Volume 72
Number 1

Sodium-potassium stimulated adenosine triphosphatase of the small intestine of man: Studies in cholera and other diarrheal diseases

NORBERT HIRSCHHORN and IRWIN H. ROSENBERG*
Dacca, East Pakistan, and Boston, Mass.

Sodium-potassium stimulated, ouabain-inhibited adenosine triphosphatase (Na-K ATPase), an enzyme implicated in active sodium transport, was demonstrated in homogenates of human jejunal and ileal mucosa. An assay was designed to measure Na-K ATPase activity in peroral biopsy specimens of jejunum in patients with cholera and other diarrheal diseases. In cholera, acute nonspecific gastroenteritis, and bacterial enteritis there was a significant depression of Na-K ATPase activity during the acute phase of the disease as compared to convalescent values. No significant depression was observed of Na-K independent ATPase activity (unstimulated ATPase) in the same biopsy specimens.

The importance of the small intestine in fluid and electrolyte homeostasis is most dramatically demonstrated by the dehydration and electrolyte disturbances which may occur when the small intestine is diseased[1] or when large segments are resected or surgically bypassed.[2] Conservation of sodium and water by the intestine depends primarily on the ability of the intestinal epithelium to actively transport sodium from the intestinal lumen to the blood

From the Pakistan-SEATO Cholera Research Laboratory, Dacca; and the Thorndike Memorial Laboratory, II and IV (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

Supported in part by Research Agreement No. 196802 between the National Institutes of Health, and the Pakistan-SEATO Cholera Research Laboratory, and in part by Public Health Service Grants FR-0076, AM-09115, and AM-5413 from the National Institutes of Health.

The Pakistan-SEATO Cholera Research Laboratory is a part of the SEATO-Cholera Research Program and is supported by the United States Department of State, Agency for International Development, the National Institutes of Health, the National Communicable Disease Center of the Department of Health, Education and Welfare, United States Public Health Services, and by the Governments of Pakistan, United Kingdom, and other SEATO nations. The NIH Cholera Advisory Committee coordinates the research program.

Received for publication Nov. 2, 1967.

Accepted for publication March 13, 1968.

*Reprint requests: Dr. Irwin H. Rosenberg, Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass. 02118.

plasma; water movement is largely dependent on sodium flux.[3] Since lumen to plasma flux of sodium occurs against an electrochemical gradient, the process requires metabolic energy[4] which is provided, in all likelihood, by adenosine triphosphate. While the molecular mechanism of active sodium transport is still not well understood, considerable evidence indicates that a membrane-bound adenosine triphosphatase, activated by sodium and potassium (Na-K ATPase), is integral to the sodium transport system.[5-8] This enzyme has been demonstrated in brush-border rich fractions of homogenates of mammalian intestinal mucosa[9-11] but not in man.

We have studied the characteristics of Na-K ATPase in human intestinal mucosa in vitro and have designed an assay which permits study of this enzyme in intestinal biopsies taken from patients with diseases in which intestinal electrolyte transport is deranged, such as cholera and gastroenteritis. The present study documents a reversible depression of intestinal Na-K ATPase activity in cholera and acute gastroenteritis without an associated depression of a nonspecific ATPase activity in the same biopsies.

Patients

All patients studied were admitted to the Pakistan-SEATO Cholera Research Laboratory (PSCRL) ward with the chief complaint of acute diarrhea. The pertinent clinical data are presented in the Appendix. The patients were divided into three groups. Group I had 10 adults with documented *V. cholera* infection; Group II was composed of 7 adults with acute, nonspecific gastroenteritis (no etiologic agent isolated and no antibody titer rise to *V. cholera*) who were studied at a time when there was little or no cholera present in the vicinity; Group III had 5 adults with bacterial enteritis proved by culture. To exclude patients with chronic malabsorption, only patients with normal xylose absorption in convalescence are included in this study.*

Methods

Biopsies. One or more biopsies were taken in the acute stage of the disease and the results compared to the findings in convalescence so that each patient could serve as his own control. The acute phase biopsy was done in all patients on the day after admission when dehydration and electrolyte imbalance had been corrected. The biopsy in convalescence was taken several days after the last liquid stool was passed (see Appendix for details). In addition, serial biopsies were performed on 6 patients. The results of the first acute and last convalescent biopsies were used to calculate the mean values for each group. All biopsies were done after an overnight fast.

