BLOOD GLUCOSE AND ERYTHROCYTE GLUTATHIONE LEVEL IN MALNOURISHED CHILDREN

Yıldız Saraçlar, M.D.,* and Şinasi Özsoylu, M.D.,**

It has been demonstrated that reduced glutathione (GSH) a tripeptide glutamyl-cysteyl-glycine, plays an important role in maintaining the integrity of the red cells.[1,2,3] It is retained in reduced form in the normal mature erythrocyte by glutathione reductase and reduced triphosphopyridine nucleotide (TPNH). This reaction is represented by the following formula (GSSG = oxidized glutathione) :

$$GSSG + TPNH + H^+ \xrightarrow{\text{glutathione reductase}} 2GHS + TPN^+$$

Red cell glucose which is equal to blood glucose is an essential substrate for TPNH regeneration and is necessary for the protection of GSH in the erythrocytes. It is well known that glucose is the only substance which can be used for energy production by mature erythrocytes. At least 90 per cent of the glucose in red blood cells is metabolized in the anaerobic Embden - Meyerhof pathway. By means of this path, two molecules of adenosine triphosphate (ATP) are generated for each molecule of glucose. When erythrocyte glucose is oxidized in the aerobic hexose monophosphate shunt, two molecules of TPNH are generated for one molecule of glucose.

The most common cause of malnutrition in children in this country is simply lack of food.[4] Fatty degeneration of the liver, which is the most important organ in the regulation of blood sugar, is a frequent finding in chronic malnutrition. It is claimed that hypoglycemia due to poor nutrition and fatty liver degeneration frequently occurs in malnourished children.[5] Since blood sugar is essential, indirectly, for erythrocyte GSH reduction, we attempted to find in this study the degree of this relation in severely malnourished children.

From Ankara University Hacettepe Faculty of Medicine and Hacettepe Children's Hospital.

* Pediatrician and fellow in Hematology.

** Hematologist and Assistant Professor of Pediatrics.

TABLE 1

AGE, SEX, DEGREE OF MALNUTRITION, BLOOD SUGAR, GLUTATHIONE SERUM PROTEIN LEVELS IN THE FIRST STUDY GROUP

Cases	Age and Sex	Weight	Degree	Blood % mgr glucose	Sugar % mg Folin Wu	GSH level/ 100 cc RBC mg	Serum Proteins gr %	
							Alb.	Glob.
I.Ç. 64/28944	9/12 M	3.800	IV	—	92	89	4.6	2.2
H.K. 64/36861	15/12 M	5.200	IV	—	112	60	4.5	2.7
S.K. 64/30854	7/12 M	3.700	IV	74	89	100	4.2	2.3
E.A. 64/34073	11/12 M	4.200	IV	—	68.5	88	—	—
S.I. 64/36832	18/12 F	6.500	III	60	—	90	3.7	2
S.S. 64/38129	18/12 F	6.800	III	82	94	46	2	2.1
R.M. 64/38985	8/12 M	4.350	III	—	112	78 GSH stability 65	1.8	1.6
M.O. 64/27850	12/12 M	5.000	III	65	85	63	2.8	2.7
M.Ş. 64/36046	6/12 M	3.750	III	94	112	74	—	—

Y.Ü. 64/27941	15/12 M	5.600	III	72	90	76	5.3	2.4
H.M. 64/28180	18/12 M	6.600	III	68	80	63	4.3	2.1
M.Y. 64/35596	12/12 M	5.000	III	83	96	77	5.1	2.3
S.A. 64/9361	18/12 M	6.200	III	56	62	145	2.3	2.3
C.B. 64/28959	20/12 M	7.600	II	—	90	125	5.	3.1
I.B. 64/30290	21/12 M	7.000	II	68	84	69	3.4	3
A.K. 64/35587	4 yrs. F	8.200	Fiftieth % ile for 9 months	41	75	73	6.1	2.7
S.G. 64/35391	5 yrs. F	10.500	25th % ile for 18 mos.	73	86	107 GSH stability 74	5.2	2.2
F.Ö. 64/37076	3 yrs. F.	5.000	25th % ile for 3 mos.	46	60	80	4.2	0.9
O.S. 64/25004	3 yrs. F.	6.600	25th % ile for 6 mos.	70	89	112	5.5	1.8

