Pediat. Res. *12:* 1033–1038 (1978)

Calcium
calcium transport
cystic fibrosis
erythrocyte membranes

Mg^{2+}-ATPase
Mg^{2+}-dependent Ca^{2+}-ATPase
Na^+, K^+-ATPase

Calcium and Sodium Transport Processes in Patients with Cystic Fibrosis. I. A Specific Decrease in Mg^{2+}-Dependent, Ca^{2+}-Adenosine Triphosphatase Activity in Erythrocyte Membranes from Cystic Fibrosis Patients

SIDNEY KATZ[34]

Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Summary

Calcium-ATPase activity (Mg^{2+}-dependent Ca^{2+}-ATPase, ATP phosphohydrolase, EC 3.6.1.3) in erythrocyte membrane preparations from cystic fibrosis (CF) patients was greatly reduced compared to erythrocyte membranes from control subjects. The K_m for calcium was found to be similar in the two groups; however, the V_{max}, the maximal rate of activation of the Ca^{2+}-ATPase, is reduced by 50% in the erythrocyte membrane preparations of the CF patients ($P < 0.001$). In contrast, the Mg^{2+}-ATPase activity of erythrocyte membranes from CF patients was unchanged compared to the control subjects. No difference in the Na^+,K^+-ATPase activity in erythrocyte membranes from CF patients compared to control patients could be observed. This indicates that the Ca^{2+}-ATPase activity noted in CF erythrocytes is not part of a generalized membrane or membrane-bound enzyme alteration. It remains to be determined whether this alteration in Ca^{2+}-ATPase activity is directly related to a defect in calcium transport in these cells and is a generalized phenomenon in CF present in cell types more directly involved in secretion.

Speculation

If the alteration in Mg^{2+}-dependent Ca^{2+}-ATPase activity is found to be specifically related to calcium transport in erythrocytes and general to other cell types in patients with CF, then it might indicate a basic alteration in the ability of these cells to transport calcium and might be important in the overall manifestation of the disease.

Although CF is manifested in a variety of abnormalities, a generally consistent finding is the increased levels of calcium and sodium in many of the glycoprotein-rich secretions of these patients (3, 9, 11, 15, 23, 28, 30). This suggests that a basic defect in CF might be associated with a calcium- and/or sodium-dependent membrane function such as secretion or transport.

The Mg^{2+}-dependent Ca^{2+}-ATPase (ATP phosphohydrolase, EC 3.6.1.3, Ca^{2+}-ATPase) has been shown to be the biochemical basis of the calcium transport mechanism in a variety of cells (20, 27). There are some reports in the literature regarding a possible alteration in the Ca^{2+}-ATPase activity in CF patients (10, 17). Horton *et al.* (17) observed that Ca^{2+}-ATPase activity was depressed in erythrocytes from CF patients compared to that in control subjects and noted that this depression was closely related to the severity of the disease. Other workers (13, 24) did not observe any alteration in the Ca^{2+}-ATPase activity in erythrocytes from CF patients.

The existence of a defect in ion transport or ATPase activity in CF has yet to be determined with certainty. In these present experiments, conditions used to characterize the sodium and calcium pump mechanisms in erythrocyte membranes (4, 5, 19) are utilized to determine whether a decrease in specific components of ATPase activity exists in CF.

In trying to detect such an abnormality, as a first step, Ca^{2+}-ATPase, Mg^{2+}-dependent, (Na^+ + K^+)-activated ATPase and Mg^{2+}-ATPase were studied in erythrocyte membranes of CF patients and control subjects.

The reasons for using erythrocyte membranes in this study are 2-fold: 1) the relative ease by which erythrocytes can be obtained from CF patients in comparison with samples from other tissues and the relative purity of the membrane preparations that can be obtained; 2) the abundant available information on cation transport and erythrocyte membrane composition and the substantial evidence that exists linking Na^+ and Ca^{2+} transport in erythrocytes to the respective ion-dependent ATP hydrolytic activities (4, 5, 19, 27). A preliminary report of this work has been presented (18).

MATERIALS AND METHODS

Erythrocyte membranes were prepared by 1:10 osmotic lysis in distilled water of fresh saline-washed erythrocytes. After centrifugation, the posthemolysate residue was washed with solutions containing ethylenediaminetetraacetate (EDTA, 1.0–10.0 mM, pH 7.4) as described previously (4). The preparation thus obtained was essentially white. The residue was made up to one-half the original packed cell volume of the washed cells and stored at 4°. The protein concentration, as determined by the Lowry technique (22), was approximately 4 mg/ml. The preparation could be stored for up to 6 days at 4° without significant loss of Ca^{2+}-ATPase or Na^+,K^+-ATPase activity. The membranes used in this study were prepared immediately after collection of the blood samples and were utilized within 48 hr. The membrane preparation was frozen and thawed twice before use to ensure complete permeability of substrates.

