush-border membrane vesicles from diabetic rats was secondary to a decrease in the rate of proton-gradient dissipation rather than an increase in  $\text{Na}^+$ - $\text{H}^+$  exchange activity. However, as shown in Table II, the intrinsic proton conductance of brush-border membrane vesicles in control and diabetic animals was not statistically different ( $P > 0.05$ , Table II), indicating that the observed enhancement of  $\text{H}^+$  gradient-dependent  $^{22}\text{Na}$  uptake in diabetes (Fig. 1) was not a result of altered  $\text{H}^+$  conductance in these vesicles, but rather was a direct consequence of increased  $\text{Na}^+$ - $\text{H}^+$  exchange.

**$\text{Na}^+$  gradient-dependent  $\text{H}^+$  influx.** Since amiloride-sensitive,  $\text{H}^+$  gradient-dependent  $^{22}\text{Na}$  uptake was increased in diabetes (as shown above), the effect of diabetes on  $\text{Na}^+$  gradient-dependent  $\text{H}^+$  influx, using acridine orange techniques (11, 29), was also examined. Vesicles were loaded with 100 mM  $\text{Na}^+$ -gluconate, 50 mM  $\text{K}^+$ -gluconate, 10 mM Tris/Hepes, pH 7.5, and were diluted into a buffer containing 100 mM N-MGG, 50 mM  $\text{K}^+$ -gluconate, 10 mM Tris/Hepes, pH 7.5, and 10  $\mu\text{M}$  acridine orange  $\pm 5 \mu\text{M}$  valinomycin.

Dilution of sodium-loaded vesicles in sodium-free media resulted in transient intravesicular acidification as observed by quenching of acridine orange fluorescence. As shown in Table III, the rate of intravesicular acidification in the absence of valinomycin was significantly enhanced in the diabetic preparations compared to respective control values. Sodium gradient-dependent influx of protons can be attributed to either conductive coupling or direct coupling via electroneutral exchange of

Table III. Effect of Streptozotocin-induced Diabetes on  $\text{Na}^+$  Gradient-Dependent Intravesicular Acidification ( $\text{H}^+$  Influx) as Assessed by Acridine-Orange Fluorescence Quenching\*

Conditions	$\text{Na}^+$ -dependent $\text{H}^+$ influx	
	Control	Diabetic
fluorescence units/min $\cdot$ mg		
No addition	$69.0 \pm 5.1$	$106.2 \pm 3.2^*$
Valinomycin	$39.8 \pm 8.3$	$76.9 \pm 4.7^*$

Values are means  $\pm$  SEM of four to six independent membrane preparations. Brush-border membrane vesicles ( $\sim 500 \mu\text{g}$  of protein) were loaded with 100 mM  $\text{Na}^+$ -gluconate, 50 mM  $\text{K}^+$ -gluconate, 10 mM Tris-Hepes (pH 7.5) and were diluted 1:80 into 150 mM N-MGG, 50 mM  $\text{K}^+$ -gluconate, 10 mM Tris-Hepes (pH 7.5), and 10  $\mu\text{M}$  acridine orange  $\pm 5 \mu\text{M}$  valinomycin.

\*  $P < 0.05$  or less compared to control.

$\text{Na}^+$  for  $\text{H}^+$ . To distinguish between these two possibilities, 5  $\mu\text{M}$  valinomycin was added in the presence of  $\text{K}^+$  on both sides of the vesicles. As shown in Table III, addition of valinomycin markedly decreased  $\text{Na}^+$ -dependent intravesicular acidification in vesicles from both control and diabetic rats, indicating the presence of significant conductive coupling of  $\text{Na}^+$  and  $\text{H}^+$  in these vesicles. The rate of  $\text{Na}^+$  gradient-dependent intravesicular acidification in the presence of valinomycin was also almost doubled (Table III) in vesicles prepared from diabetic animals compared to their control counterparts. This valinomycin-insensitive intravesicular acidification was significantly inhibited ( $\sim 85\%$ ) in the presence of 1 mM amiloride (data not shown). These findings, therefore, confirm an increase in small intestinal luminal membrane electroneutral, amiloride-sensitive  $\text{Na}^+$ - $\text{H}^+$  exchange activity associated with experimental diabetes.

**$\text{Na}^+$  gradient-dependent  $\text{H}^+$  efflux.** Inwardly directed  $\text{Na}^+$  gradient-dependent  $\text{H}^+$  extrusion was also measured to assess  $\text{Na}^+$ - $\text{H}^+$  exchange activity in these vesicles using acridine orange fluorescence technique. Vesicles were loaded with 100 mM N-MGG, 50 mM  $\text{K}^+$ -gluconate, 20 mM MES-Tris (pH 5.5) and were diluted into a buffer containing 100 mM N-MGG, 50 mM  $\text{K}^+$ -gluconate, 20 mM Tris-Hepes (pH 7.5) and 10  $\mu\text{M}$  acridine orange  $\pm 5 \mu\text{M}$  valinomycin.