Material and Methods

This study includes 27 malnourished children divided into two groups according to the duration of the fasting period. In the first group there were 19 children between the ages of six months and five years. All except two (C.B. and I.B.) had severe degrees of malnutrition (3rd or 4th grade by the definition of this hospital [4]). Blood samples were obtained before starting the hospital diet and following at least three hours of fasting. The second group consisted of eight children who, with one exception, were under two years of age and were severely (grade 3 or 4) malnourished. Their blood was drawn for investigation following at least five hours of fasting and before the hospital diet was begun. We do not know how long any of the patients had been without food prior to their arrival.

Six children without malnutrition and 18 patients from seven families with glucose six phosphate dehydrogenase (G6PD) deficiency were chosen as controls for the study and GSH determinations.

The erythrocyte GSH and G6PD activity determinations were performed by the methods of Beutler et al, [6] and Narahara - Özand, [7] respectively. Serum protein, blood sugar and blood glucose concentrations were determined according to the methods of Gornal, et al., [8] Folin - Wu, [9] and Hugget - Nixon, [10] (glucose oxidase) successively. Glutathione stability tests were done as described by Grunet and Phillips, as modified by Beutler. [11] In this test 0.1 mg sodium menadion bisulfite (Hykinone R) instead of 5 mg of acetyl phenyl hydrazine, was added to 1 cc heparinized blood as described by Zinkham. [12] Hematological indices were determined by standard methods.

Results

The results from the first group are summarized in Table 1. None of the patients in this group had true hypoglycemia (blood glucose less than 40 mg per cent), but as would be expected with the glucose oxidase method, blood sugar was lower than measured by the Folin - Wu method. Hypoalbuminemia was found in four out of 19 cases. Three of those four children (S.S., R.M., S.A.,) had edema and all the criteria of Kwashiorkor. GSH level was within normal limits and no relation could be shown between blood sugar and the severity of malnutrition. Glutathione stability tests were performed with the blood of two patients and the results were

normal. In two cases, the red blood cell GSH level was higher than normal (125 and 145 mg).

Table 2 summarizes the results in the second group of patients who fasted at least five hours before the investigation. Since the difference between the two methods in the first group was not considered significant, the blood sugar levels of these patients were determined only by the Folin - Wu method. Mild hypoglycemia (37 mg %) was found in only one case. However in all these patients erythrocyte GSH level and glutathione stability tests were within normal limits. Serum proteins were determined in four cases and none were low. Laboratory findings of the control group are given in Table 3.

As would be expected, erythrocyte GSH instability tests were abnormal in all G6PD deficient patients and in four of their mothers "carriers" (Table 4). The initial red cell GSH level was at the top level of normal in two, but was low normal in the rest. None of the patients with abnormal erythrocyte GSH stability had low hematocrit levels.

Discussion

Although GSH does not seem to be absolutely necessary for red cell viability, [13] it appears to serve an important function in red cell metabolism. Normal erythrocyte glutathione level is around 70 mg/100 ml of packed cells. [14, 15] However, levels as low as 43 mg, and as high as 160 mg/100 ml of packed cells have been reported. [6] GSH is in a dynamic state in the red cells and its half life is four days. [2]

Decreased content of GSH was the first described biochemical abnormality for the primaquine - sensitive erythrocytes. [10] However low GSH level or abnormal glutathione instability do not always indicate erythrocyte G6PD deficiency. Decreased red cell glutathione content with normal or increased G6PD and glutathione reductase activity has been found in some cases of ellyptocytosis, [17] congenital inclusion body hemolytic anemia [18] and other kinds of hemolytic anemias. [19 - 23]

Low blood sugar levels and diabetic type glucose tolerance curves have been reported in marasmus. [3, 21] It has been shown that when blood glucose is less than 35 mg %, whether this low level results from in vitro glycolysis or hypoglycemia induced by insulin, GSH instability could be produced in normal blood. [25] Glucose effect

TABLE 2

LABORATORY FINDINGS (INCLUDING BLOOD SUGAR AND ERYTHROCYTE REDUCED GLUTATHIONE LEVELS) IN THE SECOND SERIES OF MALNOURISHED CHILDREN