CF blood samples were obtained from patients attending the Vancouver Health Center Cystic Fibrosis Clinic (31, 32). Approximately 10 ml blood was drawn from each patient directly into citrate buffer. Control samples were obtained from student volunteers at the same time and under exactly the same conditions. The age range of the CF donors (male and female) was 16–27 years with a mean age of 20 years and that of the control subjects (male and female) was 22–32 years with a mean age of 27 years. The patients chosen were attending the Cystic Fibrosis Clinic for routine follow-up. All had been identified as having CF on the

basis of elevated sweat chloride. The patients had varying degrees of respiratory and digestive problems. Some of the patients were receiving pancreatic enzymes and some were receiving antibiotics.

In previous studies (19) the properties of a Mg^{2+}-dependent, Ca^{2+}-stimulated ATPase activity and the associated Ca^{2+}-dependent phosphorylation of a 150,000 mol wt component were studied in erythrocyte membranes prepared as described above. The behavior of the phosphorylated component was consistent with its role as a phosphorylated intermediate of Ca^{2+}-ATPase activity, including a similar dependence of the steady state level of the phosphorylated intermediate and Ca^{2+}-ATPase on ATP concentration, rapid turnover upon the addition of EGTA, and a good correlation between the steady state levels of Ca^{2+}-dependent phosphorylation and Ca^{2+}-ATPase activity in separate preparations possessing variable specific activity. Under these conditions only one Ca^{2+}-ATPase activity was detected and it was associated with the rapid acyl phosphate membrane phosphorylation described. The Ca^{2+}-ATPase studied is therefore believed to be associated with the calcium transport mechanism. Similarly, under these conditions, ATPase activity related to the Na^+-dependent phosphoprotein intermediate step of Na^+,K^+-ATPase was present (4, 5, 19).

To determine ATPase activity, the reaction mixture contained 25 μM $MgCl_2$, 40 mM Tris-HCl, pH 7.4, 3.4 μM [γ-^{32}P]ATP (1–3 μCi/μmol), and 0.35 ml diluted membranes (2 mg/ml) in the presence or absence of the desired Na^+ or Ca^{2+} concentration in a final volume of 0.5 ml. When calcium was present in the incubation medium it was added together with ethylene-glycolbis-(β-aminoethyl)-N,N'-tetraacetate (EGTA) in order to ensure the presence of the desired free calcium concentration. The free Ca^{2+} concentration was then determined by the association constant for the interaction of EGTA with Ca^{2+} at pH 7.4 (8). The ATPase assay at 37° was begun by the addition of the membranes. The reaction was terminated after 2 min, so that less than 20% of the substrate had been hydrolyzed by the addition of 5% trichloroacetic acid containing 5 mM ATP and 2 mM KH_2PO_4. A suspension of charcoal-trichloroacetic acid (1.5 g/10 ml) was then added to each sample, the samples incubated at 4° for 1 hr with intermittent mixing and, after centrifugation, an aliquot of the clear supernatant counted for radioactivity. This entire procedure was carried out using plastic microcentrifuge tubes (1.5 ml) and a desktop microcentrifuge (Eppendorf Co.).

The results are expressed as picomoles $^{32}P_i$ released per mg protein per min. Mg^{2+}-ATPase refers to that activity found in the absence of added Na^+ or Ca^{2+}; Ca^{2+}-ATPase activity refers to that activity present after subtraction of the Mg^{2+}-ATPase component. Na^+,K^+-ATPase is that activity found in the presence of Na^+ after subtraction of the Mg^{2+}-ATPase; this activity is insensitive to activation by K^+ at low concentrations of ATP (4, 5) and K^+ was therefore omitted from the incubation medium.

The Student *t*-test for unpaired data was used as a measure of significance; $P < 0.05$ was chosen as the criterion of significance. Standard error of the mean was used as a measure of variation. The line of best fit was drawn by visual analysis in the double reciprocal plot analysis in these studies.

[γ-^{32}P]ATP of high specific activity (80,000–120,000 mCi/mmol) and free of polyphosphate contaminants was prepared by the method of Glynn and Chappel (16) as modified by Post and Sen (25). All other chemicals used in this study were purchased from Sigma Chemical Co. (ATP, EDTA, EGTA, Trizma base) and Mallinkrodt Co. (Scintillar scintillation fluid).

RESULTS

DETERMINATION OF Ca^{2+}-ATPase ACTIVITY OF ERYTHROCYTE MEMBRANES OBTAINED FROM NORMAL SUBJECTS

In a preliminary group of studies the effect of low free calcium concentrations on the ATPase activity of erythrocyte membranes obtained from normal subjects was investigated in order to find the most suitable concentrations at which to study the possible alterations in Ca^{2+}-ATPase activity in CF erythrocytes.

