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DEFICIENCY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE  
IN NIGERIA<sup>1</sup>

by

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Since the observation by Hochwald, Arnold, Clayman & Alving (1952) that administration of primaquine induced an acute haemolytic anaemia in 10% of American negroes and the subsequent discovery that the erythrocytes of the sensitive individuals were deficient in glucose-6-phosphate dehydrogenase by Carson, Flanagan, Ickes & Alving (1956), several studies (Allison & Clyde, 1961; Allison, 1963; Motulsky, 1961; Kidson & Gorman, 1962) have attempted to define the biological role of this inherited enzyme deficiency and to elucidate the mechanism of drug-induced haemolysis in glucose-6-phosphate dehydrogenase deficient individuals. Although a good deal of information is now available the problems remain unsolved.

Studies in West Africa (Gilles & Taylor, 1961; Harris & Gilles, 1961; Allison, Charles & McGregor, 1961) have already shown that 17-24% of the male population show this enzyme deficiency. In order to initiate studies on some of the above-mentioned unresolved problems it became necessary to establish a pool of glucose-6-phosphate dehydrogenase deficient individuals who could be used as experimental subjects. This paper presents the preliminary analysis of the results of a survey designed to create such a pool.

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## METHODS

Approximately 25 ml of blood were removed from the arm vein of adults. Four ml were placed into 1 ml of acid-citrate dextrose solution and used for determination of G-6 PD while the remainder were placed into a heparinized bottle for estimation of  $O_2$  consumption, haemoglobin, packed cell volume, haemoglobin-genotype and osmotic fragility.

### G-6 PD

The method of Dawson, Thayer & Desforges (1958) was used. It was observed that a marked loss of enzyme activity occurred during dialysis of the erythrocyte haemolysate and therefore this process was eliminated. The results are expressed as change in OD at 340 m $\mu$  per five minutes per 0.1 g Hb%. Since the PCV and Hb are known, the results can readily be converted into OD 340/min/100 ml red blood cells if this method of expressing the values is preferred.

### OXYGEN CONSUMPTION OF RED BLOOD CELLS

The manometric method (Marks, Johnson, Hirschbergand & Banks, 1958; Umbreit, Burris & Stauffer, 1957) was used. Erythrocyte oxygen consumption was measured under the following conditions: 30  $\mu$ moles of glucose in 0.5 ml  $H_2O$  in the side arm of the Wasburg cup, 0.25  $\mu$ moles methylene blue in 0.5 ml distilled water, 1.0 ml of washed red cell suspension (haematocrit 50%) and sufficient Krebs-Ringer phosphate buffer to make 3.0 ml. The centre well contained 0.20 ml of 20% KOH soaked in a fold of No. 1 Whatman filter paper. Pure oxygen was used for equilibration.

### Haemoglobin

Oxyhaemoglobin method was used; 10  $\mu$ l of blood are pipetted into 4 ml of 0.04% ammonium hydroxide, the OD measured at 540 m $\mu$  in a Unicam SP 500 Spectrophotometer and the Hb values read off a calibration curve.

### OSMOTIC FRAGILITY OF RED BLOOD CELLS

Method of Dacie (1956) was followed.

### Hb genotype

The red blood cells, after washing with saline, were haemolysed with an equal volume of water. A layer of toluene was placed on the haemolysate which, after thorough mixing, was centrifuged. The haemoglobin solution was electrophoresed on Whatman No. 3 mm paper using barbitone buffer pH 8.6, ionic strength 0.05 at 200 volts for 10 hours.

### RESULTS

#### Incidence of G-6 PD deficiency

The results are shown in fig. 1 and Table 1. They confirm earlier observations on the incidence of G-6 PD deficiency in Ibadan (Gilles & Taylor, 1961) and also on the sex distribution of the deficiency. From scrutiny of the distribution pattern in males, less than 15 units are regarded as deficient, 15-40 units as partially deficient and over 40 units as normal. In males the distribution of the values is bimodal: 20% are deficient, 78% normal and only 2% intermediate. In females, on the other hand, 5% are deficient, 81% normal and 14% intermediate: unfortunately the dividing lines are not so definite and the distribution in females appears continuous rather than clearly trimodal. The results agree with the suggestion that the deficiency is inherited as a sex-linked gene with variable expression.