Cases	Age and Sex	Weight in grams	Degree of malnutrition	Serum protein A/G	Blood sugar %	GSH level /100 cc rbc	GSH level after 2 hrs incubation
R.A. 65/21233	19/12 F	7.000	II	5.5 4.1/1.4	82 mg	80 mg %	68 mg %
Z.E. 65/21183	6/12 F	3.500	IV	4.4 3/1.4	85 mg	92 mg %	78 mg %
K.Ç. 65/21669	6/12 M	4.200	III	—	37 mg	77 mg %	53 mg %
R.K. 65/21666	17/12 M	5.440	III	8.1 5.3/2.8	122 mg	101 mg %	73 mg %
A.C. 65/25875	23/12 F	6.300	III	7 4.6/2.4	85 mg	105 mg %	90 mg %
B.N. 65/26041	10/12 M	5.000	III	—	92 mg	79 mg %	62 mg %
B.A. 65/26046	4.5/12 F	2.500	IV	—	50 mg	70 mg %	53 mg %
A.K. 65/22984	7/12 M	3.800	IV	—	90 mg	100 mg %	61 mg %

TABLE 3

LABORATORY RESULTS
(Control Group)

Cases	Age and Sex	Weight	Blood sugar mg %	GSH level/ 100 cc RBC mg	Serum proteins	
					Alb.	Glob
A. G. 64/36216	5/12	7.980	98	68	4	2.4
Z. P. 62/11703	3 M	13.500	96	56	5.5	2.2
G. N. 64/43425	16/12 F	9.000	76	90	4.5	1.7
Y. T. 63/38833	18/12 M	11.300	76	71.4	4.2	2
F. Y. 64/26155	28/12 F	11.800	77.2	86	4.8	4
S. G. 64/42110	4/12 M	5.750	72	96	4	2.2

on erythrocyte GSH stability also was shown in normal newborns by Zinkham.[12] The lowest blood sugar level was 37 mg % among our patients and none of them had low erythrocyte GSH levels, and GSH instability was found to be normal in all our cases. Since most of our cases had a history of chronic diarrhea and increased blood galactose level has been observed in this condition,[24, 26] the first group of patients' blood sugar determinations were done at the same time according to both the Folin - Wu and the glucose oxidase methods. Results according to the Folin - Wu method represent the sum of the reducing substances, whereas the glucose oxidase method is specific for glucose. The difference between the two methods for the same patient was not significant. For that reason, in the second study group, blood sugar determinations were done only by the Folin - Wu method. Delayed absorption of glucose rarely causes hypoglycemia in hypothyroidism.[27] One of the patients (S.G.) with severe malnutrition had had hypothyroidism (B.E.I. = 0.17 gamma) but his blood sugar, erythrocyte glutathione values, and glutathione stability test were also normal.

The G6PD deficient group was used as an indicator for our GSH determinations and GSH stability tests. Erythrocyte GSH levels were lower than normal and GSH instability was shown to be present in all of these patients as would be expected.[11, 28]

TABLE 4

ERYTHROCYTE GLUTATHIONE LEVEL AND GLUTATHIONE STABILITY TEST RESULTS OF G6PD DEFICIENCY CASES AND THEIR FAMILIES

Cases	Age and sex	Hb. gr. %	Ret %	G6PD level units	GSH level/100 RBC	
					before incubation	after incubation
F. K.	15/12 M	8.83	1.4	0.25	60	19
Mother		14.67	1.2	9	64.5	33
G. C.	16/12 M	9.17	0.8	1.9	90	26
Mother			1.2	3	66.6	30.4
Father		18.	0.2	10.27	74	68
E. N. 63/11178		exitus		0.035		
1st sibling	5 years M	11.83	0.6	0.17	53	16
2nd sibling	3 years M	11.	0.2	8	94	80
Mother		12.	0.6	5.9	75	42
Father		17.34	—	—	81	76
E. A. 63/615	16/12 M	10.	0.8	0	70	6.1
Mother		14.	0.4	15.	68	44.3
M. A. 62/6355	2.5	11.34	0.8	4.1	81.6	29.6
Mother		11.67	0.2	5.7	76	31
Sibling	F	12.67	1.2	14	78.5	49
Y. L. 65/26058	30 year M	15.20	1	0	53	5
A. Ü. 61/1708	6 M	2.34	1.2	21	71	57
Mother	36	10.80	0.6	28	108	81
Father	45	11.85	1.6	0	69	15

