Summary

Selective red-cell aplasia or hypoplasia occurred in 9 out of 23 cases of marasmus and kwashiorkor in Kenya.

Serial bone-marrow punctures showed that the red-cell precursors were normal on admission, and the marrow became hypoplastic or completely aplastic during recovery, at a time when the serum proteins were returning to normal.

There was no single factor that could be regarded as a cause of these red-cell aplasias.

Treatment with oral or intramuscular riboflavine reactivated the marrow and was followed by reticulocytosis and a rise in haemoglobin and packed cell volume.

The implications of the marrow aplasia in marasmus and kwashiorkor are discussed.

Both the adrenal dysfunction of the "recovery syndrome" and the red-cell aplasia may be associated with riboflavine deficiency, particularly as the serum riboflavine is low in marasmus and kwashiorkor and the aplasia responds to riboflavine.

Our thanks are due to the Kenya Medical Department and the medical and nursing staff of the children's ward of the King George VI Hospital for help and assistance given to us during these investigations.

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A Government White Paper, *Disabled Persons in Government Employment*, shows that the Government employ 41,744 registered disabled persons. In the non-industrial Civil Service there are 627,127 people employed, of whom 26,554 (4.2%) are disabled. In the industrial Civil Service there are 349,711 people employed and 14,768 of them are disabled, again 4.2%. There are 452 passenger-lift attendants and 422 of them are disabled, or 93.4%. The standard percentage for the purpose of the Disabled Persons (Standard Percentage) Order, 1946, is 3%.

PRIMAQUINE-SENSITIVITY OF RED CELLS IN VARIOUS RACES IN SOUTHERN AFRICA

BY

R. W. CHARLTON, M.B., B.Sc., M.R.C.P.Ed.

AND

T. H. BOTHWELL, M.D., M.R.C.P.*

Department of Medicine, University of the Witwatersrand, Johannesburg

Some cases of drug-induced haemolytic anaemia have been shown to be due to the action of the drug on congenitally defective red blood cells (Beutler, 1959). Phenacetin, acetanilide, sulphanilamide, sulfoxone, thiazosulphone (Dern *et al.*, 1955), nitrofurantoin (Kimbro *et al.*, 1957), pamaquin, and primaquine (Dern *et al.*, 1955) belong to this group. In addition, favus beans (Sansone and Segni, 1957) and naphthalene (Zinkham and Childs, 1957) also cause haemolysis in sensitive individuals. The congenitally defective red cell has a normal life span unless exposed to one of the precipitating substances, and the trait has not been shown to lead to any other disability. Since the biochemical lesion was first characterized in subjects sensitive to the antimalarial primaquine the condition is commonly referred to as "primaquine sensitivity."

Red-cell Abnormalities in Susceptible Subjects

The pattern of haemolysis in susceptible subjects exposed to one of the precipitating agents has certain characteristic features (Dern *et al.*, 1954; Flanagan *et al.*, 1958). It begins on about the third or fourth day of administration and lasts until about the twelfth day. About half the red-cell population is destroyed, with the result that the haemoglobin and haematocrit fall to roughly half their original values. Haemolysis then ceases, and the blood picture rapidly returns to normal. This is true even when the patient continues to take the drug. The clinical picture is thus one of an acute self-limited haemolytic anaemia.

The defective red cells can be identified in several ways. The first distinguishing characteristic to be discovered was abnormal Heinz-body formation *in vitro*. Following on the clinical observation that Heinz bodies appeared transitorily in the red cells of sensitive volunteers taking primaquine (Beutler *et al.*, 1954), it was demonstrated *in vitro* that the Heinz bodies produced in sensitive cells differed from those in normal cells both in size and in number (Beutler *et al.*, 1955), and the Heinz-body test has been used as a method of predicting primaquine sensitivity.

Subsequent investigations have revealed several biochemical abnormalities in the red cells of susceptible subjects. The concentration of reduced glutathione tends to be lower than normal, though the difference is not great enough to be used as a test of sensitivity. Of more significance is the fact that the glutathione level drops sharply on incubation *in vitro* with acetylphenylhydrazine, while the level in normal blood does not change. This glutathione stability test has proved an accurate means of predicting primaquine sensitivity (Beutler, 1957).

