It is intended to follow up the cases considered to be definite, suspicious, or doubtful glaucoma, together with a random sample of those in whom no glaucoma was found, as a long-term project in the glaucoma clinic conducted by one of us (J. P. F. L.) at the Oxford Eye Hospital.

Iris colour was recorded in each patient in the survey. The results are shown in Table VI. These suggest that the colour distribution is similar in the two groups—that is, the total sample and the cases of glaucoma, although there are fewer brown eyes in the latter. This is not a significant finding, because most of our brown-eyed persons happened to be in the younger age-groups.

Summary

A survey of the intraocular pressures of a random sample of 2,000 of the population at Oxford, including all decades from 10 years of age, was undertaken. Persons with an intraocular pressure of over 20.6 mm. Hg at the first examination were subjected to aqueous-outflow studies. These results are presented and the following points made: (a) 0.9% of the total sample had raised intraocular pressure and abnormal outflow resistance; (b) 1.5% of the persons over 45 years of age fell into this group; (c) this group has been labelled "pre-glaucoma," which is regarded as a

better description at the present time than "early open-angle glaucoma"; (d) in a further 0.5% of the persons over 45 years one was led to suspect glaucoma, and in a further 1.1% the diagnosis was in doubt; (e) the importance was stressed of routine measurement of the intraocular pressure, using a Schiøtz tonometer in individuals over the age of 45 years—this simple test could be carried out by the general practitioner.

We are grateful to our colleagues at the Radcliffe Infirmary and at Littlemore, Cowley Road, and Rivermead Hospitals who allowed us access to their clinics; also to our colleagues in the R.A.M.C., the R.A.F., and the U.S.A.F. for allowing us to examine recruits. It is a pleasure to thank Dr. A. Pirie for constant encouragement and useful criticism. We are indebted to Mr. David Hewitt for handling the statistical analyses.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY LEVELS IN ENZYME-DEFICIENT GREEK INDIVIDUALS*

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A deficiency of the red-cell enzyme glucose-6-phosphate dehydrogenase (G.-6-P.D.) is considered to be responsible for many drug-induced haemolytic anaemias (Dern *et al.*, 1954; Beutler, 1959). This erythrocyte lesion is hereditary; it is transmitted through a sex-linked gene with intermediate dominance in the female (Childs *et al.*, 1958). Haemolytic reactions due to primaquine are the prototype of this group of anaemias, and have been described first in negroes; hence the term "primaquine sensitivity" (Dern *et al.*, 1954).

The presence of the same enzyme deficiency in individuals with favism justified the inclusion of the latter among this category of haemolytic anaemias (Szeinberg *et al.*, 1957; Larizza *et al.*, 1958; Zannos-Mariolea and Kattamis, 1961). The similarity of the erythrocyte defect, both in favism and in primaquine sensitivity, was proved further by the crucial experiment of Larizza *et al.* (1958)—that is, the induction of haemolysis after administration of primaquine to an individual with a past history of favism.

Recent findings have shown, however, that in Caucasians—among whom favism is mainly observed—G.-6-P.D. deficiency is more severe than in negroes (Marks and Gross, 1959), affecting not only the old red cells but also the young erythrocyte population, the liver, platelets, and white cells (Ramot *et al.*, 1959; Marks *et al.*, 1959). The clinical significance of the latter findings is at present unknown.

Data on G.-6-P.D.-activity levels in enzyme-deficient Greek individuals are very limited. They were obtained

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from Americans of Greek extraction and showed a marked decrease of enzyme activity similar to that observed in other Caucasians (Childs and Zinkham, 1959).

The purpose of this study is to determine G.-6-P.D. activity in Greek individuals with the enzyme deficiency. To this end, subjects with favism or a past history of favism were chosen. In addition, G.-6-P.D.-deficient male individuals who had never experienced any symptoms of haemolytic anaemia were included in this study. Findings in mothers of the above subjects are reported as well.

