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Effect of Erythrocyte Glucose-6-Phosphate Dehydrogenase (G-6-PD) Deficiency on Light-Induced Riboflavin Deficiency in the Neonate[1] (39986)

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Phototherapy has been adopted as a noninvasive treatment for mild hyperbilirubinemia in human newborns since it results in lowered serum bilirubin levels. We have demonstrated that this therapy may result in riboflavin deficiency in the neonate without glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (1). Riboflavin deficiency was determined by measurement of erythrocyte glutathione reductase (EGR) activity, a flavine adenine dinucleotide (FAD)-containing enzyme. Subjects with G-6-PD deficiency have significantly elevated EGR and erythrocyte FAD levels (2-4). FAD is more stable to light than riboflavin (5). We investigated whether G-6-PD deficiency would prevent the decrease in EGR activity of infants with hyperbilirubinemia undergoing phototherapy.

Subjects and methods. The study group consisted of five black neonates with G-6-PD deficiency who developed moderate hyperbilirubinemia. Four of these infants were premature.

The criteria for phototherapy were either early onset of jaundice associated with prematurity or serum bilirubin above 10 mg/dl (with normal direct bilirubin) before 72 hr of age.

The full-term infant was fed Similac formula to give 20 calories/oz. Two of the prematures received Similac 60/40 at the same caloric concentration. The remaining prematures received intravenous fluids throughout the duration of this study (Nos. 3 and 5, Table I). None of the infants received vitamin supplements during the study period.

The undressed infants with shielded eyes were in open cribs or isolettes with a bank of eight Westinghouse blue fluorescent lamps (F20T12B) of 20 W each, 56 cm from the skin surface. A Plexiglas shield over the bulbs protected them from ultraviolet irradiation. Lamps were changed every 200 hr.

Serum bilirubins were determined from venous blood by the method of Malloy and Evelyn (6).

The dye decolorization test of Motulsky and associates as described by Dacie and Lewis (7) was used for the initial screening for G-6-PD deficiency. Quantitative assays for erythrocyte G-6-PD were performed by the method of Zinkham (8). Values below 120 units/100 ml of RBC for female neonates and below 100 units/100 ml of RBC for male neonates indicate deficiency of this enzyme.

Erythrocyte glutathione reductase was determined by a modification of the method of Glatzle *et al.* which has been described in detail (9). The activity coefficient (AC) is a measure of the degree of saturation of the enzyme with the coenzyme. Normal AC values for neonates range from 0.9 to 1.2 (9).

The results of these studies were compared to those of the 21 infants with moderate hyperbilirubinemia and without G-6-PD deficiency who underwent continuous phototherapy described in detail previously (1). The two studies were run concurrently.

Results. The sex, birth weight, age at initiation of light therapy, duration of light therapy, quantitative G-6-PD values, and serum bilirubin and AC values before and after phototherapy are given in Table I.

The G-6-PD levels of the erythrocytes obtained prior to phototherapy ranged from 21 to 73 units/100 ml of erythrocytes, well

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TABLE I. DATA FOR NEONATES WITH G-6-PD DEFICIENCY BEFORE AND AFTER PHOTOTHERAPY.

Infant	Sex	Birth weight (g)	Age at light therapy (hr)	G-6-PD (units/100 ml of RBC)	Initial values		Duration of light therapy (hr)	Posttherapy values	
					Bilirubin (mg/dl)	AC		Bilirubin (mg/dl)	AC
1	F	2990	62	46	12.3	1.0	48	9.0	1.0
2	F	2410	65	21	14.5	–	90	7.5	0.95
3	M	1420	18	30	6.0	1.0	120	7.0	0.95
4	F	1900	30	28	9.5	0.9	72	5.5	0.95
5	M	1585	40	73	8.0	1.0	52	8.0	1.0

below the lower limits of normalcy for both males and females.

The initial and final AC values were all within the normal range of 0.9 to 1.2. In the case of infant No. 2 where no initial AC value was obtained, the value of 0.95 after phototherapy signified that no biochemical evidence of riboflavin deficiency occurred during the 90 hr of continuous exposure to light. The duration of phototherapy for the five infants ranged from 48 to 120 hr.

In our previous study of icteric neonates without G-6-PD deficiency, 15 of 16 infants undergoing phototherapy for 48 hr or more developed AC values of 1.3 to 2.0, indicative of riboflavin deficiency (1). The difference between the AC values in that study and those of the five neonates with G-6-PD deficiency reported here is highly significant ($P < 0.001$ by the Fisher exact test).

Discussion. The neonate is particularly susceptible to destruction of its blood riboflavin during phototherapy because of the relatively high levels of free riboflavin. It is this that creates the vulnerability since free riboflavin is considerably more sensitive to light than FAD (5). In the maternal plasma, FAD constitutes the major portion of total riboflavin. However, FAD does not pass across the placental barrier but is degraded by placental enzymes to free riboflavin which is secreted into the fetal blood. About 60% of total fetal plasma riboflavin is free riboflavin (10).

As previously shown, neonates with normal levels of erythrocyte G-6-PD subjected to phototherapy for periods over 48 hr developed riboflavin deficiency. The deficiency was measured by the decrease in saturation of EGR as determined by elevated AC values (1). The decrease in saturation of the apoenzyme with FAD coenzyme is a sensitive indicator of riboflavin deficiency in man (11).

That the EGR pathway is closely connected to the G-6-PD activity of the erythrocyte is shown in the following equations:

$$\text{glucose-6-PO}_4 + \text{NADP}^+ \xrightarrow{\text{G-6-PD}} \text{6-phosphogluconolactone} + \text{NADPH} + \text{H}^+$$

$$\text{GSSG (oxidized glutathione)} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{EGR-FAD}} 2\text{GSH (reduced glutathione)} + \text{NADP}^+$$

Neonates with erythrocyte G-6-PD deficiency have a reduced ability to make NADPH. However, their EGR-FAD levels are elevated, apparently as a compensatory mechanism to produce reduced glutathione in the presence of low levels of NADPH (2, 3). It was postulated that erythrocytes of G-6-PD-deficient subjects have an enhanced uptake of the free riboflavin which is then converted to FAD (4). As a result, the blood of the G-6-PD-deficient neonate has a higher FAD content in the erythrocytes and lower free riboflavin in the plasma. This relationship and the greater stability of FAD to light may explain the absence of riboflavin deficiency in the five neonates with G-6-PD deficiency undergoing phototherapy in the study described in this paper.

Studies in this paper and those by Meloni *et al.* (12) have demonstrated that continuous phototherapy of G-6-PD-deficient infants up to 120 hr resulted in no increase in serum bilirubin levels. Where the line between beneficial and harmful effects of phototherapy is in these neonates remains to be determined.

Summary. Five neonates with moderate hyperbilirubinemia and G-6-PD deficiency underwent phototherapy for periods of 48 to 120 hr. No biochemical evidence of riboflavin deficiency resulted. This contrasts

with evidence of riboflavin deficiency observed in 15 of 16 infants with moderate hyperbilirubinemia but no G-6-PD deficiency who were exposed to light for periods of 48 hr or longer. The elevated FAD levels of the erythrocyte of the G-6-PD-deficient infants may have protected them from the light-induced vitamin deficiency.

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