A Crosby-Kugler capsule with radio-opaque tubing was used, and its position in the upper jejunum was confirmed by x-ray. The subsequent biopsies in any given patient were obtained at a similar distance from the ligament of Treitz. The biopsy was placed in ice-cold 12 mM. Tris-2 mM. EDTA and examined under the dissecting microscope. Homogenization was carried out as described below.

Each patient received a 5 Gm. dose of D-xylose within 18 hours following each biopsy. Urinary excretion of D-xylose in the following 5 hours was measured by the method of Roe and Rice[12] and results expressed as a percentage of the dose administered.

The patients were treated with intravenous fluid and electrolytes as required. No patient received antibiotics.

*Eight patients with nonspecific gastroenteritis and persistent xylose malabsorption in convalescence had ATPase levels in the acute state which were similar to the patients reported but, in contrast to the latter, the enzyme depression persisted after recovery from diarrhea.

Preparation of enzyme.

SURGICAL SPECIMENS. Specimens of grossly normal human ileum* or jejunum† were obtained during a resection operation of contiguous structures. The samples were placed immediately in iced isotonic Tris buffer pH 7.5. The mucosa was scraped off the muscularis and homogenized in a glass homogenizing tube in an ice bath for 90 seconds in 12 mM. Tris-2 mM. EDTA (pH 7.6 at 25° C.). The homogenate was spun at 850 × g at 5° C. for 20 minutes, the supernate discarded, and the sediment resuspended in 9 volumes of 12 mM. Tris-2 mM. EDTA, separated into fractions and stored at −60° F. for later assay. To evaluate the effects of deoxycholate, the homogenate was thawed and deoxycholate was added in appropriate concentration. This preparation was stored at 4° C. for 24 hours before use.

BIOPSY SPECIMENS. Homogenization of the biopsy specimen was carried out in an all-glass micro homogenizing tube with a glass pestle. The biopsy was homogenized for 90 seconds at 0° C. in 0.125 per cent sodium deoxycholate (prepared in 12 mM. Tris-2 mM. EDTA pH 7.5 at 25° C.) and stored at 5° C. for 24 hours. The final homogenate volume ranged from 0.6 ml. to 1.1 ml. depending on the size of the biopsy.

When a loose fitting glass pestle was used, it was possible in nearly every biopsy to grind away the mucosa leaving intact a round submucosal portion which resembled a wig, with a few adhering villi on the mucosal side and a fibrous matrix on the submucosal side (as seen under the dissecting microscope). To measure the degree of dilution of mucosal ATPase activity by submucosa, assays of ATPase activities were done on homogenates of these submucosal portions as well as on the corresponding mucosal portion in 29 biopsies from patients with cholera and noncholera diarrheas in the acute and convalescent states. The results are compared below.

In Group I all assays were done on the mucosal portion only; in Group II no attempt was made to separate the mucosa from submucosa and assays were done on the total biopsy homogenate; and in 3 of 5 patients in Group III assays were done on the mucosal portions only.

Assay of ATPase activities. In all experiments 0.1 ml. of homogenate was added to 2.4 ml. of medium which contained 4 mM. magnesium: One solution contained 140 mM. sodium, 16 mM. potassium; a second solution contained no added Na^+ and K^+. The anion in each case was chloride. Both solutions had 2.5 mM. ATP (Tris or dipotassium salt) and 30 mM. Tris and the final pH of both was 7.4 at 37° C. The reactions were performed in triplicate or duplicate and stopped after 60 minutes with 1.5 ml. of 6M perchloric acid. Inorganic phosphate liberated by the reaction was measured by the method of Fiske and Subbarow,[13] and the homogenate protein was measured by the method of Lowry and associates.[14] ATPase activity was expressed in micrograms inorganic phosphorus released per milligram protein per hour (μg Pi/mg. protein/hour). Na-K ATPase activity was obtained by subtracting the activity in the absence of Na^+ and K^+ (unstimulated ATPase) from that in the presence of sodium and potassium. All glassware was acid-washed.

Standard enzyme assay. Samples of guinea pig small intestinal mucosal homogenates stored at −60° C. in 12 mM. Tris-2 mM. EDTA (pH 7.5 at 25° C.) were assayed along with human biopsies and constituted a standard assay. Results from 65 fractions taken from 5 animals gave a S.E.M. of ± 4.1 per cent of Na-K ATPase mean activity and a S.E.M. of ± 1.8 per cent of unstimulated ATPase mean activity.

Results

Identification of ATPase in surgical specimens of small intestine.