Dilution of vesicles in incubation media resulted in quenching of acridine orange fluorescence. Addition of 20 mM  $\text{Na}^+$ -gluconate (final concentration) resulted in the reappearance of fluorescence representing  $\text{Na}^+$ -dependent  $\text{H}^+$  efflux. To distinguish between conductive coupling and direct coupling, experiments were also performed in the presence of valinomycin/ $\text{K}^+$ -induced voltage clamping as described above. As shown in Table IV, the rate of reappearance of fluorescence was significantly decreased in both control and diabetic vesicles under voltage clamp conditions compared to non-voltage clamp conditions, again indicating the presence of considerable conductive coupling of  $\text{H}^+$  and  $\text{Na}^+$  in these vesicles. These findings are in agreement with previous studies by Casano et al. (11) also describing the presence of significant amounts of conductive coupling of  $\text{Na}^+$  and  $\text{H}^+$  in rat jejunal brush border membrane vesicles. In view of the presence of considerable amounts of  $\text{Na}^+$ - and  $\text{H}^+$ -conductive coupling in these vesicles, voltage clamp conditions were used for all subsequent experiments.

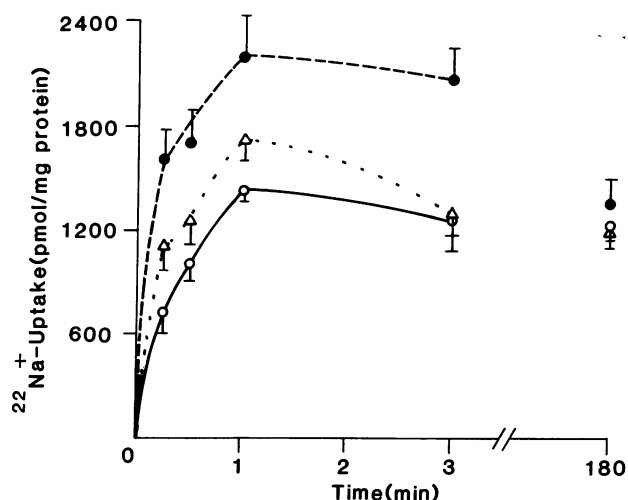


Figure 2. Effect of insulin treatment on  $H^+$  gradient-dependent time course of 1 mM  $^{22}Na$  uptake in proximal small intestinal brush-border membrane vesicles of streptozotocin diabetic rats.  $Na^+$  uptakes were measured at  $20^\circ C$  in membrane vesicles from control ( $\circ$ ), diabetic ( $\bullet$ ), and insulin-treated diabetic ( $\Delta$ ) rats at various time periods in the presence of an outwardly directed pH gradient (pH 5.5 in/7.5 out). Values represent mean  $\pm$  SEM of five to seven independent membrane preparations.

In the presence of valinomycin, the rate of fluorescence reappearance representing electroneutral, amiloride-sensitive  $Na^+-H^+$  exchange was significantly increased in diabetic vesicles compared to their control counterparts (Table IV). These findings further confirm an enhancement in  $Na^+-H^+$  exchange process in vesicles isolated from streptozotocin-diabetic rats.

**Effect of insulin treatment on  $^{22}Na$  uptake.** Many abnormalities of the diabetic state have previously been shown to be corrected by insulin therapy (7, 8, 38, 39). It was therefore of interest to examine the effect of this therapy on the enhanced  $Na^+-H^+$  exchange associated with diabetes. As shown in Fig. 2, insulin therapy for 5–7 d significantly decreased and tended to

Table IV. Effect of Streptozotocin-induced Diabetes on  $Na^+$  Gradient-Dependent  $H^+$  Efflux as Assessed by Acridine Orange Fluorescence Reappearance

Conditions	Na <sup>+</sup> -dependent H <sup>+</sup> efflux	
	Control	Diabetic
fluorescence units/s · mg		
No addition	484 $\pm$ 24	571 $\pm$ 33*
Valinomycin	193 $\pm$ 21	273 $\pm$ 21*

Values are mean  $\pm$  SEM of four or five independent membrane preparations. Brush-border membrane vesicles ( $\sim 500 \mu g$  of protein) were loaded with 50 mM  $K^+$ -gluconate, 100 mM N-MGG, 20 mM MES-Tris (pH 5.5) and were diluted 1:80 into 50 mM  $K^+$ -gluconate, 100 mM N-MGG, 20 mM Tris-Hepes (pH 7.5) and 10  $\mu M$  acridine orange  $\pm 5 \mu M$  valinomycin. After steady-state fluorescence quenching developed,  $Na^+$ -gluconate (20 mM, final concentration) from a 1 M stock solution was added, and the rate of fluorescence reappearance was measured by drawing a tangent to the initial linear portion of the fluorescence signal.

\*  $P < 0.05$  compared to control.

Table V. Serum Levels of  $1,25(OH)_2D_3$  in Various Treatment Groups

Control	$1,25(OH)_2D_3$		
	Diabetic	Diabetic + insulin	Diabetic + $1,25(OH)_2D_3$
pg/ml			
32.6 $\pm$ 4.1 (n = 11)	16.2* $\pm$ 1.7 (n = 11)	25.3* $\pm$ 4.4 (n = 5)	44.0† $\pm$ 1.5 (n = 5)

Values are mean  $\pm$  SEM of separate (n) number of animals.

\*  $P < 0.05$  or less compared to control.

†  $P < 0.05$  or less compared to diabetic.

normalize the elevated amiloride-sensitive  $^{22}Na$  uptake observed in the diabetic group at each time point (up to 3 min) to control levels. There was no significant difference observed at equilibrium point (180 min) in all the three groups, indicating that the vesicular size and number remained unchanged in each treatment group compared to controls, as previously discussed (35, 36).

