GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND NEONATAL JAUNDICE*

BY

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The reports concerning the incidence of severe jaundice in newborn infants deficient in erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) show a wide and sometimes contradictory variation. Data obtained from severely jaundiced newborns only give the impression that dangerous degrees of hyperbilirubinaemia occur frequently in G-6-PD deficient newborns (Smith and Vella, 1960; Panizon, 1960; Weatherall, 1960). Recent investigations on large numbers of unselected infants do not confirm this impression. Doxiadis, Fessas and Valaes (1961) and Fessas, Doxiadis and Valaes (1962) estimate the incidence of severe jaundice in G-6-PD deficient male and homozygous female newborns in Greece at approximately 5%. In Israel, an unduly high incidence of neonatal jaundice was found in only one of several ethnic groups with a high frequency of the G-6-PD deficiency gene (Szeinberg, Oliver, Schmidt, Adam and Sheba, 1963).

G-6-PD deficiency has been reported from Thailand (Flatz and Nelson, 1960; G. Kirk, 1960, personal communication). Frequency figures derived from a large number of individuals are not available with the exception of limited areas in North-East Thailand (Kruatrachue, Charoenlarp, Chongsuphajasiddhi and Harinasuta, 1962). Inquiries of several physicians from hospitals in Bangkok revealed that severe neonatal jaundice occurred infrequently in Thai and Chinese newborns: the few cases observed were mostly due to ABO haemolytic disease or sepsis. It was noted, however, that in some cases of icterus thought to be due to sepsis bacteriological proof was lacking and the

diagnosis was based mainly on the appearance of severe jaundice in the absence of iso-immunization. An investigation of the incidence of G-6-PD deficiency and neonatal jaundice in Bangkok seemed indicated.

Material and Methods

The data were obtained from 1,327 male and female newborns of Thai and Chinese origin, born at three Bangkok hospitals between August 1 and September 10, 1962 (672 infants from Chulalongkorn Hospital, 436 from Women's Hospital, and 219 from Bangkok Sanitarium and Hospital). A cord blood sample of each infant was examined for the activity of G-6-PD, and the infants were observed for the appearance of jaundice by one person at each hospital who was not aware of the result of the enzyme testing. The infants were classified according to the appearance and intensity of jaundice: Group 1 included all infants with no icterus or only slight and transient icterus; Group 2 were infants with distinct but moderate jaundice giving rise to no concern; Group 3 comprised infants with severe jaundice: most of the infants in this group had blood group and bilirubin determinations; the serum bilirubin levels were all over 17 mg./100 ml.

All cord blood samples were examined for G-6-PD activity by the methaemoglobin reduction test (Brewer, Tarlov and Alving, 1960). The results obtained in males were strictly bimodal, forming two groups with normal G-6-PD and with G-6-PD deficiency. In G-6-PD deficient females varying degrees of enzyme activity were recognized. All bloods found G-6-PD deficient by the methaemoglobin reduction test, and an approximately equal number of normal bloods were, in addition, examined with the dye reduction test (Motulsky, Kraut, Thieme and Musto, 1959). The use of both tests allowed the recognition of a great number of 'intermediate', presumably heterozygous females, and the separation of severely G-6-PD deficient heterozygous females from homozygous females.

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Reticulocyte counts were obtained from 141 cord bloods, and in a few cases blood smears were examined.

Results

1. Incidence of G-6-PD Deficiency in Newborns in Bangkok. 152 of the total of 1,327 infants were found to be G-6-PD deficient. There were 51 G-6-PD deficient male newborns among 680 samples from males; of the 647 females examined, 101 were G-6-PD deficient (five homozygotes, 96 heterozygotes). G-6-PD deficiency is characterized by X-chromosomal transmission. Therefore, the incidence in the male is equal to the gene frequency q ($= 0.075$). The expected frequency of heterozygous females is $2q(1-q) = 0.139$, and that of homozygous females $q^2 = 0.0056$. The number of G-6-PD deficient females found compares well with the expectation. Heterozygotes: expected 89.8, found 96; homozygotes: expected 3.6, found 5 ($\chi^2 = 1.08$, 2 d.f., $0.6 > p > 0.5$).

2. G-6-PD Deficiency and Jaundice. Table 1 shows the number of infants with different degrees of jaundice within the groups of male and female infants with normal and with deficient G-6-PD. The percentages of jaundiced infants reported in the groups from the three hospitals amount to 13.4, 14.0 and 16.2, respectively, suggesting that the criteria used in grading the jaundice were comparable.

There is no apparent excess of infants with moderate jaundice in the groups of G-6-PD deficient newborns. The group with severe jaundice is composed of 19 infants: 13 of 1,175 with normal G-6-PD, and six of 152 with G-6-PD deficiency. The difference between the groups with normal and with deficient G-6-PD is significant ($\chi^2 = 5.85$, $0.02 > p > 0.01$, with Yates' correction).