CATION REQUIREMENTS. When mucosal homogenates of ileum and jejunum were incubated with ATP without added cations, no significant hydrolysis occurred. In the presence of Mg^{++}, phosphate liberation from ATP was dem-

*Obtained through the Harvard Surgical Service, Boston City Hospital.
†Courtesy of Dr. Md. Idris, Holy Family Hospital, Dacca, East Pakistan.

onstrated.
ulated AT
This incre
mal nons
centration
140 mM.
mM. for
of both i
Fig. 1.

EFFE
Na-K AT
often en
with de
ATPase
ATPase.

EFFI
in both
is simila
testinal

EFF
by ouab
is show
and jej
10^{-7} M.
of 10^{-5}

Fig. 1.
of jej
EDTA

onstrated. An increment in ATP hydrolysis beyond this nonspecific or unstimulated ATPase occurs when Na^+ and K^+ are added to the incubation medium. This increment is designated as sodium-potassium stimulated ATPase. Maximal nonspecific ATPase and Na-K ATPase activity were found at Mg^{++} concentration of 4 mM. Optimal concentrations of Na^+ and K^+ were found to be 140 mM. and 8 mM., respectively, for the homogenate of ileum, and 80 and 8 mM. for jejunum. At optimal concentrations of cations, Na-K ATPase activity of both ileum and jejunum is linear with incubation time up to 120 minutes Fig. 1.

EFFECT OF DEOXYCHOLATE OF Na-K ATPASE (TABLE I). In preparations of Na-K ATPase from other tissues, treatment with surface active agents has often enhanced Na-K ATPase activity. The increase in Na-K ATPase activity with deoxycholate treatment appears to occur at the expense of unstimulated ATPase activity. Deoxycholate at 0.25 per cent is inhibitory to both modes of ATPase.

EFFECT OF pH ON ATP HYDROLYSIS. Maximal hydrolysis was found to occur in both ileum and jejunum at pH 7 to 7.5 as shown in Fig. 2. The pH optimum is similar to that found for Na-K ATPase activities in other mammalian intestinal preparations.

EFFECT OF OUABAIN ON Na-K ATPASE. Inhibition of Na-K ATPase activity by ouabain is a consistent characteristic of the enzyme. A dose-response curve is shown in Fig. 3, where one-half of the maximal inhibition of both ileum and jejunum Na-K ATPase activity occurs at ouabain concentration of 5×10^{-7} M, and total inhibition of Na-K ATPase activity is found at concentration of 10^{-5} M. Ouabain had no effect on unstimulated ATPase activity.

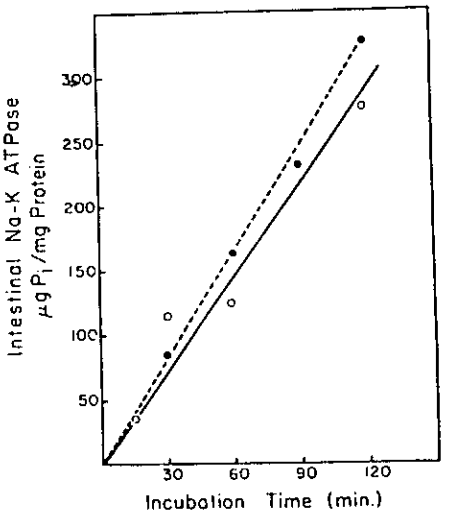

Fig. 1. Na-K ATPase activity related to incubation time in mucosa from surgical specimens of jejunum (closed circles) and ileum (open circles). Homogenate stored at -60° in Tris-EDTA until use.

Table I. Effect of storage in deoxycholate (DOC) on jejunal ATPase

Treatment	*Surgical specimens of jejunum**			*Peroral jejunal biopsies†*		
	Unstimulated ATPase	*Na-K ATPase*	*Na-K ATPase % of total*	*Unstimulated ATPase*	*Na-K ATPase*	*Na-K ATPase % of total*
None	172	129	43	252	20	7 (5)
Stored 24 hours no added DOC	168	124	42	—	—	—
Stored 24 hours in 0.0675% DOC	160	134	46	—	—	—
Stored 24 hours in 0.125% DOC	93	155	62	131	120	47 (22)
Stored 24 hours in 0.25% DOC	36	34	49	67	45	39 (4)

ATPase expressed in micrograms Pi per milligram protein per hour.
*Mean values of experiments done in triplicate.
†Mean values of convalescent phase biopsies (number of biopsies in parentheses).