**Effect of  $1,25(OH)_2D_3$  treatment on  $^{22}Na$  uptake.** It was also of interest to evaluate the effect of  $1,25(OH)_2D_3$  treatment to diabetic rats on their  $Na^+-H^+$  exchange activity. Diabetic animals were treated with  $1,25(OH)_2D_3$  at a dose of 10 ng/100 g body wt subcutaneously for 5–7 d, as described in the Methods section. Levels of  $1,25(OH)_2D_3$  were assayed in the sera of animals of the various groups at the time of sacrifice.

As shown in Table V, the diabetic state induced by streptozotocin significantly lowered  $1,25(OH)_2D_3$  levels in sera compared to controls, whereas insulin treatment as well as in vivo vitamin D treatment significantly increased  $1,25(OH)_2D_3$  levels compared to diabetic rats.

The effect of  $1,25(OH)_2D_3$  administration to diabetic rats on pH gradient-dependent (pH 5.5 inside/7.5 outside), amiloride-sensitive 1 mM  $^{22}Na$  uptake was also examined. As shown in Table VI,  $1,25(OH)_2D_3$  treatment corrected the enhanced  $^{22}Na$  uptake observed in the diabetic group to control levels. In the presence of 1 mM amiloride there was no change observed in  $^{22}Na$  uptake in any treatment group (Table VI). These findings suggest that increased  $Na^+-H^+$  exchange in diabetes may, at least in part, be secondary to decreased  $1,25(OH)_2D_3$  levels.

**Kinetics of  $Na^+-H^+$  exchange.** In order to elucidate the possible mechanism(s) of increased  $Na^+-H^+$  exchange activity in

Table VI. 1 mM  $^{22}Na^+$  Uptake into Small Intestinal Villus Brush-Border Membrane Vesicles at 5 s

Conditions	Control	Diabetic	Diabetic + $1,25(OH)_2D_3$
pmol/mg protein			
No addition	595 $\pm$ 181	1,007* $\pm$ 52	605† $\pm$ 56
+1 mM amiloride	113 $\pm$ 34	161 $\pm$ 34	116 $\pm$ 11

Values are mean  $\pm$  SEM of five to seven separate membrane preparations.

\*  $P < 0.05$  or less compared to control values.

†  $P < 0.05$  or less compared to diabetic values.

Table VII. Effect of Streptozotocin-induced Diabetes and Treatment with Insulin or 1,25(OH)<sub>2</sub>D<sub>3</sub> on Kinetic Parameters of Small Intestinal Villus Brush-Border Na<sup>+</sup>-H<sup>+</sup> Exchanger Using <sup>22</sup>Na-Uptake Technique

Parameters	Control	Diabetic	Insulin	1,25(OH) <sub>2</sub> D <sub>3</sub>
$K_{Na^+}$ (mM)	4.45±0.60	4.96±0.47	4.25±1.30	6.41±1.35
$V_{max}$ (nmol/5 s per mg)	4.11±0.58	10.12*±1.80	5.26†±1.31	6.40†±1.61

Values are mean±SEM of five to seven separate vesicle preparations. \*  $P < 0.05$  or less compared to control values. †  $P < 0.05$  or less compared to diabetic values.

diabetes and its correction by insulin and/or 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment, kinetic studies were performed to examine whether the altered activity could be explained by a change in the affinity ( $K_m$ ) of the antiporter for its substrate or an effect on its maximal velocity ( $V_{max}$ ). Amiloride-sensitive <sup>22</sup>Na uptake in the presence of an outwardly directed pH gradient (pH 5.5 inside/7.5 outside) was measured at the 5-s time period by varying <sup>22</sup>Na concentrations (1–10 mM). As analyzed by double reciprocal plots (40), as shown in Table VII, there was a significant increase in the  $V_{max}$  of <sup>22</sup>Na uptake in the diabetic group's membrane vesicles with no change in the apparent affinity ( $K_m$ ) for sodium, compared to their control counterparts. Furthermore, insulin therapy and 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment restored the  $V_{max}$  alterations observed in diabetic animal preparations to control values without any significant changes in  $K_m$  (Table VII).

## Discussion

The present studies, utilizing two distinct but complementary techniques, have demonstrated for the first time that streptozotocin-induced diabetic rats have increased amiloride-sensitive, electroneutral Na<sup>+</sup>-H<sup>+</sup> exchange activity in proximal small intestinal brush-border membranes compared to their control counterparts. Earlier studies in chemically induced diabetic animals have previously described an increase in small intestinal sodium absorption (5, 6), but did not define the mechanisms involved in this phenomenon. The present data would, therefore, indicate that increased brush-border membrane Na<sup>+</sup>-H<sup>+</sup> exchange, at least in part, may be responsible for these prior observations.

Increased intestinal Na<sup>+</sup>-H<sup>+</sup> exchange in diabetes could theoretically result from either a change in the kinetic parameters ( $V_{max}$  and  $K_m$ ) of this process and/or an alteration in the properties of a possible H<sup>+</sup> modifier site (41). In the present experiments, kinetic analyses, in fact, revealed an enhanced maximal velocity ( $V_{max}$ ) of this exchanger in diabetic preparations with no change in the apparent affinity ( $K_m$ ) for Na<sup>+</sup>. Moreover, in agreement with the results of recent studies in luminal plasma membranes of the rat colon and rabbit small intestine (16), preliminary studies from our laboratory do not support the existence of a H<sup>+</sup>-modifier site in intestinal vesicles, as Hill plot analyses of the effect of various internal pH values on <sup>22</sup>Na<sup>+</sup> uptake, failed to demonstrate positive cooperativity for protons in these preparations (unpublished observations).