Enzyme-activity levels in G.-6-P.D.-deficient infants with a history of severe neonatal jaundice unrelated to blood-group incompatibility or drug exposure are presented separately. Similar babies have been described first by Smith and Vella (1960) in Singapore and later by Italian (Panizon, 1960) and Greek authors (Doxiadis *et al.*, 1961; Zannos-Mariolea *et al.*, 1962; Fessas *et al.*, 1962); they have not been observed so far in negroes. The pathogenesis of their jaundice remains unexplained.

Levels of enzyme activity in these neonates have not been reported from Greece so far.

A correlation between biochemical findings and clinical reactions has also been attempted.

Material

The material of this study consists of the following groups:

Group I.—Forty normal children and adults, all males, used as controls.

Group II.—Twenty-two children with favism, all males except one, aged 2 to 9 years. The enzyme activity was determined during acute haemolysis.

Group IIIa.—Six male children with a past history of favism, aged 3 to 9 years.

Group IIIb.—Fifteen male individuals aged 3 to 38 years, who were found to have G-6-P.D. deficiency by the Motulsky screening test (Motulsky and Campbell-Kraut, 1962) during a large random study in the prefecture of Arta, Epirus. None of them ever experienced any haemolytic reaction in spite of the frequent ingestion of fava beans.

Group IV.—Twenty-five mothers of individuals with favism or a past history of favism who were supposedly heterozygous for the enzyme defect.

Group V.—Twenty-four mothers of G-6-P.D.-deficient males who never experienced any symptoms of increased haemolysis in spite of repeated ingestion of fava beans.

Group VI.—Three G-6-P.D.-deficient infants—one female and two males aged 18, 9, and 4 months—who had suffered from severe neonatal jaundice necessitating exchange transfusion. There was no history of blood-group incompatibility or any drug exposure either of the mother before term or of the baby.

Methods

Glucose-6-phosphate dehydrogenase activity was determined by two methods: (1) the semi-quantitative test devised by Motulsky—this procedure has been described in detail elsewhere (Zannos-Mariolea and Kattamis, 1961); and (2) a modification of the quantitative spectrophotometric method described by Zinkham *et al.* (1958). The activity of G-6-P.D. was measured after the rate of reduction of triphosphopyridine nucleotide (T.P.N.) at 340 $m\mu$. The specimen was centrifuged in the cold at 3,000 r.p.m. for 10 minutes. The supernatant plasma, white-cell layer, and a small portion of the red cells were aspirated and the remaining red cells were washed three times with chilled 0.14 M sodium chloride. After the last washing most of the saline was removed and 0.1 ml. of packed red cells was added to 0.5 ml. of digoxin solution. The mixture was shaken vigorously and kept in the refrigerator at 0° C. for 20 minutes. The resulting haemolysate was centrifuged at 5,000 r.p.m. for 10 minutes and the supernatant withdrawn.

G-6-P.D. activity was estimated in the 10-mm. cell of a Unicam spectrophotometer at 340 $m\mu$. The assay system consisted of 0.1 ml. of haemolysate, 2.75 ml. of triethanolamine buffer (5.10^{-2} M T.R.A., pH 7.6; 5.10^{-3} M E.D.T.A.), 0.1 ml. of 1.10^{-2} M T.P.N., 0.05 ml. of 4.10^{-2} M 4 G-6-P. Na (disodium glucose-6-phosphate).*

The blank solution contained all the reagents except T.P.N. and G-6-P.Na.

The reaction was started by the addition of T.P.N., and readings of optical density change were made at one-minute intervals for 10 minutes. The enzyme unit was defined as that amount in the assay system which produced an optical density change at 340 $m\mu$ of 1 unit/minute/3 ml. of reaction mixture at 25° C. The final values of G-6-P.D. activity were expressed as units of enzyme/ml. washed red blood cells. Normal values of enzyme activity are presented in the Table. Small differences in enzyme activity could be detected by this method. (Standard deviation of the method: 0.094 unit.)

In most cases the enzyme activity was determined immediately; otherwise the sample was kept in the refrigerator at 4° C. and examined within 24 to 48 hours. Acid-citrate-dextrose mixture was used as anticoagulant.

*All the reagents were supplied by Biochemica Boehringer, Mannheim.

Results

Findings are presented in the Table and the Chart.

