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A dual hereditary red blood cell defect in one family:
Hypocatalasemia and glucose-6-phosphate dehydrogenase deficiency

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Introduction

Acatalasemia is a rare hereditary abnormality with an autosomal mode of transmission, described in several Japanese (Takahara, 1952; Takahara, Hamilton, Neel, Kobaro, Ogura and Nishimura, 1960) and in two Swiss families (Aebi, Heininger, Bütler and Hassig, 1961). The term acatalasemia has been applied to the homozygous state, whereas the heterozygous carriers have been termed hypocatalasemic.

Hereditary glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is rather frequent in certain ethnic groups and is transmitted by a sex-linked gene with incomplete dominance (Childs, Zinkham, Browne, Kimbro, and Torbert, 1958; Szeinberg, Sheba and Adam, 1958). There are conflicting reports on the catalase activity in G-6-PD deficient erythrocytes, it being normal according to Beutler, Dern, Flanagan, and Alving (1955), and Szeinberg, Sheba, Hirshorn, and Bodonyi (1957), and moderately decreased according to Tarlov and Kellermeyer (1961).

A severe deficiency of both G-6-PD and catalase was found in the erythrocytes of an Iranian-born Jew, who developed a severe hemolytic reaction following contact with a fungicide, zinc ethylene bisdithiocarbamate (Pinkhas, Djaldetti, Joshua, Resnick and de Vries, 1963). A study of the patient's family was made in order to determine whether the catalase deficiency in this subject was secondary to the G-6-PD deficiency, or whether the two enzymatic defects were independent genetic abnormalities.

Material and methods

The case history of the propositus has been described in detail elsewhere (Pinkhas, Djaldetti, Joshua, Resnick and de Vries, 1963). The patient's previous history and family history were negative for hemolytic reactions or mouth ulcers. The present investigation was carried out 18 months after the hemolytic episode.

Catalase assay A. The method of Von Euler (1934), modified by Tarlov and Kellermeyer (1961), was used whenever enough blood was available. Heparinized

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venous blood was used for this assay which was performed in a cold room at 10°C. The results were expressed as enzyme concentration in moles per liter of assay solution. Normal values obtained in our laboratory range between 2.32 — 3.72 $\times$ 10^{-9} moles/liter (mean $\pm$ standard deviation 2.84 $\pm$ 0.41).

Catalase assay B. The micromethod of Miller (1958) was used only when small quantities of blood (heparinized blood obtained from a finger) were available. The principle of the method depends upon the destructive effect which hydrogen peroxide has upon catalase, and the concentrations of the reactants are arranged so that the catalase is destroyed before all the substrate is decomposed. The reaction is carried out at 37°C. and the time is long enough (75 minutes) to allow complete destruction of the enzyme. The results, denoting the quantities of H_2O_2 decomposed during the reaction, are expressed in arbitrary units per 10 micrograms of hemoglobin.

Normal values obtained in our laboratory range between 95-150 units (125 $\pm$ 13.6) being lower than those recorded by Miller (175 $\pm$ 38). The cause of this difference is being currently investigated. Preliminary studies have indicated that slight differences in the temperature of incubation have a significant influence on the final results, higher temperatures resulting in a lower activity. In a representative experiment a blood sample gave the following activities at different temperatures of incubation: at 33°C — 190 units, at 37°C — 146 units and at 41°C — 106 units. These results suggest that the rate of inactivation of catalase by H_2O_2 increases significantly with the rise of temperature in the investigated range. The temperature of the water bath used by us during routine catalase determinations fluctuated between 37-38°C.

Catalase separation and localization on starch gel. The method of Thorup, Strole and Leavell (1961) was used. The electrophoresis was carried out at 10°C for 15 hours.