Figure 1 illustrates a typical calcium concentration curve in normal erythrocyte membranes under the preparation and assay conditions utilized throughout this study. It can be noted that Ca^{2+}-ATPase activity reached a maximum at approximately 20 μM free calcium. At higher free calcium concentrations, Ca^{2+}-ATPase activity was markedly inhibited. It was observed that at free calcium concentrations, between 0.03 and 30 μM, the calcium required for half-maximal activation of Ca^{2+}-ATPase activity was approximately 0.12 μM (nine observations). This K_m value is slightly lower (higher affinity) than most of the published reports (19, 21, 27, 29) and certainly in keeping with the function of a calcium pump system that maintains internal calcium concentrations in the erythrocyte to well below micromolar concentrations (27). Another group of experiments indicated that the K_m for calcium in this range of free calcium concentrations was not affected by substrate concentration (2–2000 μM ATP) or whether the experiments were conducted under hypotonic or isotonic conditions (not shown).

After analysis of these data, six calcium concentrations (0.037–1.7 μM free calcium) were chosen to be used in all subsequent experiments since these concentrations are in the area of the calcium activation curve most sensitive to alterations in free calcium.

Ca^{2+}-ATPase, Na^+,K^+-ATPase AND Mg^{2+}-ATPase ACTIVITY IN ERYTHROCYTE MEMBRANES FROM CF PATIENTS COMPARED TO CONTROL SUBJECTS

Ca^{2+}-ATPase Activity. In all, four groups of CF patients and control subjects were investigated. In each group, erythrocyte membranes from the control subjects and CF patients were prepared simultaneously immediately after the blood collection and two to three experiments conducted the following 2 days. The erythrocyte membrane preparations from all the control subjects and CF patients in each group were assayed together in each of these experiments. The results obtained in each group were similar with a typical experiment illustrated in Figure 2. It can be seen that the Ca^{2+}-ATPase activity in the erythrocyte membrane preparations from the CF patients is greatly reduced compared to the control subjects. This reduction is apparent at every free calcium concentration tested. When data obtained in these studies is plotted in a reciprocal manner, results illustrated in Figure 3 were obtained. As can be seen, the K_m for calcium is virtually the same in the CF and the control preparations; however, the V_{max}, the maximal activation of the Ca^{2+}-ATPase, is markedly reduced.

Table 1 is a summary of the results obtained in the measurement of Ca^{2+}-ATPase activity in all of the erythrocyte membrane preparations from CF and control subjects. The K_m for calcium is similar in the two groups but the V_{max}, the maximal rate of activation of the Ca^{2+}-ATPase, is reduced by 50% in the erythrocyte membrane preparations of CF patients compared to the control subjects ($P < 0.001$).

Mg^{2+}-ATPase Activity. In subsequent studies it was decided to determine the specificity of this alteration in Ca^{2+}-ATPase activity in erythrocyte membranes of CF patients by measuring the activity of other membrane-bound, ATP-utilizing enzymes. Mg^{2+}-ATPase activity of erythrocyte membranes from CF patients was unchanged compared to the control subjects (125.7 ± 12.9 pmol $^{32}P_i$ released mg^{-1} min^{-1} compared to 130.1 ± 13.9 pmol $^{32}P_i$ released mg^{-1} min^{-1}, respectively). Mg^{2+}-ATPase represents about 30% of the total ATPase activity in these preparations and, to date, no ion transport function has been attributed to this system.

Na^+,K^+-ATPase Activity. For purposes of this study, preliminary experiments were conducted and Na^+ concentrations in the area of the Na^+,K^+-ATPase activation curve most sensitive to alterations in sodium levels chosen. In this series, three groups of CF patients and control subjects were investigated in the same manner as described for the Ca^{2+}-ATPase studies. Figure 4 illustrates a typical experiment in this group of studies. A great deal of variation from subject to subject was found but no clear-cut difference in the Na^+,K^+-ATPase activity in erythrocyte membranes from CF patients compared to control subjects could be observed. This was further verified by reciprocal plots of this data