Table II. Effect of sodium, potassium, and ouabain on ATPase activity in peroral jejunal biopsies

*Additions**	*Activity (μg Pi/mg. protein/hour)*	
	Experiment 1	*Experiment 2*
None	193	117
Na^+ (140 mEq./L.), K^+ (16 mEq./L.)	331	290
Na^+ (140 mEq./L.), K^+ (16 mEq./L.), and ouabain (10^{-3}M)	196	117

*Mg^{++} (4 mEq. per liter) in all experiments.

ATPase activity in peroral intestinal biopsies. On the basis of the above experiments an assay was designed for peroral biopsy specimens of human jejunum. In our experience very little Na-K ATPase activity could be detected in fresh homogenates of biopsy tissue in contrast to the homogenate of surgical material (possibly the scraping, homogenization, and centrifugation of the surgical material disrupted cellular membranes considerably). Storage with deoxycholate for 24 hours resulted in the appearance of Na-K ATPase activity roughly equal to unstimulated ATPase activity (Table I). Ouabain (10^{-3} M) completely inhibited the sodium and potassium stimulated activity (Table II).

ATPase activities in the mucosal and submucosal portions. Table III shows the activities in these two fractions separately and after arithmetical combination of specific activities. It can be seen that the submucosal portions had considerably less Na-K ATPase activity than the mucosal portions, but unstimulated ATPase activity was the same in both.

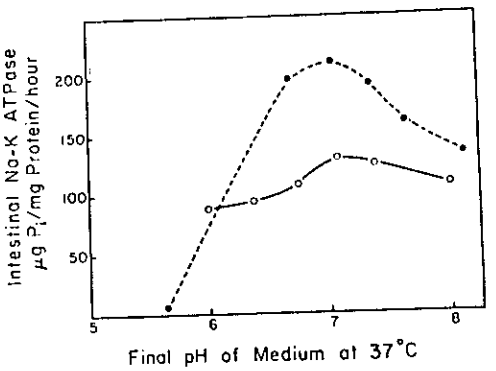

Fig. 2. Na-K ATPase activity related to final pH of medium at 37° C. in mucosa from surgical specimens of jejunum (closed circles) and ileum (open circles). Homogenate stored at -60° in Tris-EDTA until use.

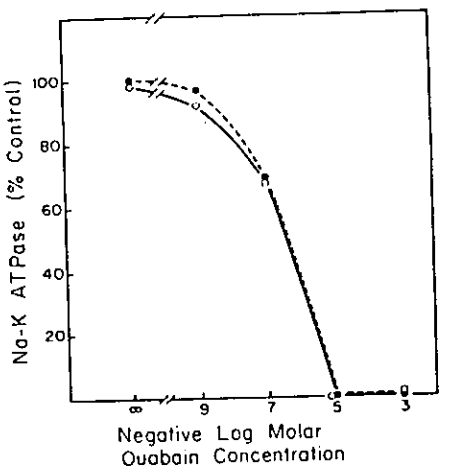

Fig. 3. Na-K ATPase activity related to varying concentrations of ouabain in mucosa from surgical specimens of jejunum (closed circles) and ileum (open circles). Homogenate stored at -60° in Tris-EDTA until use.

Table III. Mean ATPase activity in the mucosal and submucosal portions of jejunal peroral biopsies

	No. observations	*Mean protein per biopsy (mg.)**	*ATPase (μg Pi/mg. protein/hour*)*	
			Na-K ATPase	*Unstimulated ATPase*
Mucosal portion	29	1.32 ± 0.1	106 ± 5.8	125 ± 7.8
Submucosal portion	29	0.9 ± 0.06	35 ± 4.5	129 ± 7.0
Arithmetic combination	29	2.225 ± 0.52	76.2 ± 6.0	127.2 ± 7.0

*Mean ± S.E.M.

Studies in cholera and gastroenteritis.

GROUP I (CHOLERA). Ten patients were studied during an episode of acute cholera and again during the convalescent period. Biopsy ATPase activities are given on Table IV. Seven patients had depressed Na-K ATPase activity, 6 patients markedly so, in the acute state, and 3 did not. The difference between the means of acute and convalescent values for the whole group was statistically significant, and the average depression was 29 per cent. In contrast another intestinal enzyme, unstimulated ATPase, was not significantly changed in the same biopsies. Patients with lowered Na-K ATPase activity in the acute state did not differ clinically from those without it. All patients had normal absorption during the convalescent period as judged by the D-xylose test (excretion of more than 25 per cent of an oral dose).