#### G-6 PD and oxygen consumption

The results are presented in figs 2 and 3 and Table 1. The erythrocyte  $O_2$  consumption of individuals who are deficient in G-6 PD is greatly reduced. The group with intermediate values shows a wide scatter of values for  $O_2$  consumption: individuals with erythrocyte G-6 PD values greater than 40 units have normal  $O_2$  consumption, i.e. greater than  $400 \text{ l } O_2/\text{hr/g Hb}$ . Forty units therefore appears to be the critical concentration of G-6 PD in the erythrocyte: above this enzyme concentration  $O_2$  consumption is independent of enzyme concentration while below it the  $O_2$  consumption was generally reduced.

#### G-6 PD and erythrocyte osmotic fragility

No correlation was found between G-6 PD deficiency and osmotic fragility.

### G-6 PD deficiency and inheritance of Hb genotypes

No relation was noted between the inheritance of G-6 PD deficiency and the Hb genotype. Of the 20 G-6 PD deficient males, six (30%) had haemoglobin genotype AS. The combination of C gene and G-6 PD deficiency was not observed in this series of 200 individuals but a subsequent survey of a further 450 individuals includes three males with haemoglobin genotype AC and G-6 PD deficiency and one male with haemoglobin genotype AC and G-6 PD deficiency.

### Hb genotype and erythrocyte osmotic fragility

The results presented in Table 1 show that the haemoglobin C gene exerts a profound influence on the osmotic fragility of the erythrocytes, the red blood corpuscles of AC individuals being highly resistant to haemolysis. The S gene appears to have a similar but far less profound effect.

The erythrocyte osmotic fragility of 25 European males resident in Ibadan was also investigated. The sodium chloride concentration at 50% haemolysis ranged from 0.390% to 0.443% with a mean value of 0.424%.

Of the 100 male Nigerian blood donors investigated the sodium chloride concentration at 50% haemolysis was less than 0.390% in 23 (23%), and of these, eight (34.8%) had Hb genotype AA, eight (34.8%) had haemoglobin genotype AS and seven (30.4%) had haemoglobin genotype AC. This distribution of individuals with reduced osmotic fragility is to be compared with the following distributions of haemoglobin genotypes in the series:

AA	66%
AS	25%
AC	9%

The remaining two individuals whose haemoglobin genotype was AC had MCF values of 0.390% and 0.392%.

The saline osmotic fragility of 40 male Nigerian doctors and technicians was also studied. The series included three individuals of haemoglobin genotype AC and in these the MCF values were 0.342%, 0.248% and 0.372% respectively. Of the remaining 38 only two, one AS individual and one AA individual, exhibited MCF values of less than 0.390% (0.375% for the AA individual and 0.374% for the AS individual). Both subjects had normal enzyme levels (96 units and 47 units respectively).

## DISCUSSION

The energy available to the erythrocytes in mammals from the metabolism of glucose is derived from at least two metabolic pathways: the first, the Embden-Meyerhof pathway, results in the formation of lactic acid, DPNH and ATP; the second, the pentose mono-phosphate shunt results in the production of potential energy as TPNH. The DPNH and TPNH generated by the red cells are required, amongst other functions, to maintain haemoglobin in the reduced state and to maintain the level of reduced glutathione: the former function depends largely on the production of DPNH while the latter is principally dependent on TPNH production.

The rate of oxygen utilization of normal blood is relatively low compared to that of other tissues (Denstedt, 1953). A remarkable effect on oxygen consumption by the dye methylene blue was first observed by Harrop & Barron (1928), who found that in its presence oxygen uptake was increased about 20 times. The mechanism of action of methylene blue has not yet been completely elucidated but it appears that it is able to transfer  $H^+$  from both DPNH and TPNH to atmospheric oxygen.