In the present studies, administration of either insulin or 1,25(OH)<sub>2</sub>D<sub>3</sub> for 5–7 d corrected the increased Na<sup>+</sup>-H<sup>+</sup> exchange observed in the intestinal membranes of diabetic rats. These findings suggest that a number of possible mechanisms that might be involved in enhanced Na<sup>+</sup>-H<sup>+</sup> exchange in the diabetic intestine, such as alterations in serum glucocorticoid levels, membrane fluidity, metabolic acidosis, and intestinal

hypertrophy, are unlikely to play a major role in this phenomenon. For example, previous studies from our laboratory (18) had demonstrated enhanced intestinal Na<sup>+</sup>-H<sup>+</sup> exchange in response to glucocorticoid administration. Since diabetic animals have been shown to have increased serum corticosterone levels (8), it is theoretically possible that this factor might, therefore, be responsible for the present transport abnormalities. As previously noted (8), however, insulin therapy failed to influence corticosterone levels in diabetic rats and yet restored Na<sup>+</sup>-H<sup>+</sup> exchange to control levels in the present experiments.

Prior studies by our laboratory (18, 19, 42), utilizing several different experimental conditions, have established a direct relationship between alterations in intestinal membrane fluidity and Na<sup>+</sup>-H<sup>+</sup> exchange activity. In earlier studies (26), however, we have shown that intestinal diabetic membranes are less fluid than their control counterparts. Taken together, these findings would suggest that it is unlikely that the increased Na<sup>+</sup>-H<sup>+</sup> exchange noted in the current experiments in diabetic intestinal membranes was secondary to a decreased membrane fluidity.

Previous experiments in kidney brush-border membrane vesicles have also demonstrated increased Na<sup>+</sup>-H<sup>+</sup> exchange in response to metabolic acidosis (43, 44). Harris et al. (7), moreover, suggested that the enhanced renal luminal membrane Na<sup>+</sup>-H<sup>+</sup> exchange observed in streptozotocin-diabetic rats was the result of increased production of endogenous protons. While acute streptozotocin-diabetic rats have not been shown to be frankly acidotic (8), this possibility cannot be completely ruled out as a possible mechanism for enhanced intestinal Na<sup>+</sup>-H<sup>+</sup> exchange. In the current studies, however, the correction of this transport abnormality by 1,25(OH)<sub>2</sub>D<sub>3</sub> administration alone is unlikely to be solely explained by this mechanism.

In the present studies, in agreement with prior observations (1), the mucosal mass of the diabetic animal's intestines were markedly increased. This intestinal hypertrophy, however, is also unlikely to explain the increased Na<sup>+</sup>-H<sup>+</sup> exchange seen in the intestine of diabetic rats, since villus cell membrane vesicular preparations of comparable size and number were used in these studies and, furthermore, 1,25(OH)<sub>2</sub>D<sub>3</sub> corrected the transport abnormalities and yet had no effect on intestinal mucosal mass.

In agreement with earlier experiments by our laboratory (20) and others (21) in CaCo-2 and chick small intestinal cells, respectively, it would appear that Na<sup>+</sup>-H<sup>+</sup> exchange activity in the intestine of streptozotocin-induced diabetes mellitus may be modulated by serum 1,25(OH)<sub>2</sub>D<sub>3</sub> levels. Decreased serum levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> observed in this drug-induced disorder were associated with enhanced intestinal Na<sup>+</sup>-H<sup>+</sup> exchange activity and 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment of diabetic animals corrected this deficiency, concomitant with restoration of intestinal exchange activity to control values. These findings, therefore, suggest that decreased serum levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> observed in streptozotocin-diabetic rats may represent one possible mecha-

nism of enhanced  $\text{Na}^+\text{-H}^+$  exchange in small intestine of these animals. Additionally, in agreement with previous studies (22), insulin therapy corrected this decrease in serum  $1,25(\text{OH})_2\text{D}_3$  levels in diabetic rats and also normalized  $\text{Na}^+\text{-H}^+$  exchange in the present experiments.