Of the 13 infants with severe jaundice and normal G-6-PD, five had ABO haemolytic disease, one had sepsis due to proved infection with *Esch. coli*, and one had congenital leukaemia associated with Down's syndrome. In six infants no cause for the hyperbilirubinaemia could be ascertained. Of the six infants with jaundice and G-6-PD deficiency, one female was mildly deficient and suffered from ABO haemolytic disease. Two severely G-6-PD deficient heterozygous females showed marked jaundice, and two hemizygous males and one presumably homozygous female were severely icteric with bilirubin levels over 22 mg./100 ml. No other cause for the jaundice could be found in these five infants. Labour and delivery had been normal; no drugs had been given to the mother during 48 hours before delivery, or to the infants before the onset of jaundice.

TABLE 1
G-6-PD DEFICIENCY AND NEONATAL JAUNDICE IN 1,327 INFANTS

Intensity of Jaundice	Male Infants		Female Infants		Total
	Normal G-6-PD	Deficient G-6-PD	Normal G-6-PD	Deficient G-6-PD	
None or slight	492	40	425	78	1,035
Insufficient information*	45	3	45	5	98
Moderate	85	6	70	14	175
Severe	7†	2	6‡	4§	19
Totals	629	51	546	101	1,327

* None of these infants was severely jaundiced.

† Three with ABO haemolytic disease, one with sepsis, one with congenital leukaemia.

‡ Two with ABO haemolytic disease.

§ One with ABO haemolytic disease; three were heterozygous, one was presumably homozygous.

Two of the infants were born to primiparae. The mother of the severely jaundiced female newborn gave a history of two abortions and one stillbirth. In the family of the fourth infant, a heterozygous female, there was one male sibling with a history of remarkable neonatal jaundice. The mother of the remaining male infant reported that three male siblings (of four?) had been severely jaundiced during the first week of life.

3. Reticulocyte Counts. The results of reticulocyte counts in 141 cord bloods are given in Table 2. The mean for the severely G-6-PD deficient infants is somewhat higher than the mean of the group with normal G-6-PD. The difference is, however, not significant ($t = 1.06$, $p \approx 0.3$).

Smears and reticulocytes were examined in two G-6-PD deficient severely jaundiced infants. The reticulocyte counts on the fifth day of life were 3.0 and 4.8%, respectively. The smears of the peripheral blood did not show any of the changes in red cell morphology that are characteristic for the drug-induced haemolytic anaemias in G-6-PD deficient older children and adults.

Discussion

The gene frequency for G-6-PD deficiency, calculated from the incidence in 1,327 male and female newborns in Bangkok, was found to be 8%. The incidence of 7.5% in male infants was similar to that found in adult males in the same community (Flatz and Sringam, unpublished data). In females the percentage of G-6-PD deficient infants detected by the methaemoglobin reduction test was much greater than in adults of the same population (approximately 70% of the expected number

TABLE 2
RETICULOCYTES IN THE CORD BLOOD OF 141 NEWBORNS

Group	Description	No. of Infants	Reticulocyte Count		
			Mean (%)	Standard Deviation	Range
A	Females mildly G-6-PD deficient	15	3.33	—	1.2-8.3
B	Females severely G-6-PD deficient	30	3.81	1.16	1.7-7.2
C	Hemizygous males	21	3.76	1.49	1.3-8.0
B + C	Severely G-6-PD deficient infants	51	3.79	1.44	1.3-8.0
D	Infants with normal G-6-PD activity	75	3.51	1.37	1.2-8.6
Total		141	3.59	1.47	1.2-8.6

Difference group D: group B + C $0.3 > p > 0.25$.
Difference group D: group C $0.4 > p > 0.3$.

detected). This may be explained by a generally lower reduction capacity of the newborn erythrocyte.

The appearance of severe jaundice, otherwise unexplained, in five of 152 newborns with G-6-PD deficiency, as compared to an incidence of six in 1,175 infants with normal G-6-PD, suggests that deficiency of erythrocyte G-6-PD is an aetiological factor in some cases of neonatal hyperbilirubinaemia under the conditions present in Bangkok. The incidence of severe neonatal jaundice in the group of hemizygous male infants and homozygous female infants was three in 56. Despite the relatively small numbers this finding seems sufficient to calculate the risk for the group of infants with virtually complete G-6-PD deficiency at approximately 5%. This is in close agreement with the figure calculated by Doxiadis *et al.* (1961) for G-6-PD deficient newborns in Greece. A comparison with the reports from Malaya (Smith and Vella, 1960; Weatherall, 1960) and Sardinia is difficult because the number of newborns from which the reported cases were drawn is not known. The figure of 5% is, however, in contrast to the results obtained in Israel by Szeinberg *et al.* (1963).

There are three female infants heterozygous for the G-6-PD deficiency gene in the group with severe jaundice. In one of these infants the hyperbilirubinaemia is more probably due to ABO haemolytic disease. The appearance of otherwise unexplained severe jaundice in the remaining two infants suggests that heterozygous female infants are also at risk to develop dangerous hyperbilirubinaemia. Fessas *et al.* (1962) arrived at the same conclusion indirectly from the results of their family study.