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More recently several enzymatic abnormalities have been demonstrated in sensitive cells. The enzyme glucose-6-phosphate dehydrogenase has been shown to be present in quantities much below normal levels (Carson *et al.*, 1956). This enzyme catalyses the first step in the direct oxidation of glucose via the hexose-monophosphate shunt. In this reaction glucose-6-phosphate is oxidized to 6-phospho-gluconate, and the hydrogen which is removed reduces coenzyme II (triphosphopyridine nucleotide). Coenzyme II is reoxidized by glutathione, which in turn is reduced in the process (Schrier *et al.*, 1958). Glucose-6-phosphate dehydrogenase activity may be estimated in red cells, and deficiency implies primaquine sensitivity. Sensitive cells have also been shown to have increased glutathione reductase (Schrier *et al.*, 1958) and aldolase (Schrier and Kellermeyer, 1958) activity. These abnormalities are regarded at the present time as compensatory to the primary deficiency of glucose-6-phosphate dehydrogenase, the first by facilitating reduction of oxidized glutathione and the second by increasing the maximum rate of anaerobic glycolysis (Schrier and Kellermeyer, 1958). It is, however, still not clear why certain chemical agents should cause haemolysis of red cells with these various biochemical abnormalities (Beutler, 1959).

The condition is hereditary, and although further study is necessary, it appears to be transmitted by a sex-linked incompletely dominant gene (Childs *et al.*, 1958). Thus, males and homozygous females have unstable glutathione and low levels of glucose-6-phosphate dehydrogenase, while female heterozygotes show all degrees of glutathione instability and glucose-6-phosphate dehydrogenase activity. Racial distribution of the trait varies considerably. It is rare in white Americans but is found in 7 to 14% of American negroes (Beutler, 1957; Childs *et al.*, 1958). About 11% of Sephardic Jews have the trait, while it occurs rarely, if at all, in Ashkenazic Jews (Szeinberg *et al.*, 1958). It is relatively common in Greeks and Italians (Gross *et al.*, 1958; Childs *et al.*, 1958), but uncommon in Chinese (Beutler *et al.*, 1959).

Since there is no information on the incidence of the trait in Southern Africa, a survey was carried out in which blood from Bantu, Indians, Cape Malays, and Kalahari Bushmen was examined.

The Investigation

Of the 310 Bantu males tested, 129 were patients in hospital suffering from a variety of medical and surgical conditions, while 181 were healthy blood donors. The 53 Cape Malays tested were male patients in Groote Schuur Hospital, Capetown, and the 100 Indian males were patients in King Edward VII Hospital, Durban. Specimens of Bushman blood were obtained from 29 males and 41 females: 16 males and 31 females belonged to the Central Kalahari Bushman group; while 13 males and 10 females were of the Northern group. These specimens were refrigerated and flown to Johannesburg shortly after collection.

Methods

The glutathione stability test was performed by the method of Beutler (1957) as modified by Flanagan *et al.* (1958). The Heinz-body test was carried out as described by Beutler *et al.* (1955). Glucose-6-phosphate dehydrogenase activity was estimated by the screening method of Motulsky and Campbell (1961), which depends upon the decolorization of a fixed amount of the dye brilliant cresyl blue within a certain time. Glucose-6-phosphate and coenzyme II are added to the haemolysate, and the speed of the reaction thus depends on the quantity of enzyme present. The method has proved reliable and well suited to population survey studies.

Initially heparinized venous blood was used, but the necessity of performing the test within three hours of collection was a disadvantage, and in most studies the blood was collected in acid-citrate-dextrose solution. This permits a delay of at least some days before testing (Beutler, 1959). In a number of studies in which the test for glucose-6-phosphate dehydrogenase activity was used as the screening procedure the blood was obtained by digital puncture.

In all cases positive results were confirmed by applying a second test. At the beginning of the investigation glutathione stability tests were done on all specimens and glucose-6-phosphate dehydrogenase activity or the Heinz-body test was carried out on those with unstable glutathione. In later studies the screening test for glucose-6-phosphate dehydrogenase activity was used as the initial procedure and the glutathione stability test was performed on those with diminished enzyme activity.