The mechanisms underlying  $1,25(\text{OH})_2\text{D}_3$  modulation of intestinal  $\text{Na}^+\text{-H}^+$  exchange are unclear at this time. Recently, in this regard, there have been a number of studies indicating a close relationship between the  $\text{Na}^+\text{-H}^+$  antiporter and intracellular  $\text{Ca}^{2+}$  levels (45–50). For example, preliminary studies from our laboratory in CaCo-2 cells have suggested that this secosteroid may modulate  $\text{Na}^+\text{-H}^+$  activity by raising intracellular calcium levels in these cells (20). Semrad and Chang (47) have also demonstrated an inhibition of amiloride-sensitive  $\text{Na}^+\text{-H}^+$  exchange in chick enterocytes via intracellular calcium. Short-term streptozotocin-induced diabetes in rats is characterized by impaired intestinal absorption of calcium (51), secondary to low circulating levels of  $1,25(\text{OH})_2\text{D}_3$  (22). Additionally,  $1,25(\text{OH})_2\text{D}_3$  has been shown by our laboratory and others to influence intracellular calcium levels in intestinal tissue (52, 53). Based on these considerations, it is likely that  $1,25(\text{OH})_2\text{D}_3$  might modulate intestinal  $\text{Na}^+\text{-H}^+$  exchange activity in streptozotocin-diabetic rats via alterations in calcium uptake and/or release from intracellular stores, thereby, increasing intracellular  $\text{Ca}^{2+}$  levels. In vivo, the mechanism(s) by which intracellular  $\text{Ca}^{2+}$  influences  $\text{Na}^+\text{-H}^+$  exchange, may involve alterations in enterocyte  $\text{H}^+/\text{Ca}^{2+}$  and/or  $\text{Na}^+/\text{Ca}^{2+}$  exchanger activity, although Ghijssen et al. (54) have suggested that  $\text{Na}^+/\text{Ca}^{2+}$  exchange activity of rat enterocyte basolateral membranes was not regulated by  $1,25(\text{OH})_2\text{D}_3$ .  $\text{Ca}^{2+}$ -mediated effects on modulation of the  $\text{Na}^+\text{-H}^+$  antiporter have also been shown to involve protein phosphorylation by  $\text{Ca}^{2+}$ -calmodulin-dependent kinase(s) and/or by protein kinase C (9). Further studies will be required to clarify these issues.

Regardless of the mechanism(s) involved, however, it would appear that streptozotocin-induced diabetes is associated with an increase in intestinal  $\text{Na}^+\text{-H}^+$  exchange. What are the consequences of such a transport abnormality? In this regard, studies in a number of cultured cell lines have suggested that cytoplasmic alkalinization mediated by enhanced  $\text{Na}^+\text{-H}^+$  exchange may represent an early stimulus of mitogenesis (55, 56), although this is controversial (57). In view of these reports and their previous studies (58), Harris et al. (7) suggested that enhanced  $\text{Na}^+\text{-H}^+$  exchange in renal brush-border membrane vesicles of streptozotocin-induced diabetic rats may be closely related to the hypertrophy observed in whole kidneys of these animals. This assumption was also based on the fact that insulin therapy decreased  $\text{Na}^+\text{-H}^+$  exchange concomitant with a decrease in renal hypertrophy. A direct correlation between increased  $\text{Na}^+\text{-H}^+$  exchange and hypertrophic stimuli, however, was not established in these previous studies (7). Although our present studies also demonstrate a correlation between intestinal hypertrophy and  $\text{Na}^+\text{-H}^+$  exchange in diabetic rats and in insulin-treated diabetic rats, these two parameters do not seem to be interrelated in the diabetic rat small intestine, since  $1,25(\text{OH})_2\text{D}_3$  treatment to diabetic rats corrected their enhanced  $\text{Na}^+\text{-H}^+$  exchange without affecting intestinal hypertrophy.

Previous observations in kidney brush-border membrane vesicles of streptozotocin-diabetic rats have demonstrated decreased  $\text{Na}^+$ -dependent D-glucose transport concomitant with increased  $\text{Na}^+\text{-H}^+$  exchange (7, 8). Based on these findings, it was suggested that decreased  $\text{Na}^+$ -dependent D-glucose trans-

port was secondary to dissipation of the  $\text{Na}^+$ -gradient due to enhanced  $\text{Na}^+\text{-H}^+$  exchange (8). Similarly, Hopfer et al. (59) suggested that increased  $\text{Na}^+$ -glucose co-transport in intestinal microvillus membrane vesicles from diabetic rats was secondary to decreased rates of non-glucose-dependent  $\text{Na}^+$  flux. Previous results from our laboratory (26), however, have recently demonstrated that enhanced  $\text{Na}^+$ -glucose co-transport in the diabetic rat proximal small intestine was localized to luminal membranes of mid-villus and lower villus enterocytes, with no change in villus-tip enterocytes. Ghishan et al. (37) have also demonstrated that under nongradient conditions (pH 7.5 in/pH 7.5 out),  $\text{Na}^+\text{-P}_i$  co-transport in streptozotocin-diabetic small intestinal luminal membrane vesicles was decreased with no change in  $\text{Na}^+$  permeability. Additionally, in the absence of a pH gradient, no differences were observed in  $\text{Na}^+$  uptake in diabetic kidney brush-border membrane vesicles, whereas, under similar conditions,  $\text{Na}^+$ -glucose co-transport was decreased (8). These findings, taken together with our present results, suggest that other mechanism(s) might be responsible for alterations in D-glucose transport in streptozotocin-diabetic rat small intestine, rather than alterations in  $\text{Na}^+\text{-H}^+$  exchange.

In conclusion, the results of the present experiments demonstrate that the luminal membranes of the small intestine of streptozotocin-diabetic animals exhibit enhanced  $\text{Na}^+\text{-H}^+$  exchange activity, which appears at least in part, to be secondary to decreased serum levels of  $1,25(\text{OH})_2\text{D}_3$  in this experimental model. In conjunction with enhanced sodium-potassium-dependent adenosine triphosphatase activity of the basolateral membranes of these cells in diabetes (60), this increased  $\text{Na}^+\text{-H}^+$  exchange at the luminal surface may serve as one possible mechanism for enhanced  $\text{Na}^+$  absorption previously described in this pathological condition (5, 6). This increased  $\text{Na}^+\text{-H}^+$  exchange may also play an important role in maintenance of intracellular pH in the diabetic small intestine. Additional studies to further define the mechanism(s) involved in modulation of intestinal  $\text{Na}^+\text{-H}^+$  exchange by  $1,25(\text{OH})_2\text{D}_3$  as well as their possible relationship to the known structural and functional abnormalities in the intestine of diabetic animals will, therefore, clearly be of interest.