Group I.—Values of G-6-P.D. activity in the control group ranged between 3.06 and 5.20 units, with a mean of 3.98 and a standard deviation of 0.58. Six (15%) individuals had an enzyme activity between 3.06 and 3.25 units. The Motulsky test was normal in every case, with a decolorization-time well below 100 minutes.

Group II.—G-6-P.D. activity was zero in 5 (22.5%) of the favism cases and between 0.27 and 0.83 unit in the remaining 17, with the exception of one who had a value of 1.60 units. This patient had 5% reticulocytes. The mean value of this group was 0.41, and the standard deviation 0.37. The time of decolorization (Motulsky test) was above 180 minutes in all cases except 3, who decolorized in 100, 100, and 75 minutes respectively. The corresponding G-6-P.D. activity was 0.83, 1.60, and 0.55.

Group IIIa.—G-6-P.D. activity was zero in the six cases and decolorization time was above 180 minutes.

Group IIIb.—This group is comparable to group IIIa. The enzyme activity was zero in 13 (86.7%) of the 15 cases; in the remaining two it was 0.10 and 0.15 unit. The time of decolorization was above 180 minutes in all.

Group IV.—G-6-P.D. activity was below the lower value of the control group—that is, below 3.06 units—with a

Glucose-6-phosphate Dehydrogenase Activity Levels in Six Groups of Greek Individuals

Group	No. of Cases	G.-6-P.D. Activity u. ml./Packed Red Cell			Retic. Range (%)	Motulsky's Test Decol. Time Range (min.)
		Range	Mean	S.D.		
I	40	3.06-5.20	3.98	0.58	0.2-0.7	40-70
II	22	0.00-1.60	0.41	0.37	2.8-7.6	>180*
IIIa	6	0.00	—	—	0.4-1.0	>180
IIIb	15	0.00+	—	—	0.6-1.6	>180
IV	25	0.10-3.00	1.70	0.84	0.2-0.8	40->180
V	24	0.00-2.80	1.37	0.98	—	40->180
VI	3	0.0-29, 0.12	—	—	—	>180

* With the exception of 3 patients with decolorization times of 100, 100, and 75 minutes, and corresponding G-6-P.D. activity of 0.83, 1.60, and 0.55 units.
† With the exception of two individuals with G-6-P.D. activity of 0.10 and 0.15 units.

Percentage distribution of G-6-P.D. activity levels in five groups of Greek individuals.

mean of 1.70 and a standard deviation of 0.84. More analytically, very low values of 0.32, 0.11, and 0.84 unit were observed in three of the mothers; on the other hand, four mothers had high enzyme activities between 2.70 and 2.85 units, and one other mother had a value of 3.00 units. There was a fairly good correlation between the time of decolorization and the degree of enzyme deficiency. Values below 1.50 units corresponded usually to an abnormal Motulsky test.

Group V.—Findings are comparable to those of group IV. Values ranged between 0 and 2.80 units, with a mean of 1.37 and a standard deviation of 0.98. Three mothers had 0 values. High values, between 2.70 and 2.80 units, were encountered in three mothers. The correlation between decolorization time and degree of enzyme deficiency was comparable also to that of group IV.

Group VI.—This group is comparable to groups IIIa and IIIb. Values of G-6-P.D. activity were 0, 0.12, and 0.29 unit. The decolorization time was above 180 minutes.

To summarize, G-6-P.D. activity was zero as a rule in male hemizygotes during periods of rest. During haemolytic crisis a slight activity was observed in most of the cases. All heterozygous women (98%) had intermediate values; no overlapping was observed between them and the control group.

Discussion

The results of this study show that Greek male hemizygotes have a total absence of enzyme activity during periods of rest. This is in agreement with the findings of other authors obtained in Caucasians (Larizza *et al.*, 1958; Childs and Zinkham, 1959).

Very low yet significant levels of G-6-P.D. activity were found in male hemizygotes during the favic crisis. To the best of our knowledge only one large series of G-6-P.D.-deficient male Caucasian individuals studied was during that period (Larizza *et al.*, 1958). The author reports a total absence of enzyme activity; he admits, however, that low levels of G-6-P.D. activity might have been missed because of the small sensitivity of the method used.

