NEONATAL JAUNDICE ASSOCIATED WITH FAMILIAL G6PD DEFICIENCY IN ISRAEL

BY

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It is now well established that hyperbilirubinaemia and even kernicterus may occur in the newborn in association with glucose 6 phosphate dehydrogenase (G6PD) deficiency, in the absence of any of the other causes of neonatal jaundice (Panizon, 1960a, b; Doxiadis, Fessas, and Valaes, 1960; Doxiadis, Fessas, Valaes, and Mastrokalos, 1961; Fessas, Doxiadis, and Valaes, 1962; Smith and Vella, 1960; Weatherall, 1960; Flatz, Szingam, Premyothin, Penbharkkul, Ketusingh, and Chulajata, 1963).

In Israel, it is possible to determine the high incidence of G6PD deficiency in some communities, a retrospective survey of 42,000 births undertaken by Szeinberg, Oliver, Schmidt, Adam, and Sheba (1963), revealed only one case of severe neonatal jaundice that could possibly be ascribed to the enzyme deficiency. We wish to report here 8 cases of severe neonatal jaundice which occurred in G6PD-deficient infants in Jerusalem hospital (Shaare Zedek), and to submit evidence suggesting that an antiseptic dye applied to the umbilicus was the precipitating haemolytic agent.

Material and Methods

Each newborn was examined daily, and in every case of moderate or severe jaundice the serum bilirubin was determined. If the result was over 18 mg./100 ml. a detailed history was taken of the mother including her country of origin, past and family history of anemia, jaundice, or favism, and details of drugs taken in the perinatal period. Whenever Rh iso-immunization could be excluded, the blood of the infant and of the parents was examined for G6PD deficiency by the semi-quantitative method of Motulsky and Campbell as described by Doxiadis et al. (1961).

The test is based on the time taken to decolorize a solution of 'brilliant-cresyl-blue' (National Aniline Division) mixed with the blood to be examined. If decolorization occurred after 60 minutes enzyme deficiency was considered to be present, values between one and two hours suggesting heterozygosity.

For blood group studies clotted venous blood was used. The blood groups of the mothers and the babies were determined for the following red cell antigens: ABO, Rh (including C, c, D, E, e) MN, Kell, and Duffy. The direct Coombs test was performed on the blood of the infants. The serum of the mothers was screened against a panel of O red cells for the presence of antibodies belonging to the following blood group systems: MN, P, Lutheran, Rh, Kell, Duffy, Kidd, and Lewis. The red cell antigen typing of the panel cells was performed for us by Dr. A. G. Mourant of the Lister Institute, London. The screening techniques were performed using the panel cells suspended in saline, and also using the same cells treated with ficin. The saline tubes were incubated both at room temperature and at 37°C. and were followed by the indirect Coombs test. The ficinized cells were incubated at 37°C. Titers for complete and incomplete anti-A and/or anti-B were performed where indicated by the ABO groups of the mothers and babies. A dextran medium was used for the detection of the incomplete anti-A and anti-B (Mollison, 1961). The techniques used were the standard methods described by Race and Sanger (1954).

Description of Cases

The criterion of severe neonatal jaundice in this report is a total serum bilirubin level of 18 mg./100 ml. or above on one or more occasion. Exchange transfusion was performed in all but one of the cases here described. During the period July 1962 until February 1963 (incl.) there were 10 cases of severe neonatal jaundice: in 2 of these there was evidence of Rh incompatibility. G6PD deficiency was present in the other 8. The details are presented in Table 1. Most of the parents were Jewish immigrants from Kurdistan among whom the incidence, in males, of G6PD deficiency is 58.2%—the highest of any Jewish community in Israel (Sheba, Szeinberg, Ramot, Adam, and Ashkenazi, 1962). In Kurdish infants constitute less than one-fifth of the infants born in this hospital. The sudden increase in the proportion of Kurdish infants requiring exchange transfusion during the period in question is shown in the Figure. ABO
incompatibility was present in Cases 6 and 8 as shown by the titres of complete and incomplete anti A and anti B antibody respectively. In Case 7, ABO incompatibility could not be excluded as insufficient blood was available. The direct Coombs test was negative in the 8 cases, and no incompatibility in the CDE, Kell, Duffy, and MN systems could be found. Irregular blood group antibodies in the mother's serum were not detected. Details of the delivery and of drugs administered are given in Tables 2 and 3. With the exception of mild analgesics, of whose administration no record is kept, no drug was given uniformly to all the mothers. The vitamins administered to the mothers consisted of a multiple preparation without vitamin K. Although Case 1 also had signs of systemic infection, these were not considered to be responsible for his jaundice since jaundice was marked within 24 hours, whereas signs of infection appeared only on the fifth day.