## Acknowledgments

The authors thank Lynn Nelson for her excellent secretarial assistance.

This investigation was supported by National Institutes of Health grants DK-39573, DK-42086 (Digestive Diseases Core Center), and DK-26678 (Clinical Nutrition Research Unit).

## References

1. Miller, D. L., B. A. Wayne Hanson, H. P. Schedl, and J. W. Osborne. 1977. Proliferation rate and transit time of mucosal cells in small intestine of diabetic rats. *Gastroenterology*. 73:1326–1332.
2. Schedl, H. P., and H. D. Wilson. 1971. Effect of diabetes on intestinal growth and hexose transport in the rat. *Am. J. Physiol.* 220:1739–1745.
3. Lal, D., and H. P. Schedl. 1974. Intestinal adaptation in diabetes: amino acid absorption. *Am. J. Physiol.* 227:827–831.
4. Caspary, W. F. 1973. Increase of active transport of conjugated bile salts in streptozotocin-diabetic rat small intestine. *Gut*. 14:949–955.
5. Aulsebrook, K. A. 1965. Intestinal transport of glucose and sodium: changes in alloxan-diabetes and effects of insulin. *Experientia (Basel)*. 21:346–347.
6. Schedl, H. P., and H. D. Wilson. 1974. Jejunal sodium transport: effects of alloxan diabetes. *Biochim. Biophys. Acta*. 367:225–231.
7. Harris, R. C., B. M. Brenner, and J. L. Seifter. 1986. Sodium-hydrogen exchange and glucose transport in renal microvillus membrane vesicles from rats with diabetes mellitus. *J. Clin. Invest.* 77:724–733.
8. El-Seifi, S., J. M. Freiberg, J. Kinsella, L. Cheng, and B. Sacktor. 1987.  $\text{Na}^+\text{-H}^+$  exchange and  $\text{Na}^+$ -dependent transport systems in streptozotocin dia-



