Erythrocyte Enigmas in Cystic Fibrosis[1] (37070)

J. W. HADDEN, L. G. HANSEN, B. L. SHAPIRO, AND W. J. WARWICK
(Introduced by Dr. Robert A. Good)

Departments of Pediatrics and Oral Biology, University of Minnesota, Minneapolis, Minnesota 55455

Cystic fibrosis (CF) is a pathologic condition characterized biochemically, in part, by exocrine hyperexcretion of sodium and calcium. Interest in the past has naturally focused on possible abnormalities of metabolism and transport of these two ions in the cells of these patients, and much of the work has utilized the erythrocyte.

Although a previous study (1) had noted normal electrolyte content and sodium efflux in CF erythrocyte, Balfe *et al.* (2) observed that the erythrocyte of the patient with cystic fibrosis has an overall impairment of sodium efflux and that the erythrocyte of the obligate heterozygote has impaired Pump II as defined by Hoffman (3). More recently Lapey *et al.* (4) stated that the only consistent abnormality in CF and heterozygote erythrocytes is an impairment of the ouabain-insensitive sodium efflux in male patients. The findings of these studies, in sum, suggest that differences in technical factors and patient selection account for the conflicting results rather than genetic factors involved in the etiology of CF. Each of these studies (1, 2, 4) utilized an assay of sodium efflux based on iso-osmotic preloading of erythrocytes with sodium. In the present study the sodium–potassium ion exchange pump of the CF erythrocytes was analyzed by an assay of the active cation influx that occurs as a concomitant of sodium efflux.

Two recent studies indicate that calcium metabolism of CF erythrocytes may be abnormal. Horten *et al.* (5) have found calcium-dependent adenosine triphosphatase (ATPase) activity of CF erythrocyte ghosts to be reduced. Fitzpatrick *et al.* (6) have observed altered electrophoretic mobility of fragments of CF erythrocyte membranes and have related the finding to enhanced calcium binding. Schatzmann (7) has elucidated in the normal erythrocyte an active transport pump for the efflux of calcium which is presumed to be related to calcium-dependent ATPase activity of the plasma membrane; therefore, the present study was designed to analyze the calcium extrusion pump of the CF erythrocyte for possible abnormality. We report data which do not support a defect in either the sodium–potassium ion exchange pump or the calcium extrusion pump of the CF erythrocyte.

Methods. In this study, healthy CF patients free of known interfering medications and equal numbers of age and sex-matched controls were studied as pairs. Patients who were post menarchal or were receiving hormones in any form were excluded from this study. Erythrocytes were obtained from heparinized venous blood packed by centrifugation at 850*g* for 10 min and washed three times in physiologic saline. One-tenth milliliter aliquots of the final packed-cell volume were distributed to plastic tubes (Amersham–Searle) for assay.

The erythrocyte sodium–potassium ion exchange pump was assayed in thirteen pairs by the Rubidium^{86} (Rb^{86}) method of Grahame–Smith and Everest (8). Rb^{86} is used in this method as an analog of potassium because of its more favorable half-life, cost, and availability. Incubations in this assay were performed as described (8); the 0.1 ml

Requests for reprints should be addressed to: Dr. John Hadden, Box 184 Mayo Memorial, University of Minnesota Hospitals, Minneapolis, Minnesota 55455.

[1] This work was supported by grants from the National Cystic Fibrosis Research Foundation and The National Foundation–March of Dimes.

aliquots of packed cells were incubated at 37° for 2 hr in 2 ml of modified potassium-free Krebs solution containing a final concentration of 0.37 m*M* $Rb^{86}Cl$ (10 μCi) and 5.6 m*M* glucose. Following incubation the cells were washed three times with 10 ml of iced saline and counted for 10 min in a gamma counter (Nuclear Chicago). Uptake of Rb^{86} during this 2-hr incubation is linear. The definition of Pump I, Pump II, and leak are those of Hoffman (3) employed by Balfe, *et al.* (2), *i.e.*, Pump I is that ion flux inhibited by ouabain (10^{-4} *M*), Pump II is that ion flux inhibited by ethacrynic acid (10^{-4} *M*) in the presence of a maximum inhibitory concentration of ouabain (10^{-4} *M*); leak represents that ion flux which occurs in the presence of both ethacrynic acid (10^{-4} *M*) and ouabain (10^{-4} *M*). These subdivisions of the exchange pump in the erythrocyte constitute approximately 70%, 20%, and 10% of the ion fluxes, respectively (3). In ten comparative studies in which autologous plasma was used, a final concentration of 10% plasma was achieved by substitution of an equal volume of the potassium-free Krebs solution. Each patient was studied in triplicate under each condition. The data are expressed as a ratio of the patient to the paired control and of pump subdivision to total uptake as a percent.

Calcium extrusion pump activity was assayed in nine pairs by a modification of the method of Schatzmann (7). In this method, Sr^{85} is used as an analog of calcium since it is transported in the same manner and offers a more favorable, isotopic form, and the erythrocytes are loaded with the isotope in a hypotonic environment then resealed by restoration of an isotonic environment. One-tenth milliliter aliquots of packed erythrocytes were incubated in 0.5 ml distilled water containing 1 m*M* $SrCl_2$, 5 m*M* Tris–HCl (pH 7.4), 1.65 m*M* ATP, and 0.5 μCi $Sr^{85}Cl_2$ (Amersham-Searle). The tubes were agitated for 45 sec during the 120-sec incubation. The medium was then restored to isotonicity with the addition of .025 ml 3 *M* KCl and incubated at 37°. After incubation of 0, 10, and 20 min, 10 ml of cold saline was added, and the tubes were centrifuged at 700*g* for 5 min. The supernatants were removed by suction and the residual cells assayed for isotope content in a gamma counter. Extrusion in this assay is delayed while resealing of the erythrocytes occurs and is, therefore, expressed as counts extruded/.1 ml packed cells for the 10 to 20 min time interval.