Triple dye was used in this hospital as prophylactic treatment against umbilical sepsis. This dye is applied

### Table 1

**Clinical and Laboratory Findings in Eight Jaundiced Infants with G6PD Deficiency**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth date</td>
<td>30.6.62</td>
<td>5.7.62</td>
<td>16.6.72</td>
<td>23.12.62</td>
<td>10.1.63</td>
<td>5.8.62</td>
<td>30.10.62</td>
<td>4.2.63</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Birth weight (g.)</td>
<td>2,850</td>
<td>3,460</td>
<td>2,950</td>
<td>2,420</td>
<td>3,350</td>
<td>2,210</td>
<td>3,410</td>
<td>1,970</td>
</tr>
<tr>
<td>Mother's origin</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Morocco</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
</tr>
<tr>
<td>Father's origin</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Morocco</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
<td>Kurdistan</td>
</tr>
</tbody>
</table>

### Table 2

**Mode of Delivery, Postnatal Complications, and Antibiotic Treatment of Infants**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications of delivery</td>
<td>BBA*</td>
<td>Breech</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Asphyxia</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Septicaemia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Jaundice</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neomycin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Exchange transfusions</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Haemoglobin on discharge</td>
<td>12-1</td>
<td>12-7</td>
<td>?</td>
<td>8-6</td>
<td>10</td>
<td>11-8</td>
<td>?</td>
<td>11-2</td>
</tr>
<tr>
<td>Condition on discharge</td>
<td>? Neurological signs</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>

* Key for enzyme tests: + normal, - deficient, ± intermediate, ? not tested.

* Born before arrival in hospital. N = None.

### Table 3

**Drugs Administered to Mothers**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Case No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Tetracycline and dicoumarol</td>
<td>-</td>
</tr>
<tr>
<td>Pitocin</td>
<td>-</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>-</td>
</tr>
<tr>
<td>Vitamins</td>
<td>+</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>+</td>
</tr>
<tr>
<td>Sulphonamides, opiates, chlorpromazine</td>
<td>+</td>
</tr>
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</table>
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Israel.
1958;
1959
1960;
1961
1962
1963
A, first six
months of year; B, second six months of year.

while the infant is still in the delivery room and thereafter
daily in the nursery. The three premature infants
received vitamin K in a dose of 1 mg., intramuscularly.
Antibiotics were administered as shown in Tables 2 and 3.
A history of severe neonatal jaundice in previous infants
was obtained in only one family.

Discussion

In previous years between two to five exchange
transfusions were performed yearly. A sudden
increase in the need for exchange transfusion, such
as here described, suggested the possibility of a
laboratory error in the estimation of bilirubin.
Such an error would not explain why the proportion
of Kurdish infants requiring exchange transfusion
should rise suddenly. Neither the total number of
births nor the ethnic composition of the hospital
population had changed in recent years. As the
Kurdish community in Israel is unique in its particu-
larly high incidence of G6PD deficiency, suspicion
was immediately cast on this fact as being related to
the jaundice. We could demonstrate this enzyme
deficiency in all infants requiring exchange trans-
fusion in the nine months under review, apart from
two in whom Rh incompatibility was the obvious
cause. Prematurity and ABO incompatibility were
present in some cases but there was an associated
G6PD deficiency. No exchange transfusions were
required for the first two reasons alone during the
period under review.