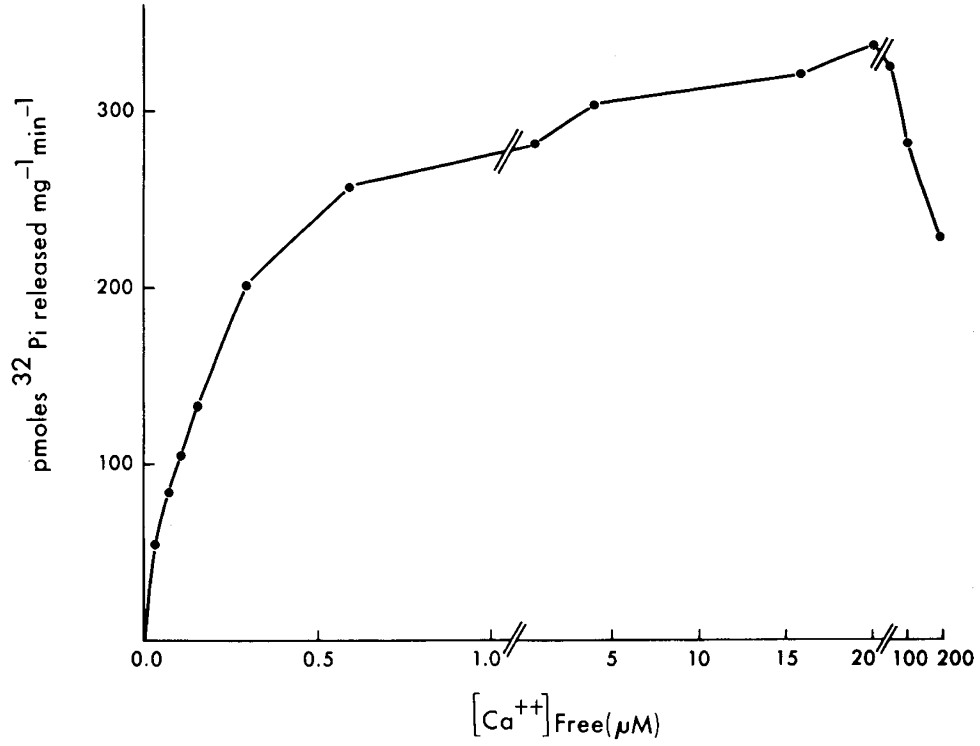

Fig. 1. Effect of Ca^{2+} on ATP hydrolysis in erythrocytes from normal subjects. Membranes (2.0 mg/ml) were initially incubated at 37° for 5 min in the presence of 0.1 mM Tris-EGTA, pH 7.4. Assays were carried out at 37° without and with varying amounts of added $CaCl_2$ (0.37–200 μM free) in a final volume of 0.5 ml containing 0.35 ml membranes, 40 mM Tris-HCl, pH 7.4, 0.0034 mM [γ-^{32}P]ATP (1–3 μCi/μmol), and 0.025 mM $MgCl_2$. The reaction was terminated with the addition of 5% TCA, 5 mM Na_2ATP, and 2 mM KH_2PO_4 and ATPase activity was measured by counting an aliquot of the clear supernatant following charcoal adsorption as described previously (6). The ATPase activity is expressed in picomoles of $^{32}P_i$ released Mg^{-1} min^{-1}. Reaction time was 2 min. The ATPase activity refers to the Ca^{2+}-stimulated component after subtraction of values measured in the absence of added $CaCl_2$. The concentrations of free calcium were calculated as described by Caldwell (8). Result shown is a typical experiment.

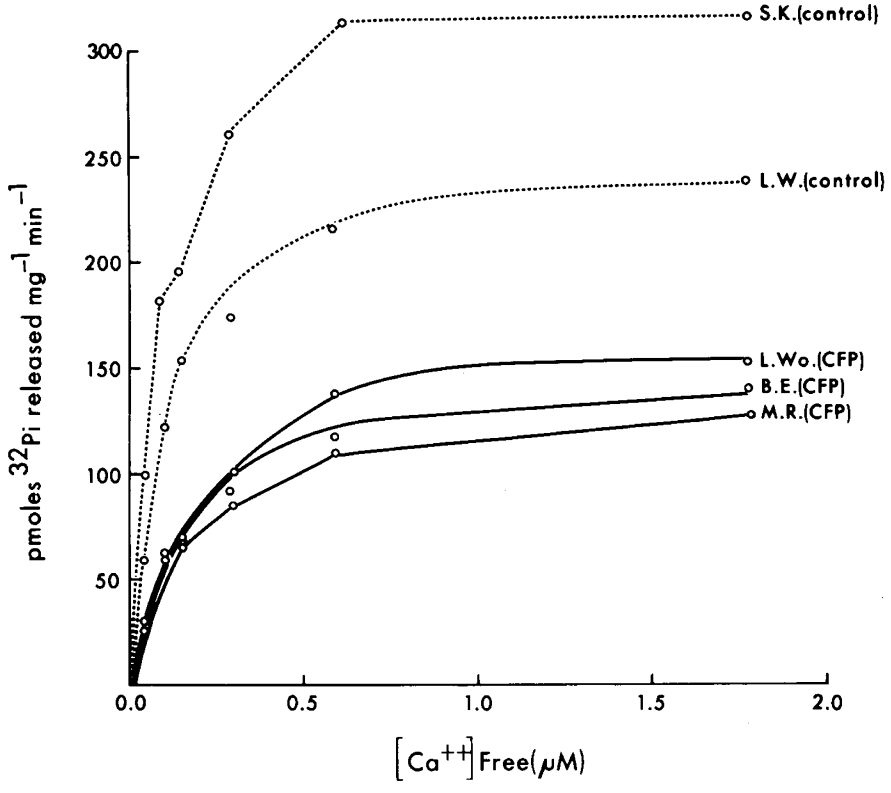

Fig. 2. Erythrocyte membrane Ca^{2+}-ATPase activity in control subjects and in CF patients. Ca^{2+}-ATPase activity expressed as picomoles of $^{32}P_i$ released mg^{-1} min^{-1} in a group of three CF patients and two control subjects. The assay was carried out as described in Figure 1 in the presence of six calcium concentrations (0.037–1.7 μM free calcium). The ATPase activity refers to the Ca^{2+}-stimulated component after subtraction of values obtained in the absence of added calcium. Results shown are a typical experiment.

