HAEMOGLOBINURIA IN SINGAPORE CHILDREN

By Wong Hock Boon, M.B.,B.S., M.R.C.P.E., F.R.F.P.S., D.C.H.

Haemoglobinuria in children has for a long time been linked with the name of Lederer; so much so that often 'Lederer's Anaemia' has been used synonymously with childhood haemoglobinuria. Lederer in 1925 first described acute haemolytic anaemia in 3 patients and in 1930 he described this condition in another 3 patients; out of these 6 patients, 2 had marked haemoglobinuria. There was an associated leucocytosis with normal osmotic fragility and after a blood transfusion, the patients recovered. Since then Patterson and Smith (1936), Greenwald (1938), Baxter and Everhart (1938) and others have published accounts of this type of acute haemolytic anaemia and have labelled them — Lederer's Anaemia. The aetiology of this type of haemoglobinuria has been obscure, but more recently, many workers considered that the majority of childhood haemoglobinurias, i.e. the so-called Lederer's Anaemia, were nothing more than the acquired idiopathic haemolytic anaemia of the auto-anti-body type, and Dacie (1954) concurs in this and found that the majority had a positive Direct Coombs Test. However, in Singapore it is noticed that the majority of such cases had a negative Direct Coombs Test, and an attempt was made to study these cases to try and elucidate the cause behind such acute haemolytic episodes. 30 cases of acute haemoglobinuria were studied in the Paediatric Unit of the General Hospital, Singapore over a period of 3 years.

CLINICAL FEATURES

The clinical features in all the 30 cases were almost similar and the following description of a case is a representative one. The patient was a boy aged 3 years who was admitted with a history of 2 days' fever, abdominal pain and vomiting. On examination, he was mildly jaundiced and was passing dark coloured urine which was found to be a haemoglobinuria. The liver was 1 f.b. palpable below the costal margin but the spleen was not palpable. There was a leucocytosis of 30.800 with the presence of some myelocytes and metamyelocytes, and there was also a reticulocytosis of 15%. His haemoglobin was 7 G% and a Heinz body preparation with methyl violet showed that nearly 100% of the cells showed these bodies. The next day his haemoglobin fell to 6 G%; a blood transfusion was given, after which his haemoglobin rose to 11 G%, the Heinz bodies disappeared and haemoglobinuria ceased. There was complete recovery. The Direct Coombs Test and the Kahn Test were negative (Figs. 1 & 2).

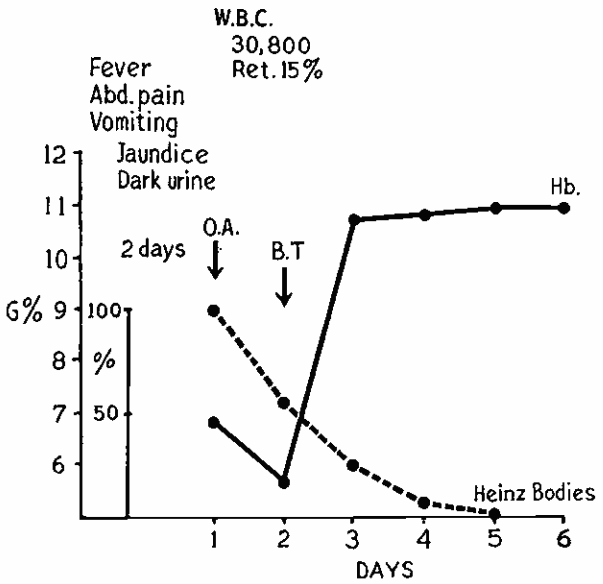

Fig. 1. Showing the progress of patient described above and the response to blood transfusion.

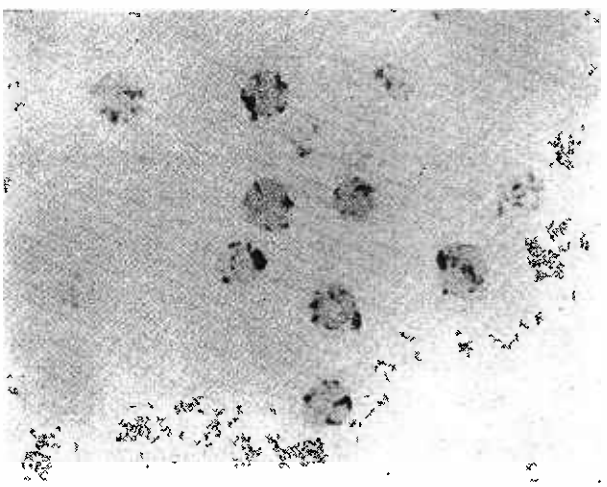

Fig. 2. Heinz bodies with methyl violet preparation present in all cells.

The incidence of the main symptoms and signs in the 30 patients on admission is seen in Table I.

TABLE I

Pallor	100 %
Fever	97.2%
'Red urine'	93.6%
Palpable liver	90 %
Jaundice	75.6%
Vomiting	68.4%
Abdominal pain	57.6%
Palpable spleen	36 %

The significant points in the Table are:—

1) Not all the patients were jaundiced on admission and only $\frac{1}{3}$ had palpable spleens. This is due to the fact that haemolysis was intravascular rather than extravascular.