Validation of Techniques.—In order to test the validity of the techniques employed two approaches were used. In the first, blood from a subject who had previously been reported as suffering from favism (Senior and Braudo, 1955) was tested. The cells were shown to form typical abnormal Heinz bodies and the concentration of reduced glutathione fell from a basal level of 51 mg./100 ml. erythrocytes to 10.6 mg./100 ml. on incubation with acetylphenylhydrazine. In addition, the screening test for glucose-6-phosphate dehydrogenase showed a marked decrease in enzyme activity. In the second test, red cells from an individual regarded as positive on the three screening tests were labelled with Cr-51 as described by Mollison and Veall (1955) and injected into the circulation of a normal compatible recipient. For eight days the rate of disappearance of radioactivity from the blood of the recipient was compatible with a normal survival of the deficient donor cells. Primaquine was then administered in a dosage of 30 mg. daily. On the third day a sharp increase in rate of disappearance of radioactivity was observed (Fig. 1), thus indicating that the cells were, in fact, sensitive to primaquine.

Results

Bantu.—In 182 Bantu males glutathione stability tests were performed as the initial test. The test was regarded as positive in five. In these subjects the average concentration of reduced glutathione after incubation with acetylphenylhydrazine was 12.3 mg./100 ml. red cells (range 8.2–18 mg./100 ml.). In the 177 negative reactors the average concentration after incubation was 54.4 mg./100 ml. (range 31–156.8 mg./100 ml.). In the five subjects with unstable glutathione the Heinz-body test was found to be positive. In a further 128 Bantu males blood samples were examined for glucose-6-phosphate dehydrogenase activity and five were found to be deficient. Glutathione stability tests were positive in each of these subjects with post-incubation values

ranging between 1 and 11 mg./100 ml. red cells. (The results of the initial 182 glutathione stability tests and of the additional five positive ones are shown in Fig. 2 and the tribal origins of all the subjects tested are shown in the Table.)

Tribal Origins of the Bantu Males Studied

Tribe	No. Tested	No. Positive
Msutu	74	2
Zulu	73	1
Xhosa	43	1
Tswana	25	2
Shangaan	18	1
Pedi	11	0
Swazi	8	1
Nyasa	7	1
Coloured (mixed origins)	13	1
Remainder (Baca, Pondo, Venda, etc.)	38	0
Total	310	10

Indians.—Blood obtained by digital puncture from 100 Indian males was tested for glucose-6-phosphate dehydrogenase activity. In no case was deficiency of the enzyme demonstrated.

Cape Malays.—The blood from 53 male Cape Malays was tested for glucose-6-phosphate dehydrogenase activity. Two specimens were deficient in the enzyme. The reduced glutathione concentrations of these bloods after incubation with acetylphenylhydrazine were 7.4 and 4.5 mg./100 ml. red cells respectively.

Bushmen.—Blood samples from 29 males and 41 females were tested for glucose-6-phosphate dehydrogenase activity. Two samples, one from a male and one from a female, were found to be deficient in the enzyme. The concentrations of reduced glutathione after incubation in these two cases were 0 and 10 mg./100 ml. red cells respectively. Both positive reactors were of the Central Kalahari group.

Comment

The results of the present investigation suggest that the incidence of primaquine sensitivity of the red cells in the Bantu of Southern Africa is approximately 3%. The 10 positive reactors detected belonged to a number of different tribes, as shown in the Table, but the numbers are not large enough for the tribal incidences to be determined. The finding of the trait in the Bantu is not surprising as pamaquin haemolysis has previously been noted in a Nyasa soldier (Mann, 1943) and in a Basuto (Smith, 1943). The incidence is, however, a good deal lower than has been reported in the majority of East African tribes, where it appears to parallel that of the sickle trait (Allison, 1960).

Although the defect was also found to be present in Cape Malays and Bushmen, the significance of the findings in the Malays is open to question, as they are not a pure racial group but represent crossbred descendants of Asiatics, Bantu, Hottentots, Bushmen, and Europeans (du Plessis, 1944).