Our findings in G-6-P.D.-deficient males confirm, therefore, previous observations by other authors that in Caucasians the degree of enzyme deficiency is much greater than in negroes. The slight increase in G-6-P.D. activity observed during the favic crisis is in accordance with the finding of Marks *et al.*, 1959, that in Caucasians old red cells do not differ very much in their G-6-P.D. activity from young erythrocytes.

No difference in enzyme activity was observed between individuals with a past history of favism (group IIIa) and males with G-6-P.D. deficiency who never experienced any symptom of increased haemolysis in spite of repeated favabean ingestion (group IIIb). From recent observations in areas with a high frequency of G-6-P.D. deficiency (Choremis *et al.*, 1962, 1963), it is concluded that favism is not very frequent among sensitive individuals. This is in agreement with the prevailing opinion that some other factor besides G-6-P.D. deficiency is required for the production of favism (Beutler, 1960; Alving *et al.*, 1960).

There were wide quantitative differences of enzyme activity in the 49 mothers (groups IV and V). Seven of them had levels between 2.70 and 2.85 units—that is, approaching low normal values—while one mother had a low normal enzyme activity (3.00 units). On the other hand, six mothers were found to have very low enzyme activity as observed in homozygous females or hemizygous

males. None of the women in the present study had ever suffered from symptoms of increased haemolysis. In view of the fact that the families of these women were not investigated, it is impossible to be sure that those with very low enzyme activity were in fact heterozygotes.

In any case, practically all heterozygous females in this study had abnormal enzyme activity. The highest values observed are still significantly lower than normal, considering the accuracy of the method (standard deviation: 0.094).

Data on G-6-P.D. activity on a large number of Caucasian heterozygous women are not available in the literature; on the other hand, it is well known that a certain number of negro female heterozygotes appear to have normal G-6-P.D. activity. The findings in this study suggest, therefore, that the greater severity of G-6-P.D. deficiency observed in Greek males as compared with negroes is also reflected in the Greek heterozygous women, in whom the diagnosis of the enzyme deficiency can be made with a high degree of accuracy. It is interesting in this respect that in a previous study by two of us the glutathione-stability method failed to detect any abnormality in 26% of mothers of children with favism (Zannos-Mariolea and Kattamis, 1961).

The Motulsky test proved to be of a high diagnostic value in Greek hemizygotes, during either favic crisis or period of rest. It was much less valuable when applied to female heterozygotes.

The findings in the three babies are comparable to those obtained in groups IIIa and IIIb. Therefore, quantitative assay of G-6-P.D. activity in these infants cannot possibly differentiate them from other G-6-P.D.-deficient individuals, and are of no help in the elucidation of the pathogenesis of their severe jaundice.

It might be interesting, however, to estimate the enzyme activity during the period of jaundice; a large series of these newborn babies might show that a statistically significant greater number of them than individuals with favism have zero values of activity.

Summary

Quantitative assay of G-6-P.D. activity in Greek individuals with the enzyme deficiency showed that there was practically total absence of enzyme activity in hemizygous males who were not in favic crisis; that small yet statistically significant levels of enzyme activity were found in hemizygous males during favic crisis; that practically all heterozygous women had values of enzyme activity significantly lower than normal; and that infants with a past history of severe neonatal jaundice not due to blood-group incompatibility or drug exposure were found to have the same degree of G-6-P.D. deficiency as other deficient subjects.

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RENAL FUNCTION IN PATIENTS WITH UNILATERAL HYDRONEPHROSIS

BY

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Few observations have been made on renal function in chronic hydronephrosis in man. Berlyne (1961) demonstrated that patients with chronic bilateral hydronephrosis may have an impaired ability to concentrate and acidify the urine, and there have been reports of isolated cases which showed excessive loss of either sodium or water in the urine (Black and Wagner, 1955; Winberg, 1959). Relief of obstruction of the urinary tract due to stone or prostatic hypertrophy may be followed by polyuria and an increased excretion of sodium (Wilson *et al.*, 1951; Roussak and Oleesky, 1954; Earley, 1956; Bricker *et al.*, 1957; Knowlan *et al.*, 1960).

