INCREASED ERYTHROCYTE GLUTATHIONE REDUCTASE ACTIVITY IN DIABETES MELLITUS

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During investigation of conditions for spectrophotometric assay of glutathione reductase in hemolyzates, several blood samples from diabetic individuals were found to have unusually high glutathione reductase activity. Further study was stimulated by Langdon's report (1960) on the inhibition of glutathione reductase of rat adipose tissue by the phenylalanyl chain of insulin.

METHODS

Hemolyzate, prepared by freezing and thawing erythrocytes which had been washed three times with 0.145 M NaCl, was diluted with 3 to 4 volumes of 0.067 M phosphate buffer, pH 7.6. After centrifugation for one hour at 30,000 x g to remove stromata, the hemolyzate was further diluted so that when 0.5 ml. was used to start the reaction, the final hemoglobin concentration would be either 0.210 gm./100 ml. (Group 1 individuals) or 0.105 gm./100 ml. (Group 2 individuals). In a volume of 3.1 ml., final concentrations also included Tris buffer (Sigma 121) 0.13 M, pH 7.6; EDTA (Eastman Kodak) 0.032 M; TPNH, enzymatically reduced (Sigma) 2.9×10^{-4} M, and oxidized glutathione (Schwarz BioResearch, Inc.) 5.3×10^{-3} M.

Reactions were run at 37° C. For Group 1, a Cary Model 14 Recording Spectrophotometer was used; for Group 2, a Beckman DU Spectrophotometer.

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Activities were determined as a function of the decrease in absorbancy at 340 mμ through 1 cm. light path occurring with oxidation of the TPNH. Activities are expressed as moles ml.$^{-1}$ min.$^{-1}$, using 6.22×10^6 sq. cm. mole^{-1} as the extinction coefficient (Horecker and Kornberg, 1948). Activities shown are maximum rather than initial rates, since there was a significant induction period even though all reactants were pre-incubated to 37° C. The variation among duplicate determinations was less than 1.5%.

After completion of the reactions, final hemoglobin concentrations were checked by mixing 1.0 ml. of reaction mixture with 9.0 ml. of Drabkin's solution (Crosby, et. al., 1954) and determining the cyanmethemoglobin at 540 mμ in a Beckman B Spectrophotometer. Enzyme activities were then corrected to an arbitrary hemoglobin reference level of 1 gm./100 ml.

RESULTS

The results obtained for individuals with maturity-onset diabetes are presented in Table 1. Diabetics who developed their disease after the age of 40, who had a history of obesity and no history of acidosis, were considered to have maturity-onset diabetes (Field, 1959).

Groups 1 and 2 differred sufficiently to require, initially, separate treatment of the data, not only because the assays were performed on different instruments at two different levels of added hemolyzate, but also because the subjects chosen were not necessarily comparable. Group 1 subjects were all male with average ages of 51 for the controls and 60 for the diabetics. Treatment was by diet alone.

Unlike Group 1, Group 2 was heterogeneous. Thus the controls include 16 males and 7 females, average age 35; the diabetics include 13 females and 7 males, average age 59. Nine of the diabetics were treated by diet alone; 11 were receiving additional treatment, 6 with insulin, 3 with tolbutamide, 1 with chlorpropamide, and 1 with phenformin. The following comparisons were

Table 1.

Glutathione Reductase Activity of Hemolyzates

(GSSG-R)*

	Group 1	Group 2	Groups 1 + 2
Normal Controls	18	23	41
Diabetics	19	20	39
Mean GSSG-R for Normal Controls	9.86 $\pm$ S.D. 1.86	9.52 $\pm$ S.D. 1.04	9.67 $\pm$ S.D. 1.45
Mean GSSG-R for Diabetics	11.38 $\pm$ S.D. 1.90	11.91 $\pm$ S.D. 1.81	11.66 $\pm$ S.D. 1.88
t Value	2.4388	5.3149	5.3090
p	< 0.02	< 0.001	< 0.001

*Values for GSSG-R in moles x 10^{-8} ml.$^{-1}$ min.$^{-1}$, referred to a hemoglobin concentration of 1 gm./ 100 ml.

therefore made: (1) The mean activities of males compared with females did not differ in either the controls or the diabetics; (2) The mean activities for 9 controls, average age 51, was not different from that for 14 controls, average age 25: (3) The mean activity for the 9 diabetics treated with diet alone, 12.19 x 10^{-8} moles ml.$^{-1}$ min.$^{-1}$, was only slightly greater than for the 11 receiving additional treatment, 11.71 x 10^{-8} moles ml.$^{-1}$min.$^{-1}$

Inspection of the data obtained for Groups 1 and 2 reveals that, in spite of the differences in the makeup of the two groups, their combination for statistical evaluation appears permissible. The combined results are given in column 3.

Another group of 9 individuals, who developed diabetes at the average age of 26, with a history of obesity in only 1, and a definite insulin requirement in 6, had a mean glutathione reductase activity, 9.81 x 10^{-8} moles ml.$^{-1}$ min.$^{-1}$, similar to the controls, 9.86 x 10^{-8}. The average age of this group at the time of the study was 37 years.

DISCUSSION

The increased activity of glutathione reductase from hemolyzates of diabetics' erythrocytes may ultimately prove to be greater than that presented in Table 1. At least 5 of the non-diabetic individuals have a family history of diabetes, and 3 of these 5 have elevated erythrocyte glutathione reductase activities but normal glucose-tolerance curves.

One possible mechanism for this increase in activity of glutathione reductase is provided by Langdon's observation concerning the inhibition of glutathione reductase by the phenylalanyl chain of insulin. With deficient quantities of insulin, this inhibition would presumably be lacking, and glutathione reductase might thereby be more active. If such an inhibitor, bound in greater amount in hemolyzates of washed normal erythrocytes than in hemolyzates of erythrocytes from diabetics, cannot be found, the increased glutathione reductase activity of the diabetics' red cells could well be intrinsic. In this case, either an increased concentration of the enzyme or a molecularly different form of the enzyme could account for the increased enzyme activity. Primarily because hemolyzates from the diabetics we have studied who developed diabetes early in life did not show elevated glutathione reductase activity, we are investigating this last possibility.

That the increased enzyme activity might be a function of cell age has not been excluded. No data on red cell life-span in the diabetic is available.

Other factors which will require explanation are: (1) The exceptions in maturity-onset diabetes, i.e., those with normal glutathione reductase activity; and the exceptions among the controls, i.e., those with elevated glutathione reductase activity and (2) Failure of those maturity-onset diabetics on treatment with insulin and other blood sugar lowering agents to have a significantly lower glutathione reductase activity than those treated with diet alone.

The known functions of glutathione reductase are the maintenance of glutathione in the reduced state and the accompanying oxidation of

triphosphopyridine nucleotide (and in some cells, diphosphopyridine nucleotide). Whether because of its function with respect to glutathione or to the pyridine nucleotides, alteration of glutathione reductase activity appears to be associated with lesions of carbohydrate metabolism. This association is suggested by the present study and also by the elevated red cell glutathione reductase activity in the primaquine-sensitive individual (Schrier, et. al., 1958). In primaquine-sensitivity, the elevated red cell glutathione reductase activity is associated with a severe deficiency of glucose-6-phosphate dehydrogenase (Carson, et. al, 1956). Activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in hemolyzates from the diabetics in this study were normal.

The usefulness of glutathione reductase in the catabolism of glucose has also been demonstrated in the reconstructed system of Couri and Racker, who accomplished oxidation of glucose-6-phosphate in vitro when, in addition to the enzymes of the pentose phosphate pathway, glutathione reductase and oxidized glutathione were added.

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