(Fig. 5) showing no alteration in K_m or V_{max} in the CF and control group.

Table 2 is a summary of all the studies carried out in this group and indicates that no difference could be found in the Na^+,K^+-ATPase activity in erythrocyte membranes from CF patients compared to the control subjects.

DISCUSSION

This study indicates that there is a highly significant decrease in the Ca^{2+}-ATPase activity of erythrocyte membranes from CF patients. The difference observed is not in the affinity of the enzyme system for calcium but in the maximal activation of the Ca^{2+}-ATPase. This indicates that either there is less enzyme per mg protein, less of a protein activator (7), or that the enzyme is altered at a site other than the catalytic site which ultimately influences the catalytic activity of the enzyme (an allosteric site). The specificity of this alteration in CF erythrocyte membranes was indicated by the fact that two other ATP-utilizing enzymes, one having an ion transport function, were not found to be altered. This would indicate that the Ca^{2+}-ATPase activity change noted in CF erythrocyte membranes is not part of a generalized membrane or membrane-bound enzyme alteration in CF. It must now be determined whether this alteration in Ca^{2+}-ATPase is directly related to a defect in calcium transport in these cells, and whether

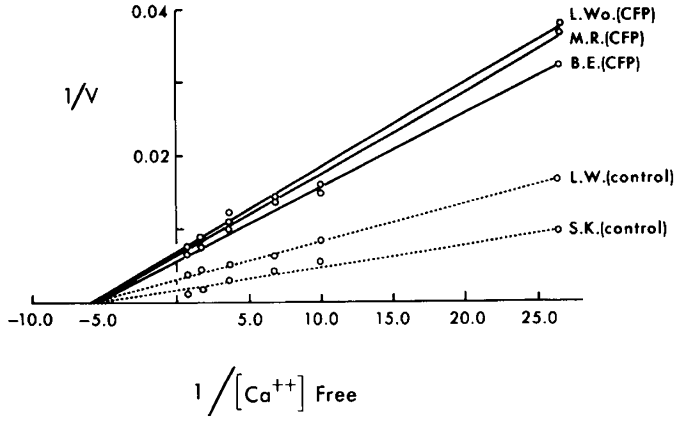

Fig. 3. K_m and V_{max} of Ca^{2+}-ATPase activity in erythrocyte membranes from control subjects and CF patients. Reciprocal plots ($1/Ca^{2+}$ free *vs.* $1/Ca^{2+}$-ATPase activity) of data shown in Figure 2.

Table 1. *Ca^{2+}-ATPase activity in control erythrocytes and in erythrocytes from patients with CF: K_m and V_{max} comparison*

	K_m (μM)	V_{max} (pmol $^{32}P_i$ mg^{-1} min^{-1})
Controls (7)[1]		
L.W.	0.14	454
S.K.	0.15	302
D.H.	0.12	296
J.Y.	0.17	337
S.O.	0.10	263
D.G.	0.08	191
D.H.L.	0.08	250
	0.12 ± 0.01[2]	299 ± 31.1
CF patients (10)		
L.Wo.	0.17	193
B.E.	0.14	178
M.R.	0.18	186
D.M.	0.19	176
M.E.	0.15	85
S.J.	0.08	152
S.S.	0.10	152
H.R.	0.07	131
S.W.	0.05	132
B.R.	0.06	133
	0.12 ± 0.02[3]	152 ± 10.41[4]

[1] Number of subjects in each group.
[2] Results shown are mean values ± SEM.
[3] Not statistically significant compared to the control subjects.
[4] $P < 0.001$ compared to the control subjects.