To minimize dependence on single observations during and after diarrhea, serial biopsies were performed in 5 patients with cholera during the course of their illness. In Fig. 4, Na-K ATPase activity is plotted against the daily stool output. The rise in Na-K ATPase activity occurred during (Patient 2) or soon after cessation of diarrhea (Patients 1, 5, and 6). A fifth patient (No. 10) showed depression of Na-K ATPase activity during diarrhea and only partial recovery thereafter.

GROUP II. Seven patients with acute nonspecific gastroenteritis had biopsies taken in the acute stage of the disease and during convalescence. The results are given on Table V. The mean values for the acute and convalescent Na-K ATPase activities were significantly different. The average depression during the acute phase was about 43 per cent. The unstimulated ATPase did not change significantly.

GROUP III. Five adults with diarrhea caused by bacterial agents were studied. Two had diarrhea associated with noncholera vibrios,[15] one had staph-

Table IV. Group I, cholera

Patient	*Na-K ATPase*			*Unstimulated ATPase*		
	Acute	*Convalescent*	*Change*	*Acute*	*Convalescent*	*Change*
1	75	187	+ 112	182	193	+ 11
2	78	161	+ 83	146	141	- 5
3	135	216	+ 81	192	230	+ 38
4	45	115	+ 70	131	144	+ 13
5	62	117	+ 55	144	75	- 69
6	47	102	+ 55	81	82	+ 1
7	122	128	+ 6	167	165	- 2
8	110	103	- 7	200	188	- 12
9	144	116	- 28	185	175	- 10
10*	112	68	- 44	104	95	- 9
Mean	93	131.3	+ 38.3	153.2	148.8	- 4.4
S.E.M.±	11.4	13.9	16.7	12.4	16.3	8.6
p	< 0.05		< 0.05	> 0.8		> 0.6

*Second acute biopsy: Na-K ATPase 46; unstimulated ATPase 147.

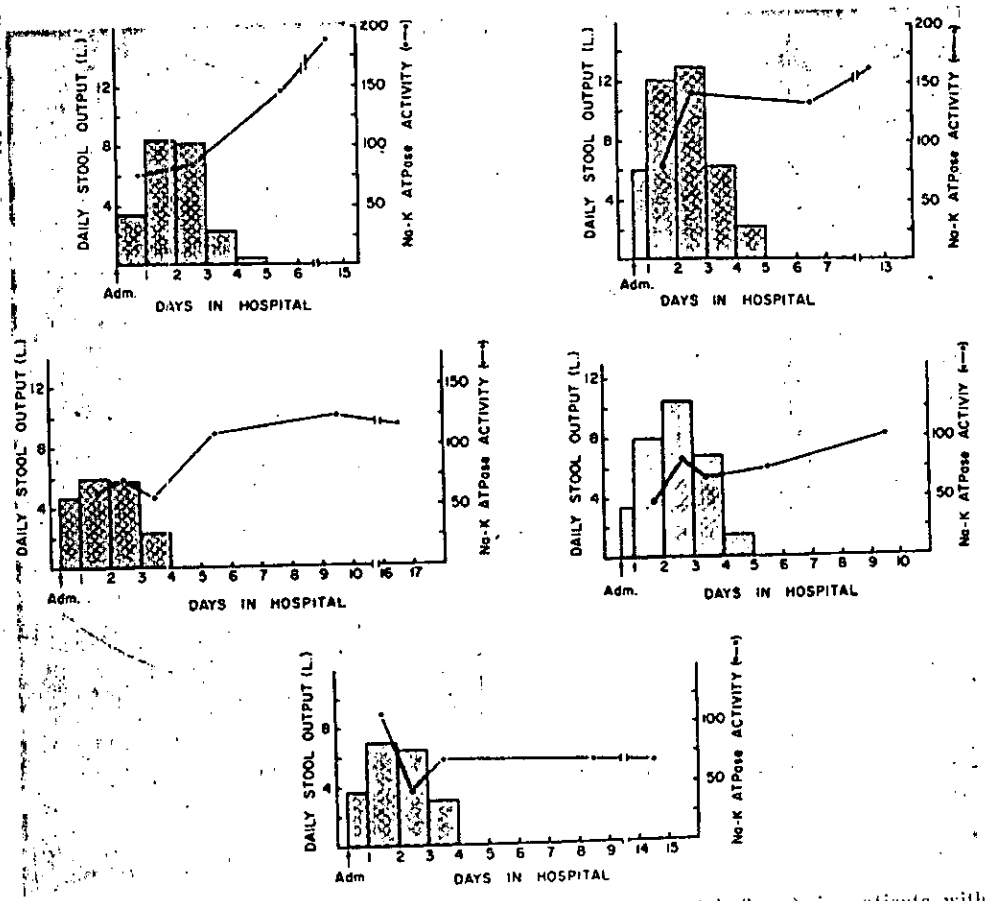

Fig. 4. Jejunal biopsy Na-K ATPase activity (μg Pi/mg. protein/hour) in patients with cholera (Group I) compared with stool output, related to time course of illness. From upper left to right: Patients Nos. 1, 2, 5, 6, and 10.