Lawrence has shown that glutathione instability could be produced by dilution of the blood with plasma or saline. [20] Hematocrit leveis were measured by the microhematocrit method and were not found to be significantly low in any of our cases including G6PD deficient group. The method for glutathione determination

as indicated by Beutler, et al [9] is not specific for GSH. As seen in Figure 1, cytein gives the same optical density changes as GSH. However, since erythrocyte free amino acid level is very low, it is not a handicap for the sensitivity of the method. We have looked for inhibitor effects of GSSG, homocystein and cysteic acid on GSH determination but none could be demonstrated.

In conclusion, we could not find hypoglycemia in severely malnourished children after three to five hours of fasting, and their erythrocyte GSH level, and GSH stability tests were normal.

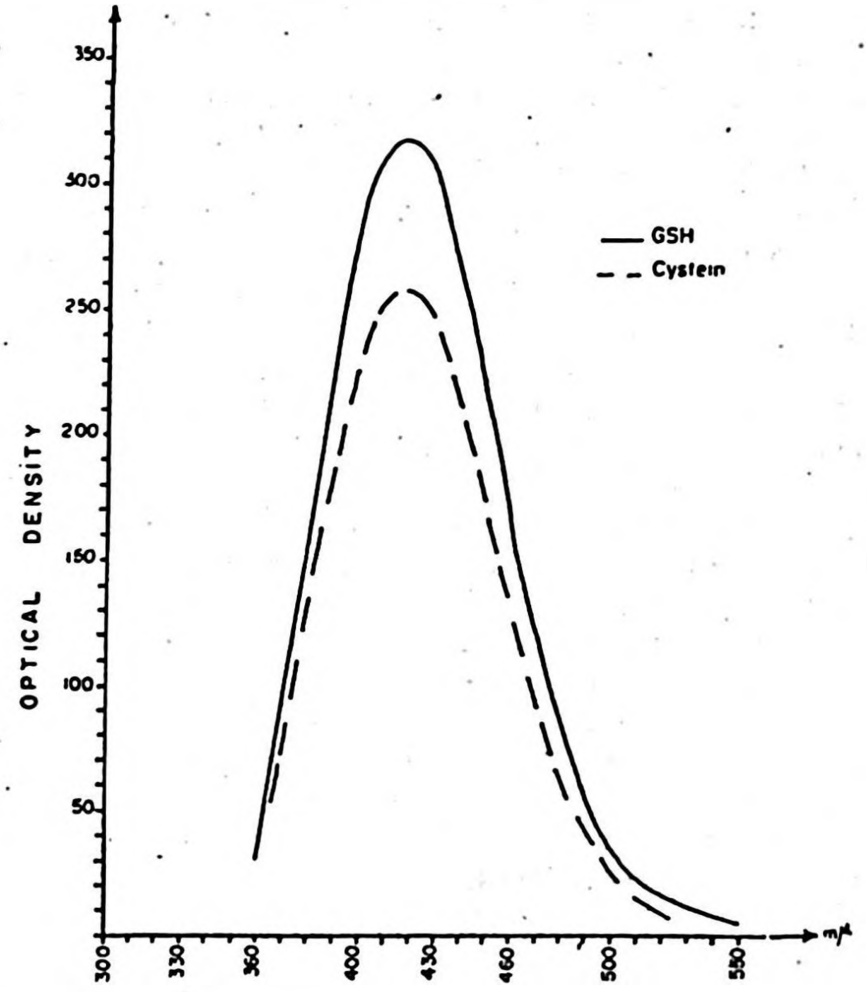

Figure 1. The optical densities of GSH and cystein at different wave lengths.

Summary

In 27 children with severe malnutrition, blood sugar levels were found to be within normal limits for this age group. Their red cell GSH levels and GSH stability tests were normal. Low erythrocyte GSH values and GSH instability were demonstrated in a G6PD deficient control group as would be expected.

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