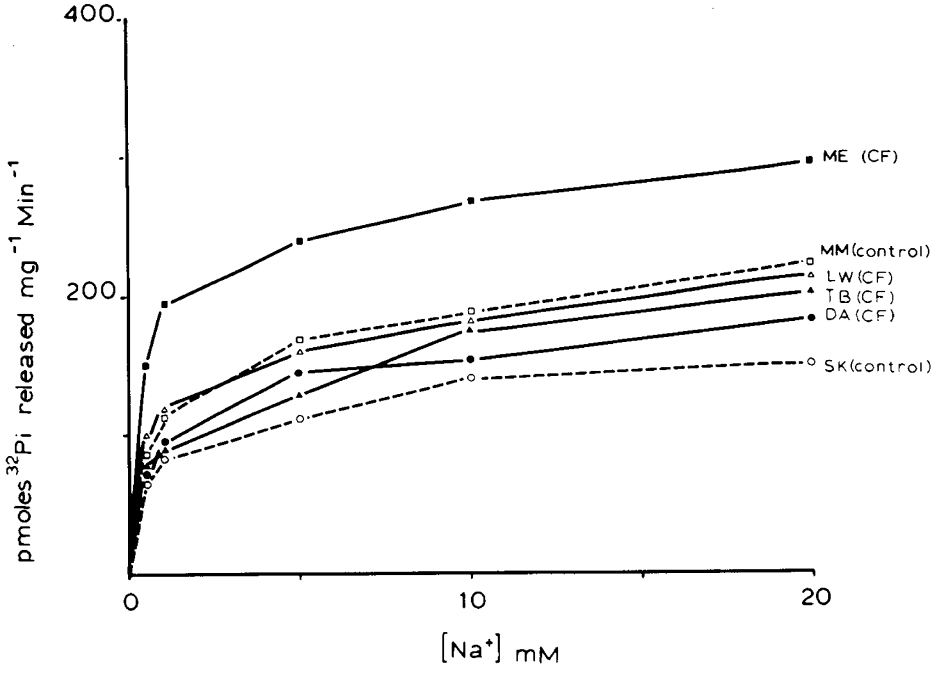

Fig. 4. Erythrocyte membrane Na^+,K^+-ATPase activity in control subjects and CF patients. Na^+,K^+-ATPase activity in a group of four CF patients and two control subjects. Activity is expressed as picomoles $^{32}P_i$ released mg^{-1} min^{-1}. The assay was carried out as described in Figure 1 in the presence of six concentrations of Na^+ (0.05–20 mM). The ATPase activity refers to the Na^+-stimulated component after subtraction of values obtained in the absence of added sodium. Results shown are a typical experiment.

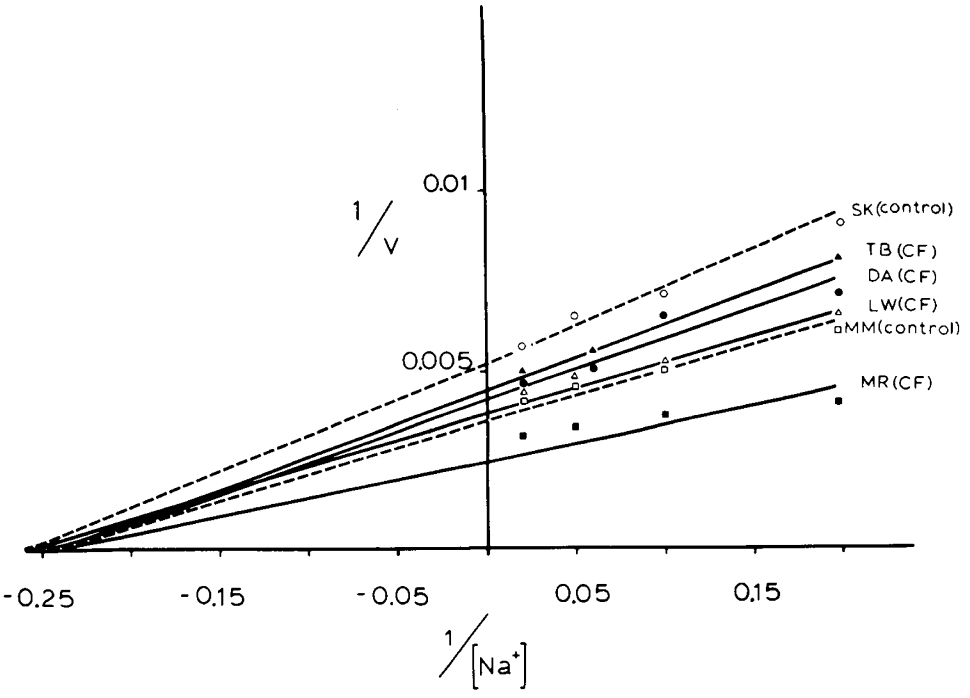

Fig. 5. K_m and V_{max} of Na^+,K^+-ATPase activity in erythrocytes from controls and CF patients. Reciprocal plots ($1/Na^+$ *vs.* $1/Na^+$-ATPase activity) of data shown in Figure 4.

Table 2. *Na^+,K^+-ATPase activity in control erythrocytes and in erythrocytes from patients with CF: K_m and V_{max} comparison*

	K_m (mM)	V_{max} (pmol $^{32}P_i$ mg^{-1} min^{-1})
Controls (6)[1]		
G.W.	4.16[2]	111
P.L.	4.16	142
D.G.	2.22	227
D.H.	2.12	384
M.M.	3.13	325
S.K.	2.95	217
	3.12 ± 0.36	234 ± 42
CF patients (8)		
A.S.	4.16	166
R.B.	4.16	166
L.W.	1.80	200
G.H.	1.50	500
T.B.	3.23	251
D.A.	3.18	346
L.Wo.	2.50	388
M.R.	2.72	455
	2.91 ± 0.35[3]	309 ± 46[3]

[1] Number of subjects in each group.
[2] Results shown are mean values ± SEM.
[3] Not statistically significant compared to the controls subjects.

this alteration in Ca^{2+}-ATPase activity is a generalized phenomenon in CF cell types more directly involved in secretion. To this end, we are presently conducting a study using cultured fibroblasts cells obtained from skin biopsies of CF patients and control subjects.