- betic rat kidneys. *Am. J. Physiol.* 252 (Regul. Integrative Comp. Physiol. 21):R40-R47.
9. Grinstein, S., and A. Rothstein. 1986. Mechanisms of regulation of the  $\text{Na}^+\text{-H}^+$  exchanger. *J. Membr. Biol.* 90:1-12.
10. Murer, H., U. Hopfer, and R. Kinne. 1976. Sodium/proton antiport in brush-border membrane vesicles isolated from rat small intestine and kidney. *Biochem. J.* 154:597-604.
11. Cassano, G., B. Steiger, and H. Murer. 1984.  $\text{Na}/\text{H}$  and  $\text{Cl}/\text{OH}$  exchange in rat jejunal and rat proximal tubular brush-border membrane vesicles: studies with acridine orange. *Pflügers Arch. Eur. J. Physiol.* 400:309-317.
12. Knickelbein, R. G., P. S. Aronson, W. Atherton, and J. H. Dobbins. 1983. Sodium and chloride transport across rabbit ileal brush-border. I. Evidence for  $\text{Na}^+\text{-H}^+$  exchange. *Am. J. Physiol.* 245 (Gastrointest. Liver Physiol. 8):G504-G510.
13. Gunther, R. D., and E. M. Wright. 1983.  $\text{Na}^+$ ,  $\text{Li}^+$  and  $\text{Cl}^-$  transport by brush-border membranes from rabbit jejunum. *J. Membr. Biol.* 74:85-94.
14. Ramaswamy, K., J. M. Harig, J. G. Kleinman, M. S. Harris, and J. A. Barry. 1989. Sodium-proton exchange in human ileal brush-border membrane vesicles. *Biochim. Biophys. Acta.* 981:193-199.
15. Foster, E. S., P. K. Dudeja, and T. A. Brasitus. 1986.  $\text{Na}^+\text{-H}^+$  exchange in rat colonic brush-border membrane vesicles. *Am. J. Physiol.* 250 (Gastrointest. Liver Physiol. 13):G781-G787.
16. Rajendran, V. M., and H. J. Binder. 1990. Characterization of  $\text{Na}/\text{H}$  exchange in apical membrane vesicles of rat colon. *J. Biol. Chem.* 265:8408-8414.
17. Knickelbein, R. G., P. S. Aronson, and J. W. Dobbins. 1988. Membrane distribution of sodium-hydrogen and chloride-bicarbonate exchangers in crypt and villus cell membranes from rabbit ileum. *J. Clin. Invest.* 82:2158-2163.
18. Dudeja, P. K., Foster, E. S., and T. A. Brasitus. 1987. Modulation of rat distal colonic brush-border membrane  $\text{Na}^+\text{-H}^+$  exchange by dexamethasone: role of lipid fluidity. *Biochim. Biophys. Acta.* 905:485-493, 1987.
19. Dudeja, P. K., Foster, E. S., R. Dahiya, and T. A. Brasitus. 1987. Modulation of  $\text{Na}^+\text{-H}^+$  exchange by ethinyl estradiol in rat colonic brush-border membrane vesicles. *Biochim. Biophys. Acta.* 899:222-228.
20. Wali, R., C. Baum, M. D. Sitrin, and T. A. Brasitus. 1989. Regulation of  $\text{Ca}^{2+}$  and  $\text{Na}^+/\text{H}^+$  exchange in  $\text{CaCo-2}$  cells by  $1,25(\text{OH})_2\text{D}_3$ . *Gastroenterology.* 96(5):A534.
21. Fuchs, R., J. Graf, and M. Peterlik. 1985. Effects of  $1,25$ -dihydroxycholecalciferol in sodium-ion translocation across chick intestinal brush-border membrane. *Biochem. J.* 230:441-449.
22. Schneider, L. E., H. P. Schedl, T. McCain, and M. R. Haussler. 1977. Experimental diabetes reduces circulating  $1,25$ -dihydroxyvitamin D in the rat. *Science (Wash. DC).* 196:1452-1454.
23. Patel, D. G. 1983. Rate of insulin infusion with a minipump required to maintain a normoglycemia in diabetic rats. *Proc. Soc. Exp. Biol. Med.* 172:74-78.
24. Brasitus, T. A., P. K. Dudeja, B. Eby, and K. Lau. 1986. Correction by  $1,25$ -dihydroxycholecalciferol of the abnormal fluidity and lipid composition of enterocyte brush-border membranes in vitamin D-deprived rats. *J. Biol. Chem.* 261:16404-16409.
25. Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. *J. Biol. Chem.* 248:2536-2541.
26. Dudeja, P. K., R. K. Wali, A. Klitzke, and T. A. Brasitus. 1990. Intestinal D-glucose transport and membrane fluidity along the crypt-villus axis of streptozocin-induced diabetic rats. *Am. J. Physiol.* (Gastrointest. Liver Physiol. 22):G571-G577.
27. Brasitus, T. A., D. Schachter, and T. Mamounas. 1979. Functional interaction of lipids and proteins in rat intestinal microvillus membranes. *Biochemistry.* 18:4136-4144.
28. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin Phenol reagent. *J. Biol. Chem.* 193:265-275.
29. Dudeja, P. K., E. S. Foster, and T. A. Brasitus. 1989.  $\text{Na}^+\text{-H}^+$  antiporter of rat colonic basolateral membrane vesicles. *Am. J. Physiol.* 257 (Gastrointest. Liver Physiol. 20):G624-G632.
30. Murer, H., U. Hopfer, and R. Kinne. 1976. Sodium/proton antiport in brush-border membrane vesicles isolated from rat proximal intestine and kidney. *Biochem. J.* 154:597-604.
31. Ives, H. E., V. J. Yee, and D. G. Warnock. 1983. Mixed type inhibition of the renal  $\text{Na}^+\text{-H}^+$  antiporter by  $\text{Li}^+$  and amiloride: evidence for a modifier site. *J. Biol. Chem.* 258:9710-9716.
32. Lee, H. C., and J. G. Forte. 1978. A study of  $\text{H}^+$  transport in gastric microsomal vesicles using fluorescent probes. *Biochim. Biophys. Acta.* 508:339-356.
33. Reenstra, W. W., D. G. Warnock, V. J. Yee, and J. G. Forte. 1981. Proton gradients in renal cortex brush-border membrane vesicles. Demonstration of a rheogenic proton flux with acridine orange. *J. Biol. Chem.* 256:11663-11666.
34. Warnock, D. G., W. Reenstra, and V. J. Yee. 1982.  $\text{Na}^+/\text{H}^+$  antiporter of brush-border vesicles: studies with acridine orange uptake. *Am. J. Physiol.* 242 (Renal Fluid Electrolyte Physiol. 11):F733-F739.
35. Brasitus, T. A., P. K. Dudeja, M. J. G. Bolt, M. D. Sitrin, and C. Baum. 1989. Dietary triacylglycerol modulates sodium-dependent D-glucose transport, fluidity and fatty acid composition of rat small-intestinal brush-border membranes. *Biochim. Biophys. Acta.* 979:177-186.