Table V. Group II, acute nonspecific gastroenteritis

	Na-K ATPase			*Unstimulated ATPase*		
Patient	*Acute*	*Convalescent*	*Change*	*Acute*	*Convalescent*	*Change*
1	18	114	+96	104	102	- 2
2	19	95	+76	152	179	+27
3	70	107	+37	129	120	- 9
4	80	112	+32	80	101	+21
5	68	91	+23	105	127	+22
6	75	95	+20	118	109	- 9
7	65	84	+19	114	91	-23
Mean	56.4	99.7	+43.3	114.6	118.4	+ 3.9
S.E.M.±	9.2	4.0	10.7	7.9	6.8	6.8
p		< 0.001	< 0.01		> 0.7	> 0.5

Table VI. Group III, bacterial enteritis

Patient's diagnosis	*Na-K ATPase*			*Unstimulated ATPase*		
	Acute	*Convalescent*	*Change*	*Acute*	*Convalescent*	*Change*
Diarrhea with non-cholera vibrios*	74	112	+ 38	98	92	6
Diarrhea with non-cholera vibrios*	55	96	+ 41	132	106	- 26
Shigella B dysentery	24	166	+ 142	133	94	- 39
Staphylococcal enteritis	36	114	+ 78	63	159	+ 96
Typhoid fever†	116	131	+ 15	93	124	+ 31
Mean	61	123.8	+ 62.8	103.8	115	+ 11.2
S.E.M.±	16.2	11.9	22.2	13.2	12.4	24.3
p		< 0.02	< 0.05		> 0.5	> 0.6

*Submucosa included in homogenate of biopsy.
†Second acute biopsy Na-K ATPase 71; unstimulated ATPase 120.

ylococcal enteritis, one had shigella B dysentery, and one had salmonella D septicemia with copious diarrhea (15.7 L. over 5 days) from which no pathogen was isolated. All had depression of Na-K ATPase activity in the acute state with recovery during convalescence (Table VI), although the patient with typhoid showed this depression only in the second acute biopsy. The mean change was 51 per cent.

Serial biopsies were performed on the patient with staphylococcal enteritis. The highest convalescent value of Na-K ATPase activity was not achieved for this patient until one week after diarrhea ceased.

Examination of the specimens under the dissecting microscope showed no consistent morphological difference between the acute and convalescent biopsies. Although histological examinations were not done on the biopsies reported here, microscopic studies on biopsies from similar patients with cholera and with other, nonspecific, diarrheas showed the epithelial cells to be normal in both acute and convalescent biopsies, and the minimal increase in inflammatory cell infiltrate in the acute biopsies was so subtle as to require statistical verification. The details of these microscopic studies are reported elsewhere.[16]

Discussion

Cholera is perhaps the most dramatic of diarrheal diseases in that fluid losses are commonly severe enough to result in either shock or fatal dehydration; in the treated patient, losses may exceed the total body weight over the course of the disease.[1] Although ample evidence indicates that loss of fluid into the intestine is not due to denudation or destruction of intestinal epithelium,[17, 18] the relative importance of increased fluid movement into the intestine versus decreased sodium and water reabsorption from the intestine remains a subject of controversy. Phillips[1] suggested a mechanism for the latter possibility; he

postulated that inhibition of active sodium transport in the small intestine by some product of *V. cholerae* could account for the copious, sodium-rich diarrhea.

Skou[19] was the first to suggest that an enzyme requiring sodium, potassium, and magnesium ions for the hydrolysis of ATP was involved in the active transport of sodium. His recent review[5] summarized eight requirements of a sodium transport system, all of which are shared by the Na-K ATPase enzyme. In the present study we have demonstrated the presence of an ATPase in both ileum and jejunum of human intestine, which is stimulated by sodium-potassium and inhibited by ouabain. The Na-K ATPase of human intestinal epithelium, like that of other tissues, shares the cardinal characteristics of the sodium transport system. While it has not been unequivocally proved that Na-K ATPase of the intestine, or of other tissues, is responsible for sodium transport, the indirect evidence is convincing and we therefore thought it important to measure the activity of this enzyme in the small intestine during sodium-losing diarrhea.