The present investigation was designed to study the function of chronic hydronephrosis in humans in more detail. For this purpose patients with unilateral disease were selected so that the function of the hydronephrosis could be compared with that of the normal kidney. These patients also had no uraemia or electrolyte disturbance, so that dysfunction of the kidney could not be ascribed to extrarenal influences. A preliminary account of this work has already been published (Platts and Williams, 1962).

Clinical Material

Eight females and four males were investigated. They were selected only in so far as they all had unilateral hydronephrosis and were admitted for operation to relieve the obstruction. The diagnosis was made by excretion urography and retrograde pyelography, which showed dilatation and retention of the dye in the pelvis. Ten of these patients had an obstruction at the pelvi-ureteric junction which was relieved by an Anderson-Hynes plastic repair (Anderson and Hynes, 1949). Aberrant vessels were found in only two patients, and they were not thought to be the cause of the obstruction, which was presumed to be of the so-called "functional" type. One similar patient (Case 10) has not yet been operated upon. One patient (Case 11) had a gross hydronephrosis and hydroureter which were relieved by reimplanting the lower end of the ureter into the bladder.

Nine of these patients presented with pain, three with haematuria, two with symptoms referable to hypertension (Cases 3 and 4), and one with frequency and nausea. All those who have been operated upon have been relieved of symptoms referable to the urinary tract. Five patients were hypertensive at the time of investigation (Table I). The highest blood-urea concentration was 46 mg./100 ml. (Table II). In two patients the urine from the hydronephrosis was infected.

An attempt was made to grade the severity of the hydronephrosis by measuring the area of the pelvis and calices as seen on either the excretory urogram or the retrograde pyelogram, and where possible the thickness of the renal parenchyma was measured on the films (Table I). At the time of operation an estimate of the thickness of renal tissue and the volume of the pelvic fluid was made.

Methods

Clearance studies were made on each kidney after catheterization of each ureter and insertion of a Foley catheter into the bladder under sacral anaesthesia, using 2% procaine.

Leakage around the ureteric catheter was common at first, but when further holes were made in the catheters this was largely prevented. The catheter was inserted into the hydronephrotic sac, its position being obvious from the copious flow of urine. On the normal side the catheter was located in the upper ureter.

TABLE I.—Clinical Features of Hydronephrotic Patients

Case No.	Sex and Age	Pelv. Area (sq. cm.)	Pelv. Vol. (ml.)	Tissue Thickness (cm.)	B.P.	Urine Protein	Urine Infection	Urine-reducing Substance
1	F 36	21	50	2.0	150/90	Trace	Yes	Nil
2	F 41	22	—	1.4	180/100	—	No	—
3	M 58	38	77	—	180/100	Nil	—	Nil
4	F 32	46	45	0.5	210/120	+	—	—
5	F 28	50	—	—	120/90	++	—	—
6	F 39	55	165	0.8	130/90	Nil	—	—
7	F 19	59	45	1.2	140/90	—	—	—
8	F 38	84	145	—	120/75	++	Yes	—
9	M 13	45	150	1.2	130/80	Nil	No	—
10	M 28	101	—	—	170/100	—	—	—
11	M 42	V. large	—	—	130/90	++	—	—
12	F 32	94	500	0.8	180/110	Nil	—	—

TABLE II.—Plasma Components in Patients with Unilateral Hydronephrosis

Case No.	Blood Urea Creatinine		Na	K	Cl	mOsm./kg
	mg. 100 ml.					
			(mEq/l.)			
3	35	—	142	4.0	101	—
5	33	0.89	140	4.9	105	—
1	46	1.03	149	3.9	108	292
2	—	—	148	—	105	282
4	42	1.16	148	—	109	300
6	22	—	142	4.2	106	282
7	12	—	—	3.7	—	—
8	12	0.76	135	4.7	104	272
9	30	—	132	4.0	94	—
10	—	—	149	4.9	108	—
11	36	—	—	—	107	—
12	18	0.87	137	3.7	100	—