36. Kinsella, J., and B. Sacktor. 1985. Thyroid hormone increases  $\text{Na}^+\text{H}^+$  exchange activity in renal brush-border membranes. *Proc. Natl. Acad. Sci. USA.* 82:3606-3610.
37. Ghishan, F. K., S. Borowitz, and A. Mulberg. 1985. Phosphate transport by jejunal brush-border membrane vesicles of the streptozotocin-diabetic rat. *Diabetes.* 40:723-727.
38. Brasitus, T. A., and P. K. Dudeja. 1985. Correction of abnormal lipid fluidity and composition of rat ileal microvillus membranes in chronic streptozotocin-induced diabetes by insulin therapy. *J. Biol. Chem.* 260:12405-12409.
39. Olsen, W. A., and I. H. Rosenberg. 1970. Intestinal transport of sugars and amino acids in diabetic rats. *J. Clin. Invest.* 49:96-105.
40. Lineweaver, H., and D. Burk. 1934. Determination of enzyme dissociation constants. *J. Am. Chem. Soc.* 56:658-666.
41. Aronson, P. S., J. Nee, and M. A. Suhm. 1982. Modifier role of internal  $\text{H}^+$  in activating the  $\text{Na}^+\text{-H}^+$  exchanger in renal microvillus membrane vesicles. *Nature (Lond.).* 299:161-163.
42. Dudeja, P. K., E. S. Foster, and T. A. Brasitus. 1986. Regulation of  $\text{Na}^+\text{-H}^+$  exchange by transmethylation reaction in rat colonic brush-border membranes. *Biochim. Biophys. Acta.* 859:61-68.
43. Kinsella, J., T. Cujdik, and B. Sacktor. 1984.  $\text{Na}^+\text{-H}^+$  exchange in isolated renal brush-border membrane vesicles in response to metabolic acidosis: the role of glucocorticoid. *Proc. Natl. Acad. Sci. USA.* 81:630-634.
44. Tsai, C. J., H. E. Ives, R. J. Alpern, V. J. Yee, D. G. Warnock, and F. C. Rector. 1984. Increased  $V_{\text{max}}$  for  $\text{Na}^+\text{-H}^+$  antiporter activity in proximal tubule brush-border vesicles from rabbits with metabolic acidosis. *Am. J. Physiol.* 247 (Renal Fluid Electrolyte Physiol. 16):F339-F343.
45. Manganel, M., and R. J. Turner. 1990. Agonist-induced activation of  $\text{Na}^+/\text{H}^+$  exchange in rat parotid acinar cells is dependent on calcium but not on protein kinase C. *J. Biol. Chem.* 265:4284-4289.
46. Grinstein, S., and J. D. Goetz. 1985. Control of free cytoplasmic calcium by intracellular pH in rat lymphocytes. *Biochim. Biophys. Acta.* 819:267-270.
47. Semrad, C. E., and E. B. Chang. 1987. Calcium-mediated cyclic AMP inhibition of  $\text{Na}/\text{H}$  exchange in small intestine. *Am. J. Physiol. (Cell Physiol.* 21):C315-C322.
48. Semrad, C. E., E. J. Cragoe, Jr., and E. B. Chang. 1990. Inhibition of  $\text{Na}/\text{H}$  exchange in avian intestine by atrial natriuretic factor. *J. Clin. Invest.* 86:585-591.
49. Donowitz, M., J. Wicks, S. Cusolito, and G. W. G. Sharp. 1984. Cytosol free  $\text{Ca}^{2+}$  in the regulation of active intestinal  $\text{Na}$  and  $\text{Cl}$  transport. In *Mechanisms of Intestinal Electrolyte Transport and Regulation by Calcium*. M. Donowitz, editor. Alan R. Liss, Inc., New York. 171-189.
50. Favus, M. J., V. Tembe, M. D. Tanklefsky, K. A. Ambrosic, and H. N. Nellans. 1989. Effects of quinacrine on calcium active transport by rat intestinal epithelium. *Am. J. Physiol.* 257:G818-G822.
51. Schneider, L. E., and H. P. Schedl. 1972. Diabetes and intestinal calcium absorption in the rat. *Am. J. Physiol.* 223:1319-1323.
52. Wali, R. K., C. L. Baum, M. D. Sitrin, and T. A. Brasitus. 1990.  $1,25(\text{OH})_2$  vitamin  $\text{D}_3$  stimulates membrane phosphoinositides turnover, activates protein kinase C, and increases cytosolic calcium in rat colonic epithelium. *J. Clin. Invest.* 85:1296-1303.
53. Lieberherr, M., B. Grosse, P. Duchambon, and T. Druke. 1989. A functional cell surface type receptor is required for the early action of  $1,25$ -dihydroxyvitamin  $\text{D}_3$  on phosphoinositide metabolism in rat enterocytes. *J. Biol. Chem.* 264:20403-20406.
54. Ghijssen, W. E. J. M., M. D. DeJong, and C. H. VanOs. 1983. Kinetic properties of  $\text{Na}^+/\text{Ca}^{2+}$  exchange in basolateral plasma membranes of rat small intestine. *Biochim. Biophys. Acta.* 730:85-94.
55. Fine, L. G., B. Badie-Dezfooley, A. G. Lowe, A. Hamjeh, J. Wells, and S. Salehmoghaddam. 1985. Stimulation of  $\text{Na}^+\text{-H}^+$  antiport is an early event in hypertrophy of renal proximal tubular cells. *Proc. Natl. Acad. Sci. USA.* 82:1736-1740.
56. Moolenaar, W. H., Y. Yarden, S. W. deLaat, and J. Schlessinger. 1982. Epidermal growth factor induces electrically silent  $\text{Na}^+$ -influx in human fibroblasts. *J. Biol. Chem.* 257:8502-8506.
57. Grinstein, S., D. Rotin, and M. J. Mason. 1989.  $\text{Na}^+/\text{H}^+$  exchange and growth factor-induced cytosolic pH changes: role in cellular proliferation. *Biochim. Biophys. Acta.* 988:73-97.
58. Harris, R. C., J. L. Seifter, and B. M. Brenner. 1984. Adaptation of  $\text{Na}^+\text{-H}^+$  exchange in renal microvillus membrane vesicles. Role of dietary protein and uninephrectomy. *J. Clin. Invest.* 74:1979-1987.
59. Hopfer, W. 1975. Diabetes mellitus: changes in the transport properties of isolated intestinal microvillus membranes. *Proc. Natl. Acad. Sci. USA.* 72:2027-2031.
60. Fedorak, R. N., M. E. Gaon, and M. Field. 1986. Modification of intestinal  $\text{Na}/\text{K}\text{-ATPase}$  in experimental diabetes and with *in vitro* addition of glucagon. *Gastroenterology.* 90:A1412.