2) Many of the above signs and symptoms resemble rather closely those of infective hepatitis, a much more common condition with which it may be confused.

Haematologically, the haemoglobin varied from 3-10 G%, with a reticulocytosis and a polymorphonuclear leucocytosis often with the presence of myelocytes and metamyelocytes. The serum bilirubin seldom exceeded 3 mg. per 100 ml. of blood and were all of the indirect variety. The plasma showed the presence of free haemoglobin or methaemalbumin and examination of the urine showed presence of free haemoglobin and large amounts of urobilinogen. The Kahn Test was negative in all cases.

AETIOLOGY

The interesting feature about these cases was that in only 2 cases was the Direct Coombs Test positive, i e. only 2 of the 30 cases were of the auto-antibody type. The Direct Coombs Test was negative in all the remaining 28 cases.

Therefore, in the latter 18 cases, the erythrocytes were tested for the presence or absence of an enzyme glucose-6-phosphate dehydrogenase which is responsible for the production of reduced glutathione which in turn is responsible for the integrity of the red cell. Fig. 3 is a simplified diagram of the relevant biochemical reactions in the normal erythrocyte.

1. Glucose + ATP $\xrightarrow{\textit{hexokinase}}$ glucose-6-phosphate + ADP
2. Glucose-6-phosphate + Triphosphopyridine nucleotide (TPN) $\xrightarrow{\textit{glucose-6-phosphate dehydrogenase}}$ 6-phosphogluconate + TPN(H)
3. Glutathione + TPN(H) $\xrightarrow{\textit{glutathione reductase}}$ Reduced glutathione + TPN

Fig. 3. Simplified biochemical reactions in normal erythrocyte. Hence, it can be seen that glucose-6-phosphate dehydrogenase is necessary for the reduction of TPN which in turn aids in the reduction of glutathione. Therefore if there is a deficiency of glucose-6-phosphate dehydrogenase, there would be diminished amounts of reduced glutathione which would then result in decreased stability of the erythrocyte, so that if there is a trigger mechanism in the form of drugs, chemicals, poisons or sepsis, acute haemolysis may occur.

The method of estimation of glucose-6-phosphate dehydrogenase is essentially the screening method of Motulsky and Campbell (1958) and modified by Vella (1959). Essentially, the blood of the patient was mixed with a substrate of glucose-6-phosphate and triphosphopyridine nucleotide in 'Tris' buffer and brilliant cresyl blue was used as indicator. The tubes were layered with paraffin oil and placed in an incubator at 37°C instead of a water bath as used by Vella (1959). If the cresyl blue was decolourised within 60-70 minutes, the enzyme was present and if decolourisation did not occur by 120 minutes, it was deficient and if decolourisation occurred in about 100 minutes, the reading was deemed to be intermediate.

The results are summarised in Table II.

TABLE II

Direct Coombs Test positive:	2
Direct Coombs Test negative:	28
Enzyme studies done in 18 patients.	
Deficient G-6-P-D: 13 (1 Hb H and 1 Hb Q)	
G-6-P-D present:	5

It is seen therefore that 13 out of the 18 had deficient enzymes, i.e. 72%. 2 of the 13 had associated haemoglobinopathies, 1 a Hb H disease and the other Hb Q disease. 5 of the 18 have normal enzymes, and the aetiology of this last group is still obscure.

As mentioned above, haemolysis in the enzyme deficient patients would occur only if a trigger mechanism was present and in the present series it was found that the drugs commonly used were Sulphonamides and Phenacetin, one of the patients being a new born infant who had Sulphanilimide powder applied to his umbilical cord. He had acute haemoglobinuria with Heinz bodies in the peripheral blood and subsequently died of kernicterus. Many of these 13 patients had also taken native drugs and herbal brews and it has not been possible, so far, to analyse the active principles which might have been responsible for the haemolysis.

DISCUSSION

In direct contrast to the aetiology of haemoglobinurias seen in some Western Countries (Dacie (1954)) which is usually of the auto-antibody type, it is seen that in Singapore only a few are of the auto-antibody type with a positive Direct Coombs Test, and that the majority (about $\frac{3}{4}$) have drug-induced acute haemolysis on a deficient glucose-6-phosphate basis and that there are about 25% of these cases where the aetiology is still obscure.

Further study of these 13 absent enzyme cases showed that the majority are males (Fig. 4). This is to be expected as this enzyme deficiency is transmitted in a sex-linked manner, males being more affected than females (Childs et alia (1958)), and that many of the latter have intermediate valves. Age incidence is shown in Fig. 5,

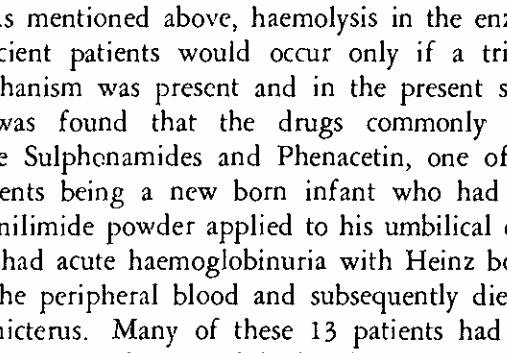

Fig. 4. Showing the male preponderance in haemoglobinuria due to deficient enzyme.