Glucose-6-phosphate dehydrogenase activity. G-6-PD activity was determined spectrophotometrically by a slightly modified method of Kornberg and Horrecker (1955), (Ramot, Szeinberg, Adam, Sheba and Gafni, 1959). The normal range of G-6-PD activity in erythrocytes by this method is 10-19 units/g. hemoglobin. When only small amounts of blood obtained from a finger were available, the G-6-PD activity in the erythrocytes was assayed by the screening method of Motulsky (1960) as described by Doxiadis, Fessas, and Valaes (1961). The latter method, which is reliable for the detection of subjects with fully expressed enzyme deficiency, fails, however, in the detection of a significant proportion of heterozygous females.

Hemoglobin estimation. Hemoglobin in hemolysates, used for assays of catalase and G-6-PD activities was determined as alkaline oxyhemoglobin, read at 450 mμ in a Beckman D.U. spectrophotometer (Anderson, Kalckar, Kurashi and Isselbacher, 1957).

Results

Red blood cell catalase activity. Out of 52 members of the 5 generations on whom a family history was obtained (fig. 1), blood examination was performed on 39 subjects, all but one (III, 5) belonging to the generation of the propositus (IV, 3) and the next generation. As seen in table I and figure 1, severe hypocatalasemia (7-9 per cent of

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Table 1. Catalase and glucose-6-phosphate dehydrogenase deficiency in the investigated family

Pedigree position	Sex	Catalase activity		G-6-PD activity	
		Method A 10^{-9} mol/L assay mixture	Method B units/10 μg Hb	Spectrophotometric method units/g. Hb	Motulsky screening test
III, 5	♀		146		Normal
IV, 1	♀		140		Normal
IV, 2	♂	2.37		13.5	Normal
IV, 3	♂	0.25	14	0.7	Deficient
IV, 4	♀	2.71	120	6	
IV, 5	♀		151		Normal
IV, 6	♂	1.38	67	13.6	Normal
IV, 7	♂	2.61	120	13.4	
IV, 8	♀		140		Normal
IV, 9	♂	2.56	117	10.1	
IV, 10	♀		56		Normal
IV, 11	♀		146		Normal
IV, 12	♂	2.44	64	14.2	
IV, 13	♀		140		Normal
V, 1	♀		140		Normal
V, 2	♀		140		Normal
V, 3	♀		146		Normal
V, 4	♀		129		Normal
V, 5	♂		140		Normal
V, 6	♂	144.	62	0.9	Deficient
V, 7	♂	1.43	75		Normal
V, 8	♀		67		Normal
V, 9	♀		73		Deficient
V, 10	♂		157		Normal
V, 11	♀		73		Normal
V, 12	♀		129		Normal
V, 13	♂		118		Normal
V, 14	♀		78		Normal
V, 15	♂		95		Normal
V, 16	♀		67		Normal
V, 17	♂		78		Normal
V, 18	♂		151		Normal
V, 19	♂		84		Normal
V, 20	♂		140		Normal
V, 21	♀		134		Normal
V, 22	♀		73		Normal
V, 23	♀		78		Normal
V, 24	♀		146		Normal
V, 25	♂		73		Normal

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mean normal activity) was found in the propositus only, whereas intermediate catalase deficiency (49-67 per cent of mean normal activity) was found in 15 other members of the investigated family. These included all the four children of the propositus, two of his four first cousins, his second cousin as well as eight children of the