The pathogenetic mechanism connecting G-6-PD deficiency and neonatal hyperbilirubinaemia remains

obscure. Known haemolysing agents do not seem to play a role in the five cases observed. Fava beans are neither grown nor eaten in Thailand. Despite an appreciable number of G-6-PD deficient individuals in the population haemolysis due to food components does not seem to occur. All infants included in these investigations were delivered in the obstetrical wards and kept in the nurseries of the participating hospitals. Therefore, factors deriving from local customs, as suggested by Smith and Vella (1960) in Malaya, cannot have contributed to the development of hyperbilirubinaemia.

It is of great interest to know whether the factor additional to G-6-PD deficiency in the causation of the jaundice affects the infant prenatally or post-natally. Panizon and Mela (1961) reported three G-6-PD deficient infants with cord blood bilirubins near 3 mg./100 ml.; they took this slight rise as an indication of a prenatal onset of haemolysis and hyperbilirubinaemia. Considering the effective clearance of unconjugated bilirubin by the placenta, which can be surmised from animal experiments (Schmid, Buckingham, Mendilla and Hammaker, 1959), one has to assume that marked haemolysis has to take place in order to raise the cord blood bilirubin.

There is, of course, the possibility that all newborns with G-6-PD deficiency are subject to much increased haemolysis before birth, and that only a few (e.g. those with a low bilirubin conjugating capacity) develop hyperbilirubinaemia. The very slight difference between the cord blood reticulocyte counts of normal and of G-6-PD deficient newborns does not support this assumption. Moreover, the two G-6-PD deficient infants examined at the height of jaundice had reticulocyte counts within the limits of normal for the first days of life (Washburn, 1941). Similar findings reported by Panizon

and Mela (1961) seem to exclude major prenatal haemolysis (comparable to the events in Rh haemolytic disease) in G-6-PD deficient infants as an obligatory or frequent occurrence.

In two families there was a history of severe neonatal jaundice in more than one child. This is in accord with familial predilection for neonatal hyperbilirubinaemia found in families with G-6-PD deficiency by Fessas *et al.* (1962). These authors interpret the familial incidence as an indication for the presence of an additional independent genetic factor which determines the appearance of hyperbilirubinaemia in G-6-PD deficient newborns. The effect of this hypothetical 'hyperbilirubinaemia gene' could be an impairment or delay in the maturation of the hepatic bilirubin conjugating system.

It is to be expected that this hypothetical genetic factor is present in newborns with normal G-6-PD at a frequency similar to that in G-6-PD deficient infants. In the present investigations five infants with otherwise unexplained severe jaundice were found among 152 G-6-PD deficient newborns. On the other hand only six cases of unexplained hyperbilirubinaemia were found among 1,175 infants with normal G-6-PD activity. Assuming that the six cases in normal infants are also due to the genetic hyperbilirubinaemia factor, the most likely explanation for the much higher incidence in G-6-PD deficient infants would be a factor common to them, e.g. the G-6-PD deficiency itself. The enzyme defect may cause a moderate shortening of the erythrocyte life span unaccompanied by an appreciable rise of the reticulocytes. Decreased red cell survival has been described in the absence of haemolysants in G-6-PD deficient adults (Brewer, Tarlov and Kellermeyer, 1961).

This mechanism would explain several observations: the high familial incidence of hyperbilirubinaemia in G-6-PD deficient newborns is determined by the enzyme defect, and not by the 'hyperbilirubinaemia factor'. In infants with normal G-6-PD who carry the genetic factor predisposing to hyperbilirubinaemia severe jaundice will easily develop if other, often non-genetic, factors causing a shortening of the erythrocyte survival are present (e.g. infections, extreme hypoglycaemia, enclosed haemorrhage). Consequently, a familial occurrence of neonatal jaundice is not to be expected in families of infants with hyperbilirubinaemia due to the hypothetical genetic factor when associated with normal G-6-PD. In none of the six cases of unexplained jaundice with normal G-6-PD was a history of hyperbilirubinaemia in siblings obtained. In families with other genetically determined con-

ditions causing mild haemolysis but not obligatory hyperbilirubinaemia (e.g. ABO-iso-immunization, hereditary spherocytosis) an unexpectedly high incidence of neonatal jaundice due to the combination of the factor causing mild haemolysis and the factor decreasing bilirubin clearance should be found.

The practical conclusions for areas with a high incidence of G-6-PD deficiency are obvious: arrangements for early recognition and treatment of neonatal hyperbilirubinaemia must be made if the risk for newborns is found to be appreciable.

Summary

Examinations were carried out on 1,327 unselected newborns in Bangkok for erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD): 152 infants were found to be deficient.

The incidence of otherwise unexplained severe jaundice was 5% in the group of hemizygous male and homozygous female infants, and approximately 2% in heterozygous female infants.

No significant difference was found between the cord blood reticulocyte counts of infants with normal G-6-PD activity and G-6-PD deficient infants.

Some aspects of the incidence and the pathogenesis of neonatal hyperbilirubinaemia associated with G-6-PD deficiency are discussed.

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