An alteration in calcium transport activity in CF may have a number of possible implications that may explain some of the manifestations of the disease; Ca^{2+} is involved in many secretory systems including macromolecular secretions (12). An alteration in calcium pump activity would thus affect the secretion of glycoproteins. A number of workers have also recently shown that altered extracellular calcium levels will affect glycoprotein fluidity (viscosity) and thus mucus clearance, an important and central problem in CF (2, 6, 14). Calcium level alterations may greatly influence Na^+ reabsorption and Na^+-Ca^{2+} exchange systems (1, 26).

In this present study, no attempt was made to correlate the results obtained with the severity of the disease state due to the relatively small size of each group studied.

The results of this study are opposed to those of McEvoy *et al.* (24). These latter workers observed that the K_m for calcium was considerably decreased in CF erythrocytes but that the V_{max} was unaffected in the disease state. Since McEvoy *et al.* (24) did not employ calcium buffers to allow for quantitation of the free calcium concentrations, evaluation of this finding is not possible. These workers also reported that the Ca^{2+}-ATPase measured in their studies was extremely labile with storage for 24 hr at 4°, resulting in complete loss of this activity. In contrast, the membranes prepared in this present series maintained Ca^{2+}-ATPase activity for up to 6 days when stored at 4°. This indicates a major difference in the membrane preparations used in these two studies.

The observation that Na^+,K^+-ATPase activity of CF erythrocyte membrane preparations is unaltered in comparison with the control subjects agrees with that of Cole and Dirks (10) who used a different method of erythrocyte membrane preparation and different incubation conditions. Cole and Dirks (10), however, noted a significant decrease in a "ouabain-insensitive" component of ATPase activity. These workers did not use EDTA in their erythrocyte membrane wash solutions nor were calcium binders present in the ATPase assay. It is thus likely that this ouabain-insensitive component contained Ca^{2+}-ATPase as well as Mg^{2+}-ATPase activity. Feig *et al.* (13) also reported no alteration in Na^+,K^+-ATPase activity or Mg^{2+}-ATPase activity in erythrocyte membrane preparations of CF patients. These workers, however, also found no alteration in Ca^{2+}-ATPase activity. Differences in this present study and that of Feig *et al.* (13) in erythrocyte membrane preparations and ATPase assay conditions may have led to this discrepancy in results.

CONCLUSION

In this present study, Ca^{2+}-ATPase was found to be significantly reduced in erythrocyte membrane preparations of CF patients

compared to control subjects. It must now be determined whether a similar alteration in Ca^{2+}-transport is present in CF erythrocytes. It also must be determined whether this alteration is present in other CF cell types in order to indicate the relevance of these findings to the overall manifestation of the disease.