We have demonstrated in biopsy specimens depressed Na-K ATPase activity in 7 patients with cholera. A similar depression of enzymatic activity was seen in other forms of gastroenteritis with diarrhea. Noncholera diarrheas in East Pakistan may be as severe as cholera and perhaps share some mechanisms of pathogenesis.[20]

Interpretations of these observations must take into account the limitations of this study. First, assay of an enzyme in a single biopsy may not necessarily reflect the enzyme level over the remainder of the intestine. Second, although we presume that the convalescent values reflected premorbid enzyme activity, this is not yet proved. Third, ATPase activity in such a biopsy homogenate represents a composite of enzymes from a mixed cellular population; the assay cannot distinguish among membrane sites whose function may vary with respect to the transport of sodium. Should there occur in cholera and other diarrheas a large influx of leukocytes into the lamina propria of the intestine, leukocyte ATPase activity, with perhaps a large nonspecific ATPase to Na-K ATPase activity ratio,[21] would be indistinguishable from intestinal ATPase in the assay. This would influence not only the ratio of Na-K ATPase to nonspecific ATPase, but also the nonenzyme protein content of the biopsy. This seems an unlikely explanation of the data since individuals in East Pakistan without acute diarrheal disease show a chronic inflammatory cell infiltrate,[22] and the increase in cellular infiltrate during acute diarrhea is so subtle that it is only apparent when a large number of biopsies are examined in the absence of information concerning the clinical status of the patient.[16] Separation of mucosal and submucosal fractions should further minimize any error introduced by inflammatory cells.

The significance of depressed Na-K ATPase in acute diarrheal disease is uncertain. It is most important to distinguish between an enzyme depression which merely reflects the altered condition of diseased epithelium and one that has functional significance. If the Na-K ATPase depression demonstrated in this study has any functional significance, it is unlikely that the decrease in enzyme activity is a major factor in sodium and water malabsorption. A quantitative relationship of enzyme depression to diarrhea was lacking. Diarrhea, however, may be associated with injury to membrane enzymes; functions

dependent on these enzymes may then be impaired. For example, reversible depression of jejunal disaccharidases and impaired lactose hydrolysis occur in acute diarrhea.[23] Similarly, depression of an enzyme which may be involved in sodium absorption from the gut could contribute to the formation of diarrheal fluid. In this study, damage to membrane enzymes was not entirely nonspecific as unstimulated ATPase, which has not been implicated in sodium transport, was not significantly impaired. The possibility exists, therefore, that the depression of Na-K ATPase in cholera and other enteric disease may contribute to a worsening of, if not the initiation of, the intestinal fluid loss.

Final interpretation of the functional significance of these observations must await further information on the role of Na-K ATPase in sodium transport by intestine and more definitive data on the mechanisms of diarrhea in cholera and other enteric infections.

The authors acknowledge the technical assistance of Mr. J. R. Saha, Miss Ayesha Siddiqea, and Mr. S. M. Alam and the suggestions of Drs. John Lindenbaum, Robert A. Phillips, and Charles S. Davidson.

