Acta Paediatrica Academiae Scientiarum Hungaricae Vol. 8 (3), pp. 323—326 (1967)

The Role of Erythrocyte Glucose-6-Phosphate Dehyrogenase Deficiency in Icterus gravis neonatorum

By

E. SULYOK and P. CHOLNOKY

Department of Paediatrics, University Medical School, Pécs

(Received October 1, 1966)

Some drugs are known to induce haemolysis in sensitive persons [1, 9, 6, 23, 35, 29, 19]. In such cases the study of erythrocyte enzymes often revealed a deficiency of glucose-6-phosphate dehydrogenase (G-6-PD), the enzyme which catalyzes the first step of direct oxydation of glucose [1, 7]. In normal erythrocytes this process provides the cell with reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$), formerly called reduced triphosphopyridine nucleotide (TPNH), the compound necessary for maintenance of a normal level of reduced glutathion; this in turn plays an important role in the integrity of the red cell membrane. The deficiency is genetically determined and transmitted as a sex-linked gene with intermediate dominance [5, 8]. The deficiency occurs in most races, but with various frequencies [3, 8, 14, 17, 30, 36], the gene frequency is rather high in Negroes, while the defect is practically absent in Scandinavians. The clinical manifestations may be different in Negroes and non-Negroes [24].

SZABÓ and VIRÁG [28] reported on a case in Hungary and they performed a screening study involving 404 persons both healthy and with various diseases. They also gave a detailed description of the biochemical background of the disease.

The disorder sometimes leads to severe haemolytic jaundice during the early neonatal period [6, 10—15, 18, 21, 24—26, 32, 33]. We have attempted to assess the role of the defect in icterus gravis.

MATERIAL

The material consisted of all newborns with non-obstructive jaundice admitted to our department between January 1, 1959, and December 31, 1965. Patients with a birth-weight less than 2500 g were excluded; all neonates had had a maximal bilirubin level higher than 14 mg per 100 ml (determined by the method of JENDRASSIK and GRÓF). The enzyme determination took place between March 1, 1966 and April 10, 1966, thus the youngest patient was 4 months, the oldest one 7 years old at the time of investigation.

METHOD

We applied TÖNZ's qualitative method [31]. It is based on differential staining of haemoglobin and methaemoglobin in the erythrocyte. The normal red cell is able to reconvert methaemoglobin to haemoglobin by means of $NADPH_2$ sufficiently produced by the direct oxydation of glucose. G-6-PD deficient cells are not able to

do so, therefore they can be distinguished from the normal cells. In normal persons this procedure results in a film of haemoglobin-containing, well-stained erythrocytes (Fig. 1). In the hemizygous G-6-PD deficient male and in the homozygous deficient female there are only "empty" cells to be seen (Fig. 2). According to LYON's hypothesis [22] there are two different cell populations in the heterozygous female: enzyme activity in each cell depends on whether the paternal or maternal X-chromosome was genetically active in it.

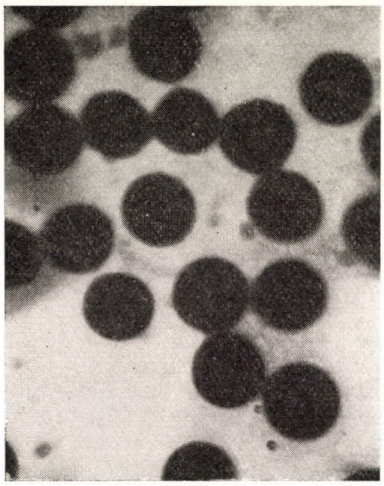

FIG. 1

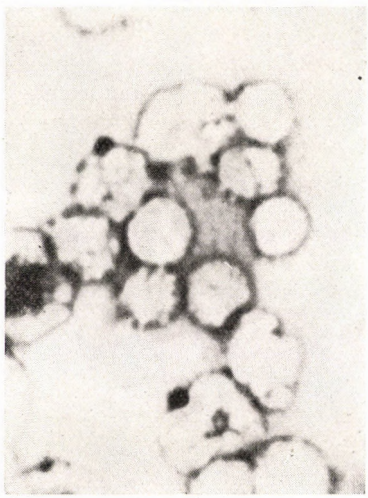

FIG. 2

Apart from this morphological approach, we determined the methaemoglobin reducing capacity in each blood sample in order to avoid possible subjective errors in the evaluation of blood films; after adding $NaNO_2$ the methaemoglobin — haemoglobin ratio was measured before and after incubation in the presence of methylene-blue and glucose.