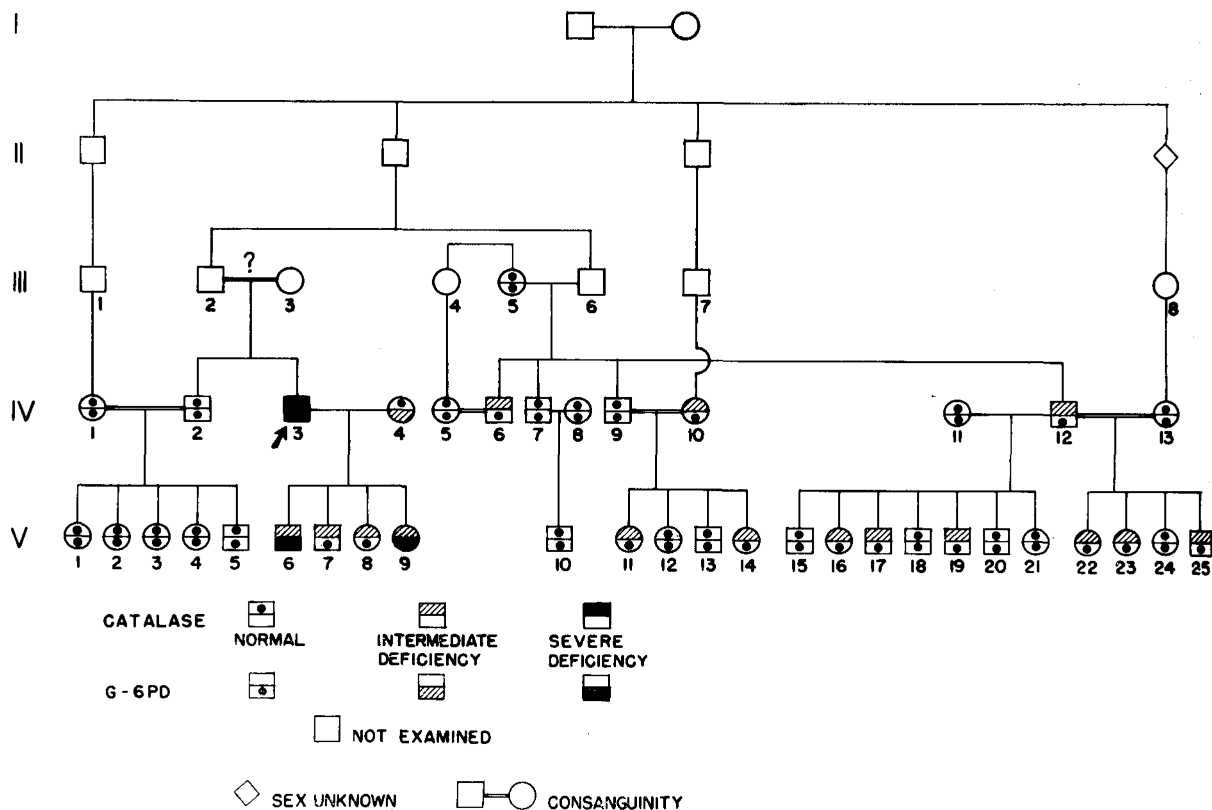

Fig. 1. Pedigree of investigated family

said cousins. Unfortunately, the parents of the propositus were not available for examination. According to the information obtained, the parents were related to each other, but the type of consanguinity was not known.

The finding of many cases with decreased catalase activity in this family, as opposed to the absence of such cases in a large sample of population examined at random (about 200 subjects), suggested a genetic origin of the catalase deficiency. The family pattern was compatible with autosomal inheritance of the defect. The male propositus and one of his male first cousins (IV, 12) transmitted the abnormality to both male and female offspring. The number of affected male and female members of the family was equal (eight males and eight females). There was no significant

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difference in the range of catalase activity in the erythrocytes of the intermediate males (62-84 units, method B). and females (56-78 units, method B).

The data suggested incomplete dominance of the gene, resulting in intermediate catalase deficiency in heterozygous subjects, and very low activity in the homozygous state. If it is assumed that the propositus with severe catalase deficiency was homozygous for the defect, his wife being normal, all their children ought to have been heterozygous. Indeed all four children of this couple were found to have intermediate catalase activity. Fifty per cent of children of couples, in which one of the partners is intermediate and presumably heterozygous, and the other is normal, ought to be heterozygous. The actual findings for two such families was eight intermediates amongst 15 children.