REFERENCES

1. Phillips, R. A.: Water and electrolyte losses in cholera, Fed. Proc. 23: 705, 1964.
2. Jackson, W. P. U.: Massive resection of the small intestine, *in* Avery-Jones, editor: Modern trends in gastroenterology, New York, 1958, Paul B. Hoeber, Inc., ser. 2, p. 243.
3. Curran, P. F.: Ion transport in the intestine and its coupling to other transport processes, Fed. Proc. 24: 993, 1965.
4. Curran, P. F.: Na, Cl and water transport by rat ileum in vitro, J. Gen. Physiol. 43: 1137, 1960.
5. Skou, J. C.: Enzymatic basis for active transport of Na^+ and K^+ across cell membrane, Physiol. Rev. 45: 596, 1965.
6. Post, R. L., Merritt, C. R., Kinsolving, C. R., and Albright, C. D.: Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte, J. Biol. Chem. 235: 1796, 1960.
7. Dunham, E. T., and Glynn, I. M.: Adenosine triphosphatase activity and the active movements of alkali metal ions, J. Physiol. 156: 274, 1961.
8. Bonting, S. L., and Canady, M. R.: Na-K activated adenosine triphosphatase and sodium transport in toad bladder, Am. J. Physiol. 207: 1065, 1964.
9. Taylor, C. B.: Cation-stimulation of an ATPase from the intestinal mucosa of the guinea pig, Biochim. et biophys. acta 60: 437, 1962.
10. Berg, G. G., and Chapman, B.: The sodium and potassium activated ATPase of intestinal epithelium. I. Location of enzymatic activity in the cell, J. Cell. & Comp. Physiol. 65: 361, 1965.
11. Rosenberg, I. H., and Rosenberg, L. E.: Localization and characterization of ATPase in guinea pig intestinal epithelium, Comp. Biochem. Physiol. 24: 975, 1968.
12. Roe, J. H., and Rice, E. W.: Photometric method for determination of free pentoses in animal tissues, J. Biol. Chem. 173: 507, 1948.
13. Fiske, C. H., and Subbarow, Y.: The colorimetric determination of phosphorus, J. Biol. Chem. 66: 375, 1925.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the folin phenol reagent, J. Biol. Chem. 193: 265, 1957.
15. McIntyre, O. R., Feeley, J. C., Greenough, W. B., III, Benenson, A. S., Hasan, S. I., and Saad, A.: Diarrhea caused by non-cholera vibrios, Am. J. Trop. Med. & Hygiene 14: 412, 1965.
16. Kent, T. H., and Lindenbaum, J.: Correlation of jejunal function and morphology in patients with acute and chronic diarrhea in East Pakistan, Gastroenterology 52: 972, 1967.

17. Fresh, J. W., Versage, P. M., and Reyes, V.: Intestinal morphology in human and experimental cholera, Arch. Path. 77: 529, 1964.
18. Gangarosa, E. J., Beisel, W. R., Benyajati, C., Sprinz, H., and Piyaratn, P.: The nature of the gastrointestinal lesion in Asiatic cholera and its relation to pathogenesis: A biopsy study, Am. J. Trop. Med. & Hygiene 9: 125, 1960.
19. Skou, J. C.: The influence of some cations on an adenosine triphosphatase from peripheral nerves, Biochim. et biophys. acta 23: 394, 1957.
20. Lindenbaum, J., Greenough, W. B., III, Benenson, A. S., Oseasohn, R., Rizvi, S., and Saad, A.: Non-vibrio cholera, Lancet 1: 1081, 1965.
21. Block, J. B., and Bonting, S. L.: Sodium-potassium activated adenosine triphosphatase and cation transport in normal and leukemic human leukocytes, Enzym. Biol. et Clin. 4: 183, 1964.
22. Lindenbaum, J., Alam, A. K. M. J., and Kent, T. H.: Subclinical small-intestinal disease in East Pakistan, Brit. M. J. 2: 1616, 1966.
23. Hirschhorn, N., Saha, J. R., and Siddiqea, A.: Jejunal disaccharidase activity in acute diarrhea and convalescence. In preparation.

Appendix

Summary of clinical findings (means; ranges in parentheses)

	Patient group		
	Group I	*Group II*	*Group III*
	Cholera	*Acute nonspecific gastroenteritis*	*Bacterial enteritis*
Number	10	7	5
Age (years)	24.2 (12-40)	29 (19-50)	25 (19-30)
Weight, convalescent (Kg.)	39.8 (31.3-48.3)	44 (36.4-54)	40.1 (36.3-45.3)
Admission systolic B.P.	25 (0-100)	64 (0-80)	72 (0-120)
Number with fever over 100° F., p.r.	1	5	3
Plasma protein (Gm. %)			
Admission	11 (8.8-13.0)	10.3 (7.4-12.1)	10.3 (7.7-12.0)
After rehydration	7.8 (7.0-8.3)	7.4 (7.0-8.3)	7.4 (6.6-7.9)
Total stool volume (L.) in hospital	24.4 (3.3-58.0)	1.1 (0-3.6)	4.8 (0.2-15.7)
Number with occult blood in stool	4	4	4
Number with intestinal parasites	7 (3 multiple)	4 (1 multiple)	3 (1 multiple)
Xylose absorption (% excretion in urine)*			
Acute	15.6 (9-22)	25.4 (10-44)	19 (10-34)
Convalescent	33 (25-46)	34.3 (26-41)	31 (24-37)
Biopsy			
First acute biopsy hours after onset	38 (31-52)	30 (23-41)	35 (25-60)
Last convalescent biopsy (days after last liquid stool)	8 (4-13)	8 (6-13)	8 (4-12)

*Normal 25 per cent.