RESULTS

118 cases have been examined. 41 had A-0, 5 B-0, 3 A-B, 29 Rh incompatibility, 13 had both types of incompatibility. In 23 cases (19.4 per cent) there was no blood group antagonism between mother and child (Table I). In none of these cases were we able to demonstrate a partial or complete deficiency of G-6-PD.

DISCUSSION

In populations where the defect is common, the enzymopathy plays an important role among the causes of neonatal jaundice. We do not exactly know how this deficiency leads to haemolysis in the newborn. It can be ascribed to drugs taken by the mother during late pregnancy, to unknown maternal factors passing the placenta, to the process of delivery (hypoxia, acidosis) or to drugs administered to the newborn (e.g. vitamin K).

The closest relation between neonatal jaundice and G-6-PD deficiency has been described in Greece. DOXIADIS et al. found the defect in 4.6 per cent of the newborns. Jaundice developed in 34 per cent of the affected newborns whereas in those with normal enzyme activity the incidence amounted to 9.1 per cent [12].

TABLE I

Incompatibility	Males	Females	Both sexes
A—0	22	19	41
B—0	3	2	5
A—B	3	0	3
Rh	16	13	29
Rh and AB0	8	5	13
None	21	3	24
Uncertain	3	0	3
Total	76	42	118

Although our material is not sufficient for drawing conclusions valid for the general population of Hungary, it still permits the exclusion of a gene frequency of 0.031 or higher at the 5 per cent level, and of 0.045 higher at the 2 per cent level, had the sample randomly been selected. Our material was, however, composed assortatively, it had been chosen from the general population by the presence of a frequent sign of the enzyme defect, namely neonatal jaundice. We may therefore assume that the gene frequency is much lower than that given above. In spite of this, we feel that the defect must be sought for in every case of neonatal jaundice of unknown origin.

SUMMARY

118 cases of previous Icterus gravis neonatorum were reinvestigated for G-6-PD deficiency. None of these exhibited the disorder. It has been concluded that the occurrence of this enzymopathy must be rather low in Hungary; the gene frequency is probably lower than 4.5 per cent.

Addendum. Since submitting this manuscript a similar survey has been published by *J. Rosta et al.* (Acta paediat. Acad. Sci. hung. **8,** 41, 1967.) Their examinations on 70 mature infants suffering from icterus gravis of unexplained origin also led to the conclusion that G-6-PD deficiency does not seem to be a frequent cause of neonatal jaundice in Hungary.

REFERENCES

1. BEUTLER, E., DERN, R. J., FLANAGAN, C. E., ALVING, A. S.: The hemolytic effect of primaquine. VII. Biochemical studies of drug-sensitive erythrocytes. J. Lab. clin. Med. **45,** 286 (1955).
2. BEUTLER, E., DERN, R. J., ALVING, A. S.: The hemolytic effect of primaquine. IV. The relationship of cell age to hemolysis. J. Lab. clin. Med. **44,** 439 (1954).
3. BEUTLER, E.: The glutathione instability of drug-sensitive red cells. A new method for the in vitro detection of drug sensitivity. J. Lab. clin. Med. **49,** 94 (1957).
4. BEUTLER, E., YEH, M. K. Y., NECHELES, T.: Incidence of the erythrocyte defect associated with drug-sensitivity among oriental subjects. Nature (Lond.) **183,** 684 (1959).
5. BROWNE, E. A.: The inheritance of an intrinsic abnormality of the red blood cell predisposing to drug-induced hemolytic anemia. Bull. Johns Hopkins Hosp. **101,** 115 (1957).
6. CAPPS, F. P., GILLES, H. M., JOLLY, H., WORLLEDGE, S. M.: G-6-PD deficiency and neonatal jaundice in Nigeria. Their relation to the use of prophylactic vitamin K. Lancet **2,** 379 (1963).
7. CARSON, P., FLANAGAN, C. L., ICKES, C. E., ALVING, A. S.: Enzymatic deficiency in primaquine-sensitive erythrocytes. Science **124,** 494 (1956).
8. CHILDS, B., ZINKHAM, W., BROWNE, E., KIMBRO, E., TORBERT, J. V.: A genetic study of a defect in glutathione metabolism of the erythrocyte. Bull. Johns Hopkins Hosp. **102,** 21 (1958).
9. DERN, R. J., BEUTLER, E., ALVING,

