Red Cell Glutathione and Glutathione Reductase in Cystic Fibrosis[1] (37552)

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Following histochemical studies of salivary glands and olfactory nasal mucosa in which pentose phosphate pathway (PPP) enzymes were localized in sites of active ion transport (1, 2) we speculated that a relationship exists between enzyme systems of the PPP and ion transport. As a test of this hypothesis and in order to learn more about cystic fibrosis (CF) we contrasted glucose-6-phosphate dehydrogenase (G6PD) and NADP and NADPH in red blood cells (RBC) of non-CF control individuals and CF patients (3). NADPH/NADP ratios were decreased and G6PD activity was increased in the CF group. In order to further examine pathways related to the PPP in CF we have assayed glutathione reductase (GR) activity in RBC of CF patients. Beutler (4) has reported the stimulation of GR activity of normal RBC by riboflavin. Since CF patients frequently are supplemented with vitamins, we assayed GR also in a group of CF and control subjects with similar vitamin ingestion. In addition, we assayed total glutathione (GSSG and GSH) in whole blood. We report significantly higher levels of RBC GR activity and total blood glutathione in individuals with CF in comparison with control individuals.

Materials and Methods. For GR assays, venous blood was collected into heparinized syringes and within 1 hr centrifuged at 1935*g* for 10 min. RBC were collected and washed with 3 vol of cold saline and centrifuged. Washing and centrifugation were repeated. The RBC were hemolyzed by freezing and thawing. To 0.2 ml of hemolyzed RBC was added 3.8 ml of distilled water. The hemolyzate was centrifuged at 18,800*g* for 30 min. GR in the supernatant was determined from the change in OD at 340 nm at 37° in hemolyzates to which GSSG and NADPH in Tris buffer and EDTA was added (5). Hemolyzate hemoglobin was determined at the end of the reaction by comparison at 540 nm with a standard hemoglobin. Samples from 18 CF patients and from 18 non-CF controls were assayed. Of these, 7 pairs of individuals were matched for vitamin supplementation.

Total glutathione was determined on whole blood from an additional 18 CF patients and 18 controls using the technique of Tietze (6). Finger puncture blood (0.1 ml) was hemolyzed in 9.9 ml of ice-cold 0.01 *M* potassium phosphate buffer containing 0.005 *M* EDTA (pH 7.5). To 0.67 ml of the buffer used for hemolysis was added 0.10 ml of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) 240 mg/ml), 0.01 ml of GR (100 units/ml), 0.02 ml of hemolyzate and 0.20 ml NADPH (1 mg/ml in PO_4 buffer). The change in OD at 412 nm at 25° after adding the NADPH was recorded against a no-hemolyzate control and compared to a glutathione standard. Hemoglobin was determined as for GR assays. All the CF patients in this study were ambulatory and active.

Results and Discussion. GR of RBC may be considered part of the PPP because of its specificity for NADPH (7). Since we had already found variations from control samples in G6PD levels and NADPH/NADP ratios in CF RBC it was appropriate to assay for GR and glutathione in these cells. Results of

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TABLE I. Glutathione and Glutathione Reductase in Cystic Fibrosis Patients and Controls.

		N	Mean	SD	*t* test	*p*
RBC Glutathione Reductase[a]	CF	18	13.48	1.82	4.154	<0.001
	Control	18	11.25	1.27		
Total Whole blood Glutathione[b]	CF	18	2462.39	258.33	3.438	<0.001
	Control	18	2174.89	243.25		

[a] NADPH (μmoles oxidized/min/g hemoglobin) at 37°.
[b] Hemoglobin (μg/g).

the GR and glutathione assays are summarized in Table I. CF RBC clearly demonstrated greater levels of GR activity ($p < 0.001$). Moreover, whole blood total glutathione was increased in CF ($p < 0.001$).