The oxygen consumption measured with methylene blue in the system represents the maximum metabolic activity of the erythrocytes under the experimental conditions described. Under the conditions employed in the present study the maximum oxygen consumption of normal red cells is approximately 550 l/hr/g Hb while the oxygen consumption of G-6 PD deficient erythrocytes is 200 l/hr/g Hb. G-6 PD catalyzes the oxidation of glucose-6 phosphate to 6 phospho-gluconic acid which is the first step of the direct oxidative pathway of glucose metabolism and it is to be assumed that in the absence of this enzyme, this system of glucose metabolism cannot be operated by the red cells. Thus under the experimental conditions described two thirds of the energy available is derived from the direct oxidative pathway and the remaining one third from the Embden-Meyerhof glycolytic pathway. The studies of erythrocyte metabolism by Murphy (1960) have shown that the relative proportions of the energy derived from the two systems depend on the oxygen concentration of the equilibrating gas and on other factors.

In seeking clues for the mechanism of action of drugs which induce haemolysis in G-6 PD deficient individuals, insufficient attention has been paid to the metabolic component which appears to be independent of the direct oxidative pathway. In G-6 PD

deficient erythrocytes the energy derived from this metabolic component may be adequate, under normal conditions, for the maintenance of the integrity of the red blood corpuscles. Any drug which inhibits or interferes with this metabolic component may produce haemolysis of those erythrocytes which cannot operate the pentose monophosphate shunt because they lack G-6 PD.

The profound influence which the haemoglobin C gene exerts on red cell osmotic fragility is of some interest. Haemoglobin C differs from haemoglobin A in the beta chain (Hunt & Ingram, 1958) and the results of the osmotic fragility tests suggest that inheritance of the beta chain of haemoglobin C may be associated with a beta chain thalassaemia, i.e. diminished rate of production of this Hb component. On the other hand, the MCHC values of the AC subjects were normal and for this reason the reduced osmotic fragilities in AC individuals may not be associated with deficient haemoglobinization of the red cells but may be due to a low concentration of total proteins and other osmotically active constituents of the red cells. This problem deserves further study.

The finding of increased osmotic resistance of the red cells of the Nigerian population in the lower socio-economic group as compared with those in the upper socio-economic group shows the importance of parallel investigations in these two groups in many African communities. Such parallel investigations provide a relatively easy procedure for sorting out differences that may have genetic bases from others that are due to socio-economic factors such as under-nutrition or parasitization. It is evident from this study that, after due regard had been taken of the effect of the abnormal haemoglobins, the high incidence of reduced saline osmotic fragility of the red blood corpuscles of Nigerians has a strong socio-economic basis, though an inherited defect (thalassaemia) can not be entirely ruled out, since the anomaly exists, but at a greatly reduced incidence, in the upper socio-economic group as well.

Further studies are necessary in order to elucidate the factors responsible for the difference in osmotic fragility. Preliminary analysis of the data collected during this survey indicates that the MCHC of the subjects with low saline osmotic fragility is not reduced as compared with the values found in the group with normal saline fragility and for this reason, as in the case of AC subjects, further study of the concentration of total proteins and other osmotically active constituents of the red cells is being undertaken.

TABLE 1. ANALYSIS OF RESULTS FOR Hb, PCV, ERYTHROCYTE G-6 PD ACTIVITY, ERYTHROCYTE O<sub>2</sub> CONSUMPTION AND SALINE OSMOTIC FRAGILITY OF RED CELLS IN 100 NIGERIAN ADULT MALES

	All (100)	G-6 PD >15 AA (52)		G-6 PD <15 AA (14)		G-6 PD >15 AS (19)		G-6 PD <15 AS (6)		G-6 PD >15 AC (9)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PCV %	44	44	3.3	44	3.1	43	2.9	45	2.7	43	4.1
Hb g/100 ml	14.2	14.2	1.18	14.3	1.45	14.0	0.5	14.4	1.07	14.6	1.05
O <sub>2</sub> consumption ml/hr/g Hb	465	518	203	585	174	509	59	54	59	87	34
G-6 PD Δ <sup>D</sup> 340/5 mins/0.1 g Hb%	35	22	4.4	18.4	0.405	0.397	0.019	0.016	0.022	0.022	0.022
NaCl concentration at 50% haemolysis g/100 ml	0.402	0.411	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015