Although none of the Indians tested showed evidence of glucose-6-phosphate dehydrogenase deficiency, it is evident that the trait does occur in India, as there are several reports of haemolysis due to pamaquin in Indian subjects (Manifold, 1931; Amy, 1934; Sein, 1937; Proc. Conf. Med. Spec., 1944; Dimson and McMartin, 1946). However, most of these patients were Northern Indians, while the Indians of Natal are for the most part descendants of immigrants from Madras (Shafa'at Ahmad Khan, 1946). It is thus possible that the gene frequency varies in different parts of India.

Summary

Red blood cells genetically deficient in glucose-6-phosphate dehydrogenase and with unstable glutathione haemolyse when exposed to certain drugs, chemicals, and favus beans.

The trait has been demonstrated in approximately 3% of Bantu males and has also been found to be present in Cape Malays and Bushmen. It has not been observed in 100 Indian males from Natal.

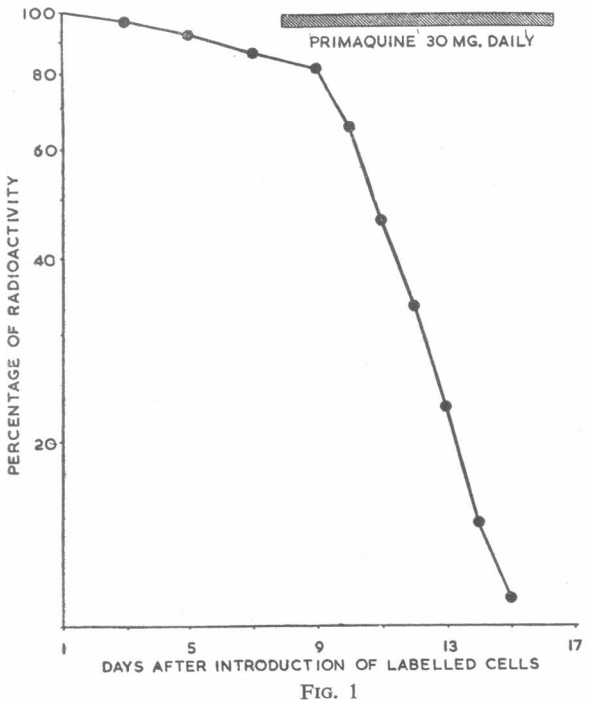

FIG. 1

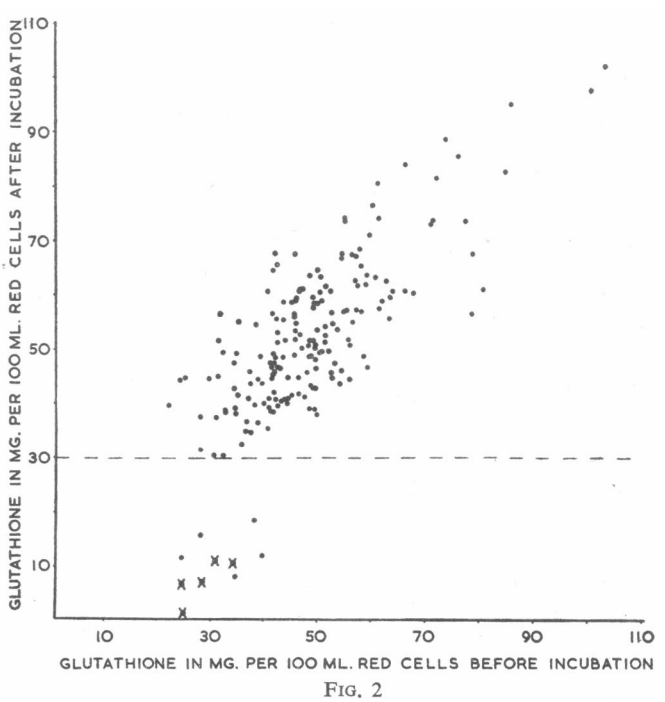

FIG. 2

FIG. 1.—Rate of disappearance of radioactivity from blood before and during administration of primaquine. FIG. 2.—Results of glutathione stability tests.