REFERENCES AND NOTES

1. Baker, P. F.: Transport and metabolism of calcium ions in nerve. In: J. A. V. Butler and D. Noble: Progress in Biophysics and Molecular Biology, p. 179 (Pergamon Press, New York, 1972).
2. Bettelheim, F. A.: On the aggregation of a calcium precipitable glycoprotein from human submaxillary saliva. Biochim. Biophys. Acta, *263:* 702 (1971).
3. Blomfield, J., Warton, K. L., and Brown, J. M.: Flow rate and inorganic components of submandibular saliva in cystic fibrosis. Arch. Dis. Childhood, *48:* 267 (1973).
4. Blostein, R.: Relationship between erythrocyte membrane phosphorylation and adenosine triphosphate hydrolysis. J. Biol. Chem., *234:* 1957 (1968).
5. Blostein, R.: Sodium-activated adenosine triphosphatase activity of the erythrocyte membrane. J. Biol. Chem., *245:* 270 (1970).
6. Boat, T. F., Wiesmann, U. N., and Pallavicini, J. C.: Purification and properties of the calcium-precipitable protein in submaxillary saliva of normal and cystic fibrosis subjects. Pediat. Res., *8:* 531 (1974).
7. Bond, G. H. and Clough, D. L.: A soluble protein activator of (Mg^{2+} + Ca^{2+})-dependent ATPase in human red cell membranes. Biochim. Biophys. Acta, *232:* 592 (1973).
8. Caldwell, P. G.: In: A. W. Cuthbert: A Symposium on Calcium and Cellular Function, p. 10 (Macmillan and Co. Ltd., London, 1973).
9. Chernick, W. S., Barbero, G. J., and Parkins, G. M.: Studies on submaxillary saliva in cystic fibrosis. J. Pediat., *59:* 890 (1961).
10. Cole, C. H., and Dirks, J. H.: Changes in erythrocyte membrane ATPase in patients with cystic fibrosis of the pancreas. Pediat. Res., *6:* 616 (1972).
11. Di Sant'Agnese, P. A., and Talamo, R. C.: Pathogenesis and physiopathology of cystic fibrosis of the pancreas: Fibrocystic disease of the pancreas (mucoviscidosis). New Engl. J. Med., *277:* 1344 (1967).
12. Douglas, W. W.: Stimulus-secretion coupling: The concept and clues from chromaffin and other cells. Brit. J. Pharmacol., *34:* 451 (1968).
13. Feig, S. A., Segal, G. B., Kern, K. A., Osher, A. B., and Schwartz, R. H.: Erythrocyte transport function in cystic fibrosis Pediat. Res., *8:* 594 (1974).
14. Forstner, J. F., and Forstner, G. G.: Effects of calcium on intestinal mucin: Implications for cystic fibrosis. Pediat. Res., *10:* 609 (1976).
15. Gibson, L. L., Matthews, W. J., Minihan, P. T., and Patti, J. A.: Relating mucous calcium and sweat in a new concept of cystic fibrosis. Pediatrics, *48:* 695 (1971).
16. Glynn, I. M., and Chappel, J. B.: A simple method for the preparation of ^{32}P-labelled adenosine triphosphate of high specific activity. Biochem. J., *90:* 147 (1964).
17. Horton, C. R., Cole, W. Q., and Bader, H.: Depressed (Ca^{++})-transport ATPase in cystic fibrosis erythrocytes. Biochem. Biophys. Res. Commun., *40:* 505 (1970).
18. Katz, S.: Ca^{2+}-ATPase activity in erythrocyte membranes from normal volunteers and from patients with cystic fibrosis. In: Cystic Fibrosis Club Abstracts, Eighteenth Annual Meeting, San Francisco, p. 9 (1977).
19. Katz, S., and Blostein, R.: Ca^{2+}-stimulated membrane phosphorylation and ATPase activity of the human erythrocyte. Biochim. Biophys. Acta, *389:* 314 (1975).
20. Katz, A. M., Tada, M., and Kirchberger, M. A.: Control of calcium transport in the myocardium by the cyclic AMP-protein kinase system. Advan. Cyclic Nucl. Res., *5:* 453 (1975).
21. Lee, K. S., and Shin, B. C.: Studies on the active transport of calcium in human red cells. J. Gen. Physiol., *54:* 713 (1969).
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall., R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 266 (1951).
23. Mandel, I. D., Eriv, A., Kutsher, A., Denning, C. Thompson, R. H., Kessler, W., and Zegarelli, E.: Calcium and phosphorus levels in submaxillary saliva: Changes in cystic fibrosis and asthma. Clin. Pediat., *8:* 161 (1969).
24. McEvoy, F. A., Davies, R. J., Goodchild, M. C., and Anderson, C. M.: Erythrocyte membrane properties in cystic fibrosis. Clin. Chim. Acta, *54:* 195 (1974).
25. Post, R. L., and Sen, A. K.: Methods of Enzymol., *10:* 762 (1967).
26. Russell, J. M., and Blaustein, M. P.: Calcium efflux from barnacle muscle fibers: Dependence on external cations. J. Gen. Physiol., *63:* 144 (1974).
27. Schatzmann, H. J.: Dependence on calcium concentration and stoichiometry of the calcium pump in human red cells. J. Physiol. (London), *235:* 551 (1973).
28. Wiesmann, U., Pallavicini, J. C., Handwerger, D., Swerdlow, H., and di Sant'Agnese, P. A.: Effect of flow rate on electrolytes, carbohydrates and proteins in submaxillary saliva of normal patients and patients with cystic fibrosis. In: Cystic Fibrosis Club Abstracts, Ninth Annual Meeting, Atlantic City, p. 12 (1968).
29. Wins, P., and Schoffeniels, X.: Studies on red cell ghost ATPase systems: Properties of a (Mg^{++} + Ca^{++})-dependent ATPase. Biochim. Biophys. Acta, *120:* 341 (1966).
30. Zipkin, I., Hawkins, G. R., Damergis, J. A., Gugler, E., Swerdlow, H. and di Sant'Agnese, P. A.: Selected constituents in the parotid and submaxillary saliva of individuals with cystic fibrosis. In: Cystic Fibrosis Club Abstracts, Eleventh Annual Meeting, Atlantic City, p. 57 (1970).

31. The author wishes to thank Miss Julie Yamamoto and Louisa Roelofs for excellent assistance on this project. The author wishes to also thank Drs. Lawrence Wong and A. G. F. Davidson and the staff and patients of the Vancouver Health Centre for Children for their cooperation in obtaining blood samples necessary for this study.
32. The use of human volunteers reported in this paper was approved by a screening committee for research involving human subjects of the Faculty of Medicine, University of British Columbia. Informed consent was received by the parents of the children involved.
33. This research was supported by a grant from the Canadian Cystic Fibrosis Foundation.

34. Requests for reprints should be addressed to: S. Katz, Faculty of Pharmaceutical Sciences, University of British Columbia, 2075 Wesbrook, Vancouver, BC (Canada).

35. Received for publication August 18, 1977.
36. Accepted for publication February 2, 1978.

0031-3998/78/1211-1033$02.00/0

Printed in U. S. A.