% of total

Deficient in G-6 PD

AA	20
AS	66
AC	25
	9

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FIG. 1

DISTRIBUTION OF ERYTHROCYTE G-6 PD ACTIVITY IN 100 ADULT NIGERIAN MALES AND 100 ADULT FEMALES

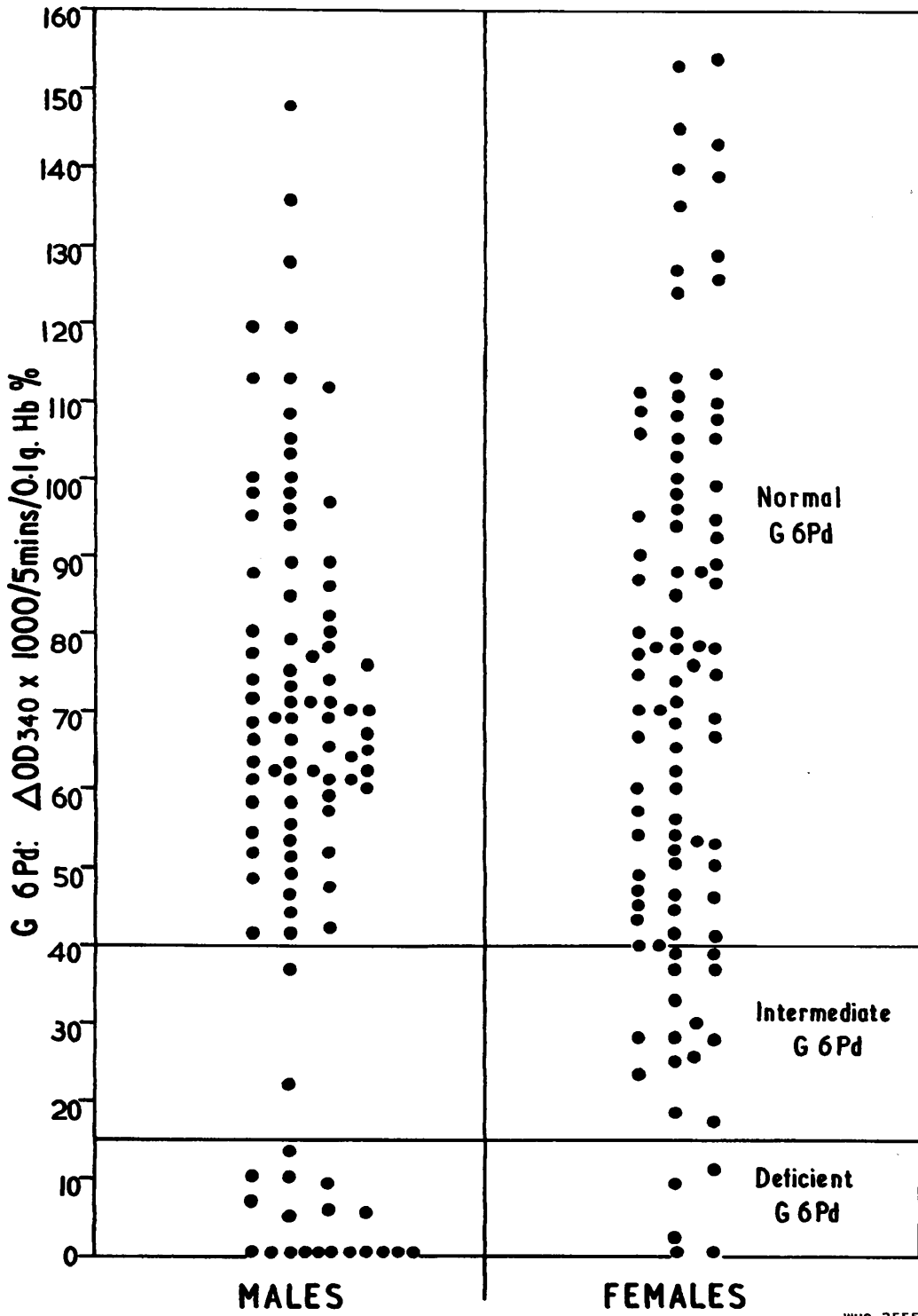


FIG. 2

ERYTHROCYTE O<sub>2</sub> CONSUMPTION AND G-6 PD ACTIVITY IN THE MALE SUBJECTS

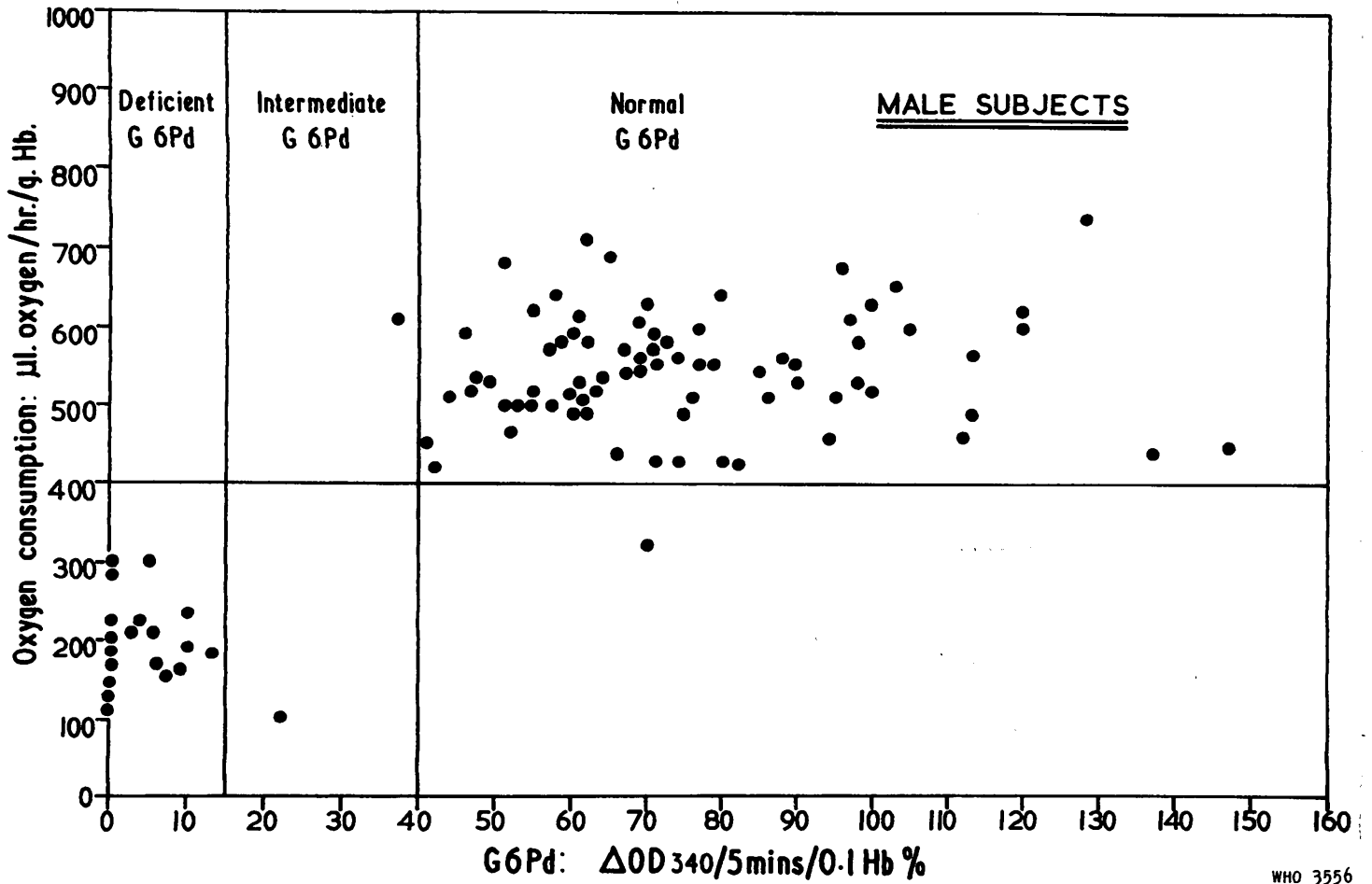