Results and Discussion. The comparison of Rb^{86} uptake in erythrocytes from thirteen CF patients and age, sex-matched controls are shown in Table IA. The mean total uptake of the two groups is identical. The error terms are large on the raw data because variation in isotope activity was uncontrolled from one day to another; however, as paired studies, the standard error term for the triplicated measurements for each individual is only 6% of the mean. The fractional influxes of Rb^{86} based on the classification of Hoffman (3) show no differences between CF and Control. Identical studies were performed in the presence of 10% heparinized, autologous

TABLE I. Erythrocyte Studies in Patients with Cystic Fibrosis.

			CF mean ± SEM	Normal mean ± SEM
A)	Sodium Potassium Ion Exchange Pump			
	Number		13	13
	Total uptake Rb^{86} in CPM/2 hr incubation		11,404 ± 3,588	11,686 ± 3,721
	Fractional uptake in percent of total	Pump I	70.2 ± 2.8	71.3 ± 2.2
		Pump II	13.6 ± 1.7	12.6 ± 1.6
		Leak	16.2 ± 1.4	16.1 ± 1.4
B)	Calcium Extrusion Pump			
	Number		9	9
	Total uptake Sr^{85} at 0 time in CPM		5,719 ± 686	5,435 ± 624
	Sr^{85} efflux in CPM/10 minute period		2,260 ± 390	2,320 ± 490

plasma in an effort to show possible effects of "Spock" or other serum factors and no differences between the groups were manifest. Similarly, subanalyses of the data on the basis of age and sex were unrevealing.

Analysis of calcium extrusion pump activity in nine pairs of CF and age, sex-matched normals assayed by Sr^{85} extrusion is shown in Table IB. The uptake of isotope during the period of hypotonic loading appeared to be equivalent for the two groups and the amounts extruded in the period measured are identical.

The erythrocytes of patients with cystic fibrosis circulate in a milieu markedly different from the normal and are potentially affected by a plethora of factors including anoxia, infection, malabsorption, liver disease, vitamin and antibiotic therapy, and altered hormonal status. For the many who have utilized this cell in an effort to gain insight into the primary genetic defect present in CF, the problems of patient selection and manipulation to control these secondary variables have been considerable. Although the means of RBC counts, hematocrit, hemoglobin, erythrocyte indices, and reticulocyte counts performed on 47 CF patients in the clinical laboratory of the University of Minnesota were found to be within the range of control values for the age group, individual patients are frequently abnormal in one or more of these indices. In the present study patients were carefully selected on the basis of normal erythrocyte indices and of relative health and freedom from therapeutic manipulation.

Our study of active potassium influx via the ion exchange pump in these patients does not indicate a defect in this transport, either intrinsic to the cell or secondary to an influence of plasma. We offer, therefore, corroborative support for those studies of sodium efflux (1, 4) which did not reveal a consistent abnormality of ion exchange transport which might relate to the primary genetic abnormality present in CF. Discrepant findings would appear to us to derive from secondary features of the disease manifested in the erythrocytes of selected patients.

Furthermore, our study of calcium transport indicates this function to be normal. The implication that this function might be abnormal rested primarily on the observation that calcium-dependent ATPase activity was reduced in CF erythrocyte ghosts (5). Since calcium transport functions appear to be normal the reduced calcium-dependent ATPase activity described (5) may result from a function of the ATPase unrelated to transport.

Summary. Cystic fibrosis (CF) is characterized by exocrine hyperexcretion of sodium and calcium, and several studies have suggested abnormalities of these transport functions in the CF erythrocyte. The present study analyzed the functions of the sodium-potassium ion exchange pump and the calcium extrusion pump of the erythrocytes of CF patients and found them to be equivalent to those of paired age and sex-matched normals, therefore, no evidence could be found to support a primary genetic defect in the CF erythrocyte.

1. Lobeck, C., "Third International Conference on Research on Pathogenesis of CF of the Pancreas," p. 107. Wickersham Printing, Lancaster, Pennsylvania (1966).

2. Balfe, J., Cole, C., and Welt, L., Science **162**, 689 (1968).

3. Hoffman, J., Amer. J. Med. **41**, 666 (1966).

4. Lapey, A., and Gardner, J., Pediat. Res. **4**, 478 (1970).

5. Horton, C., Cole, W., and Bader, H., Biochem. Biophys. Res. Commun. **40**, 505 (1970).

6. Fitzpatrick, D., Landon, E., and James, V., Nature New Biol. **235**, 173 (1972).

7. Schatzmann, H. J., and Vincenzi, F. F., J. Physiol. **201**, 369 (1969).

8. Grahame-Smith, D., and Everest, M., Brit. Med. J. **1**, 286 (1969).

Received Oct. 4, 1972. P.S.E.B.M., 1973, Vol. 142.