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COMPLICATIONS OF CHICKEN-POX

BY

CHAVA E. ROTEM, M.D., M.R.C.P., M.R.C.P.Ed.
*Formerly Senior Medical Registrar, Leicester Isolation Hospital and Chest Unit**

Varicella is a highly infectious disease of children, usually mild in nature, without complications or sequelae. Occasionally, however, the virus of varicella may give rise to a number of complications of varying severity which may occur singly or in association, affecting (in descending order of frequency) the respiratory system, the nervous system, the urinary system, and the skin. Cases of visceral chicken-pox have been reported by Johnson (1940), Nicolaides (1957), and McLachlan (1957). Haemorrhagic chicken-pox is usually associated with other complications and is of grave prognosis; two such cases were seen at the Leicester Isolation Hospital; another had been described by Cohen and Bansmer (1947).

Mostly single cases or complications affecting one system only are described in the literature. This paper describes all the complications of varicella seen in this hospital over a ten-year period.

Survey of Material

Twelve patients with complications of varicella were admitted to this hospital during 1950–9. The hospital admits infectious cases from an area including Leicester City and County, and Rutland, with a total population of about 1,000,000.

*Present address: EMMS Hospital, Nazareth, Israel.

Table I shows the distribution of cases by age and sex, and the fatalities that occurred.

Table II shows the various complications encountered in order of descending frequency. No cases of nephritis or nephrosis were seen. Five patients had symptomless albuminuria and mild pyuria for a short time.

TABLE I

No. of Cases	Age Groups	Sex		Fatalities	
		M	F	M	F
5	1–10	2	3	1	1
1	11–20	1			
2	31–40	2		1	
2	41–50	2			
2	61–85	1	1		
Total 12		8	4	2	1

TABLE II

Complications	No. of Cases		Total	Fatal	Remarks
	M	F			
Disseminated pneumonia	4	2	6	—	Two cases associated with profuse rash, 1 with zoster, 1 with varicella gangrenosa, 1 with meningo-encephalitis, 1 with haemorrhagic varicella and visceral lesions
Encephalitic symptoms	3	2	5	1	Fatal case had haemorrhagic chicken-pox
Albuminuria and leucocytes in urine	4	1	5	—	None had any symptoms referable to genito-urinary system. All urine abnormalities cleared spontaneously
Localized skin complications	2	1	3	—	One had varicella gangrenosa. Two had superinfection with *Staph. aureus*. All were mild
Herpes zoster . .	1	1	2	—	One had superinfection with *Staph. aureus* and pulmonary infarcts. The other had disseminated pneumonia
Pulmonary infarcts	2	—	2	1	Fatal case had thrombosis of pelvic veins
Visceral lesions	—	1	1	1	Varicella haemorrhagica

Pulmonary Complications

Pulmonary complications were the most common. Four of the six cases of disseminated pneumonia were admitted for other complications. None had any symptoms referable to the chest and the diagnosis was made radiologically. The case reports of the other two cases are given below.

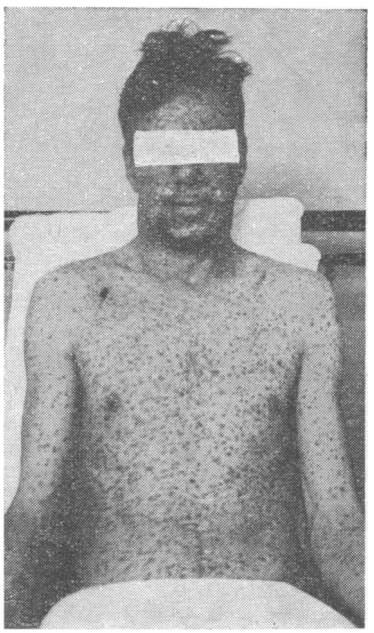

FIG. 1.—Case 3 on May 21, 1950.

Case 3. — Man aged 44; probable source of infection —own child with uncomplicated varicella two weeks prior to illness. May 14, 1950: onset with malaise. May 15: first spots, with rapid increase in spread and density. May 16: upper abdominal pain aggravated by deep breathing; no vomiting, but loss of appetite, normal stools. May 20: admission to hospital. On examination—profuse rash