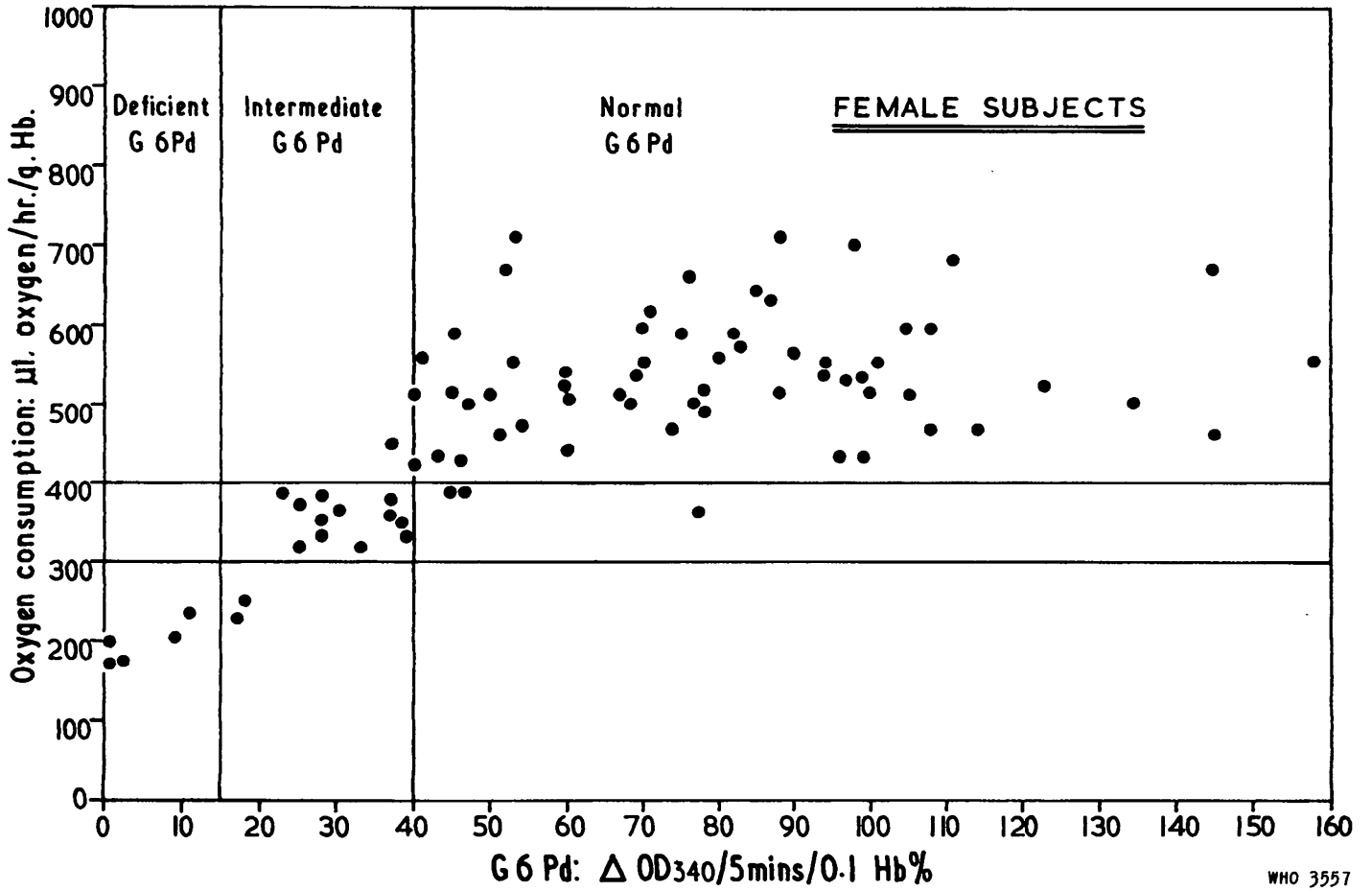


FIG. 3

ERYTHROCYTE O<sub>2</sub> CONSUMPTION AND G-6 PD ACTIVITY IN THE FEMALE SUBJECTS



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