A. S.: The hemolytic effect of primaquine. II. The natural course of the hemolytic anemia and the mechanism of its self-limited character. J. Lab. clin. Med. **44,** 171 (1954).
10. Doxiadis, S. A., Fessas, P. H., Valaes, T.: Erythrocyte enzyme deficiency in unexplained kernicterus. Lancet **2,** 44 (1960).
11. Doxiadis, S. A.: A new aetiological factor of severe neonatal jaundice. Lancet **1,** 297 (1961).
12. Doxiadis, S. A., Valaes, T., Karakalis, A., Starrakalis, D.: Risk of severe jaundice in G-6-PD deficiency of the newborn. Differences in population groups. Lancet **2,** 1210 (1964).
13. Doxiadis, S. A., Valaes, T.: The clinical picture of G-6-PD deficiency in early infancy. Arch. Dis. Childh. **39,** 545 (1964).
14. Engelson, G., Kjelman, B.: Red cell G-6-PD and glutathione stability in neonatal hyperbilirubinaemia. Acta paediat. scand. **52,** 82 (1963).
15. Flatz, G., Thanangkul, O., Simarak, S., Manmontri, M.: G-6-PD deficiency and jaundice in newborn infants in Northern Tailand. Ann. paediat. (Basel) **203,** 39 (1964).
16. Gross, R. T., Hurwitz, R. E.: The pentose-phosphate pathway in human erythrocytes. Pediatrics **22,** 453 (1958).
17. Gross, R. T., Hurwitz, R. E., Marks, P. A.: An hereditary enzymatic defect in erythrocyte metabolism: G-6-PD deficiency. J. clin. Invest. **37,** 1176 (1958).
18. Gross, R. T.: G-6-PD activity in hemolytic disease of the newborn. Amer. J. Dis. Child. **102,** 591 (1961).
19. Kimbro, E.: The mechanism of hemolytic anemia induced by nitrofurantoin (Furadantin). Bull. Johns Hopkins Hosp. **101,** 118 (1957).
20. Kirkman, H. N., Riley, H. D.: Congenital non-spherocytic hemolytic anemia. Amer. J. Dis. Child. **102,** 313 (1961).
21. Levin, S. E., Freiman, I., Charlton, R. W.: G-6-PD deficiency and neonatal jaundice in South African Bantu infants. J. Pediat. **65,** 757 (1964).
22. Lyon, M. F.: Sex-chromatin and gene action in the mammalian X-chromosome. Amer. J. hum. Genet. **14,** 135 (1962).
23. Newton, V. A., Bass, J. C.: Glutathione sensitive chronic non-spherocytic hemolytic anemia. Amer. J. Dis. Child. **96,** 501 (1958).
24. O'Flynn, M. E. D., Hsia, D. Y.: Serum bilirubin levels and G-6-PD deficiency in newborn American Negroes. J. Pediat. **63,** 160 (1963).
25. Panzion, F.: L'ictère grave du nouveau-né associé à une deficience en G-6-PD. Biol. Neonat. (Basel) **2,** 167 (1960).
26. Shahidi, N. T., Diamond, L. K.: Enzyme deficiency in erythrocytes in congenital non-spherocytic anemia. Pediatrics **24,** 245 (1959).
27. Smith, G., Vella, F.: Erythrocyte enzyme deficiency in unexplained kernicterus. Lancet **1,** 1133 (1960).
28. Szabó, L., Virág, I.: A vörösvérsejtek glucose-6-phosphat dehydrogenase enzym-defektusa. Orv. Hetil. **105,** 2318 (1964).
29. Szeinberg, A., Sheba, C., Hirshorn, N., Bodonyi, E.: Studies on erythrocytes in cases with past history of favism and drug-induced acute hemolysis. Blood **12,** 603 (1957).
30. Szeinberg, A., Sheba, C., Adam, A.: Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes. Blood **13,** 1043 (1958).
31. Tönz, O.: Zur Problematik der Heterozygotie beim G-6-PD. Mangel. Ann. paediat. (Basel) **204,** 24 (1965).
32. Weatherall, D. J.: Enzyme deficiency in hemolytic disease of the newborn. Lancet **2,** 835 (1960).
33. Yue, P. C. K., Strickland, M.: G-6-PD deficiency and neonatal jaundice in Chinese male infants in Hong-Kong. Lancet **1,** 350 (1965).
34. Zinkham, W. H., Childs, B.: Effect of naphthalene derivates on glutathione metabolism of erythrocytes from patients with naphthalene hemolytic anemia. J. clin. Invest. **36,** 938 (1957).
35. Zinkham, W. H., Childs, B.: Effect of vitamin K and naphthalene metabolites on glutathion metabolism of erythrocytes from normal newborns and patients with naphthalene hemolytic anemia. Amer. J. Dis. Child. **94,** 420 (1957).
36. Zinkham, W. H., Lenkard, R. E., Childs, B.: A deficiency of G-6-PD activity in erythrocytes from patients with favism. Bull. Johns Hopkins Hosp. **102,** 169 (1958).

Dr. E. Sulyok
Gyermekklinika
Pécs, Hungary