Hyperbilirubinaemia in newborn infants may be
due to increased cell destruction or to inhibition of
the conjugating capacity of the liver. That increased
haemolysis had occurred in our cases can be assumed
by the early onset of jaundice in some cases and the
low haemoglobin values on discharge. The sudden
appearance of a comparatively large number of cases
of jaundice of this type, which heretofore was almost
non-existent in Israel (Szeinberg et al., 1963), led to a
search for changes in treatment which might have
given rise to haemolysis in enzyme-deficient infants.

As jaundice appeared within 24 hours in five
infants, a hypothetical haemolytic agent in the
breast milk could not have been the precipitating
factor. None of the mothers ate beans at the time
of delivery and the cases were not confined to the
season in which favism occurs. It was suggested to us by
Professor E. Goldschmidt that a likely cause was the
antisepic dye used in treating the umbilicus.

The reason for implicating this substance is as follows:
as jaundice due to enzyme deficiency was confined to
our hospital, the provoking agent must be confined
to that hospital. The other hospitals in this town
use either 'brilliant green' alone or no dye at all,
whereas in our hospital a 'triple dye' was used to
prevent umbilical sepsis, as recommended in the
control of staphylococcal infections in newborn
The B.P.C. preparation of 'triple dye' consists of a
mixture of brilliant green, crystal violet, and
proflavine hemisulphate, but instead of the last, the
closely related ethacrynic lactate ('Rivanol') was
used. By April 1963 the evidence against this dye
was strong enough to warrant using brilliant green
alone, which was the practice in a neighbouring
hospital where G6PD deficiency jaundice was not
occurring in newborn infants. In the nine months
followings the discontinuation of its use, only one
Kurdish infant had severe neonatal jaundice.

In order to test the potential haemolytic properties
of our 'triple dye', it was incubated with enzyme-
deficient red cells. There was no abnormal
decrease of reduced glutathione and we, therefore,
failing to confirm such a haemolytic effect in vitro
(E. Heimann-Hollaender and S. Freier, 1963, unp-
published observations). This, however, does not
exclude the possibility that one of the breakdown
products of these dyes present in the patient's plasma
may be responsible for haemolysis. The amount of
substance that can be absorbed through the umbilicus
is obviously very small, but cases of G6PD-deficient
jaundice are on record where the offending substance
was probably a comparably small amount of
naphthalene present in clothes impregnated with
this substance (Zinkham and Childs, 1958; Dawson,
The evidence is, therefore, strongly in favour of an
external factor being the cause of G6PD-deficient
neonatal jaundice in Israel.

The rarity of severe neonatal jaundice due to
G6PD deficiency in African infants in America and
in infants of Oriental Jews in Israel as opposed to the
high incidence of such jaundice in Greece, Italy, and Singapore, has given rise to the suspicion that some, as yet unrecognized, toxic substance is responsible for increased haemolysis in those countries. Our findings support such a theory. On the other hand Fessas et al. (1962) have adduced evidence for a familial tendency for enzyme-deficient neonatal jaundice. They suggest that a second genetic factor inherited independently of G6PD deficiency may exist in these families. As deficiencies of other enzymes, namely acid phosphomonoesterase and catalase, are known to occur in the erythrocytes of some G6PD deficient subjects (Tarlov and Kellermeyer, 1961; Oski, Shahidi, and Diamond, 1963) this possibility must be seriously considered. The relative importance of hereditary as opposed to environmental factors in enzyme-deficient jaundice in newborn infants, therefore, awaits further evaluation.

Summary

Eight infants with severe neonatal jaundice associated with deficiency of G6PD were observed in a Jerusalem hospital. Incompatibility of the ABO groups of mother and infant possibly contributed to the jaundice in three cases. In spite of the high incidence of G6PD deficiency among Kurdish Jews in Israel, neonatal jaundice of this type is known to be uncommon in this country. An antiseptic preparation applied to the umbilicus was considered to be the most likely cause of the haemolysis in our cases.

We wish to thank most sincerely Professor E. Goldschmidt for her help at all stages of this work, and Miss Lia Cividalli for carrying out the Motulsky test.

References