GR means of subgroups in which vitamin consumption of CF subjects and their controls was equal for 2 wk prior to collection were not significantly different from the inclusive groups. However, mean activity in the vitamin control subgroup was about 15% greater than in the remainder of controls. Mean activity in the CF subgroup was essentially identical to the remainder of CF subjects. No attempt was made to control the type of vitamin used. In fact, 2 of the 7 pairs matched for vitamin supplementation took no vitamins during the 2 wk period prior to collection of blood. Multivitamin capsules used were those ordinarily taken by the subjects and were not the same in all pairs. The vitamin subgroups were used merely to determine whether the increased levels of GR activity in the CF sample could be attributed to their more uniform use of vitamin supplements than the general population. We found no evidence to support this since the difference between CF and controls persisted in the vitamin matched pairs.

Jacob and Jandl (8) reported that the rate of PPP metabolism in RBC is directly proportional to the cellular concentration of GSSG. They suggested that the major proportion of PPP activity is regulated by the need for NADPH generated by the PPP for maintenance of cellular sulfhydryl groups. The present findings together with our earlier report (3) also seem to demonstrate the mutual activities of the PPP and GR. Furthermore, the coincident increase in G6PD and GR activities and the decrease in NADPH/NADP ratios in CF RBC suggests that although PPP activity may be increased in CF the NADPH generated by it is actively consumed in the GR reaction, presumably to maintain cellular GSH. Less than 0.5% of RBC glutathione exists in the oxidized state and glutathione is virtually absent in extracellular fluids (6, 9). Accordingly, the increase in total glutathione in whole blood in CF may be attributed to an increase in RBC GSH.

We determined RBC count, hematocrit, hemoglobin, erythrocyte indices and reticulocyte counts on 47 CF patients (Table II). The mean values were found to be no different from normal values for a comparable age group (10, 11). Accordingly, it is unlikely that elevated glutathione and GR in CF is attributable to an erythrocytosis secondary to pulmonary disease. Specifically, reticulocyte counts of the CF patients were within the normal range. This finding would mitigate the possible role of a younger population of RBC secondary to liver and spleen disease as being responsible for increased GR activity.

TABLE II. Erythrocyte Values in 47 Cystic Fibrosis Patients.

	Mean ± SEM
RBC count	4.65 ± 0.2 × 10^6/mm^3
HCT	40.1 ± 0.5%
Hb	13.5 ± 0.2 g/100 cm^3
Mean corpuscular vol	82.9 ± 0.7 μm^3
Hb	28.1 ± 0.3 pg
Hb con	33.7 ± 0.3%
Reticulocyte count	1.57 ± 0.9%

Moreover, increased activity of GR cannot be attributed to cell age since GR activity of erythrocytes is not affected by their ages (7).

The relationship between the reported findings and CF is not yet clear. However, GSH is thought to be important to membrane integrity of the cell (7, 12). Furthermore, a clear relationship between sulfhydryl compounds, including GSH, and ion permeability in RBC has been demonstrated (13–15). Meister (16) recently published a significant paper on the enzymology of amino acid transport. In it he emphasized that a major function of glutathione is its role in amino acid transport and possibly in carbohydrate and cation transport as well. Since an ion or molecular transport defect may be the basic lesion in CF (17, 18) our findings may be directly related to its pathogenesis.

The data reported here support our earlier findings that alterations of PPP enzyme systems occur in CF RBC. These systems may be related to maintenance of membrane integrity and/or transport. However, the relative distance of these findings from the primary defect in CF is not known. Until the basic abnormality in CF is determined it will be unclear whether increased glutathione and GR are compensatory changes or are fundamental to the disease. In either case these differences between CF and control RBC appear to be sufficiently distinct that they may shed light on both CF and on RBC biology.

Summary. Red blood cell glutathione reductase and whole blood total (oxidized and reduced) glutathione were assayed in cystic fibrosis subjects and non-cystic fibrosis controls. Glutathione reductase activity and total glutathione levels were greater in the cystic fibrosis sample. These findings are consistent with previous data reporting increased pentose phosphate pathway activity and decreased NADPH/NADP ratios in cystic fibrosis erythrocytes. They support the notion that pentose phosphate pathway alterations occur in cystic fibrosis. These data may indicate also the possible role of sulfhydryl group changes in membrane transport abnormalities of the disease.

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