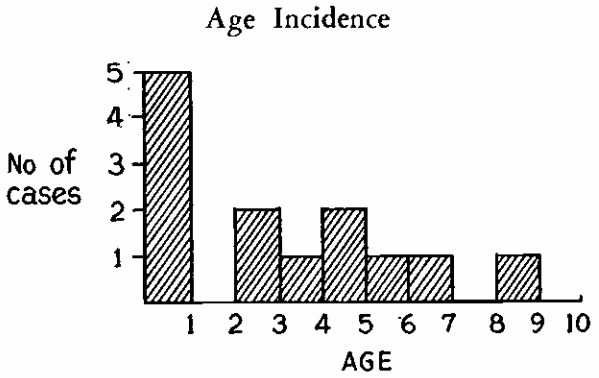

Fig. 5. Showing age incidence of haemoglobinurias due to deficient enzyme.

and there does not seem to be any age predilection — the increased number in infancy is due probably to the fact that a simultaneous study was being carried out on jaundice in the first week of life and this selection probably accounts for the slight increase in this age group. There were 10 Chinese, 2 Jews and 1 Malay. It is significant that there were 2 Jews in this series as they form only a very small minority of the population in Singapore. However, it is mentioned that 20% of non-Ashkenazic Jews from Iraq and Persia have deficient enzymes and it has also been shown that Favism in Jews is due to this enzyme deficiency, the trigger being the Favia bean (Szeinberg et alia (1958)). In this country, Vella (1959) and Wong (1960) have tested random samples of the various races in Singapore with reference to the incidence of enzyme deficiency, and Table III shows the combined figures of the 2 workers.

TABLE III

Adult (*Blood donors*)	*No.*	*Deficient enzymes*
Chinese :	200	2.5%
Indians :	63	4.7%
Europeans :	72	0%
Malays :	95	0%
Infants (*Cord blood*)		
Chinese :	205	2.0%
Indians :	106	0.9%
Malays :	61	1.6%

The above figures are significant only for the Chinese as the number of samples of the other

races are too few. Approximately 2% of the Chinese population have a deficiency of this enzyme.

Drug-induced haemolytic anaemia was noted for as long as 30 years ago when it was noticed that some Negroes given the anti-malarial drug Plasmoquine developed haemoglobinuria but it was only later (Beutler et alia (1955), Carson et alia (1956)) that it was discovered that this occurred in Negroes because of deficient glucose-6-phosphate dehydrogenase.

The treatment of these patients is usually simple. The offending drug is withheld, and if the haemoglobin level is very low, then a blood transfusion is given, when the haemoglobin level will rise (Fig. 1) and haemoglobinuria will cease. According to Beutler (1959), it is only the older cells which undergo haemolysis while the younger ones are relatively resistant, so that if a blood transfusion is given in time, the patients should survive. In the present series all the 13 patients recovered, some with blood transfusion and some without. However, the possibility of anuria has always to be considered.

Fig. 6 illustrates the family studies done on 4 patients and shows clearly the mode of inheritance. It also shows the frequency with which intermediate values are met with in females.

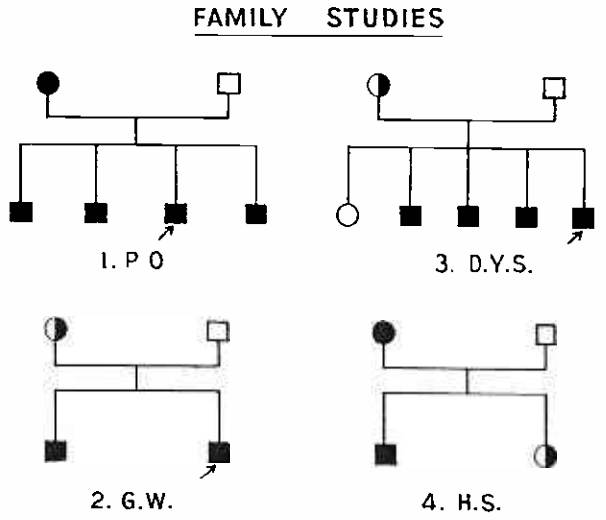

Fig. 6. Showing family studies of patients with deficient A-6-P-D. Fully hatched=deficient. Semi-hatched=intermediate. Plain=normal. Circle=female. Square=male.

SUMMARY

1. The clinical features of 30 cases of acute haemoglobinuria are described.
2. About 75% are drug-induced on a deficient enzyme basis, very few are of the auto-antibody type and in 25% of cases, the aetiology is still obscure.
3. The management of these cases is briefly described and the incidence of this defect in the various races in Singapore mentioned.
4. The genetics of this condition is briefly described.

ACKNOWLEDGEMENT

I am grateful to Dr. Quah Quee Guan, L.M.S., M.R.C.P.E., D.C.H. for allowing me to study some of the cases under her care, and to Mr. Chua Teck Seng for technical assistance.

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