Starch-gel electrophoresis of catalase. Catalase in the hemolysates prepared from the erythrocytes of the propositus (IV, 3), of one of his cousins (IV, 6) with intermediate

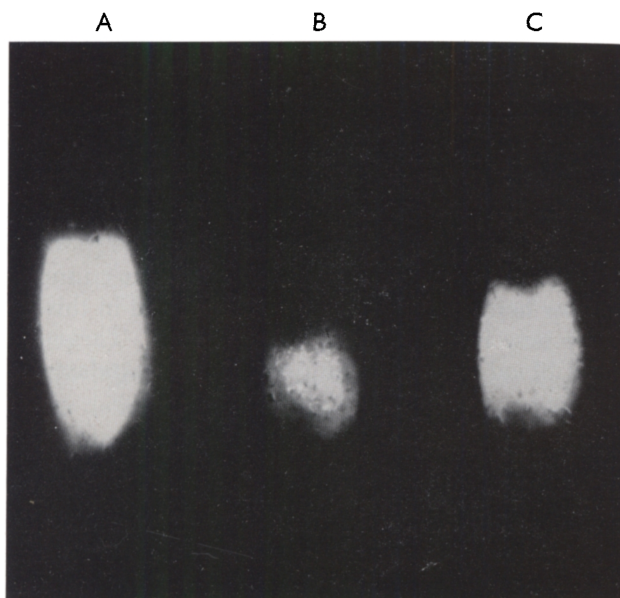

Fig. 2. Starch-gel electrophoresis of hemolysates, stained for catalase. A: normal subject (IV, 4); B: propositus (IV, 3); C: intermediate subject (IV, 6)

catalase activity, and the normal wife (IV, 4) of the propositus, was localised on starch-gel after electrophoresis and staining by a method specific for this enzyme. As can be seen from fig. 2, also by this method a slight catalase activity was detected in the erythrocytes of the propositus. The quantitative difference between the catalase activity of the erythrocytes from the presumed heterozygous subject and that of a normal subject is clearly demonstrated.

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Glucose-6-phosphate dehydrogenase activity. G-6-PD deficiency was found in the immediate family of the propositus only. The propositus was fully enzyme deficient (0.7 units/gm. Hb.), his wife (IV, 4) was probably heterozygous, having intermediate G-6-PD activity (6.0 un./gm. Hb.). One of their sons was enzyme deficient, while the second son had a normal activity. One of the daughters was enzyme deficient as determined by the screening method of Motulsky. The other daughter gave a normal result by this method, but from the analysis of the family tree she should have been heterozygous or homozygous (because of the enzyme deficiency in her father and the heterozygous state of her mother). This discrepancy may possibly be explained by the inability of the screening method to detect a large proportion of heterozygotes.

Independent segregation of the enzymatic defects. The above results suggest that the two enzymatic defects segregated independently in this family, the G-6-PD deficiency being sexlinked, and the catalase deficiency autosomally transmitted. Thirteen members of the family had intermediate hypocatalasemia, not associated with G-6-PD deficiency, and one member (IV, 4), the wife of the propositus, had intermediate G-6-PD deficiency, but was normocatalasemic. The propositus had a full deficiency of both enzymes, while one of his sons (V, 6) had a full G-6-PD deficiency and only intermediate hypocatalasemia.

Hematological findings. Several members of this family had somewhat low hemoglobin values, ranging from 9.0 to 12 gm. per cent, but no significant difference was observed between the intermediate hypocatalasemic and the normal subjects. The only exception was a one-year old hypocatalasemic girl (V, 14) who had a hemoglobin of 7.5 gm. per cent, a red blood cell count of 3.300.000 per cu.mm. and hypochromia in the blood smear. Low hemoglobin values, such as found in this family, are common in villages, peopled by new immigrants in this country. Target cells or spherocytes were not found in the blood smears of any of the hypocatalasemic members.

Discussion

The mode of transmission of the inherited catalase deficiency in the presently studied family is similar to that found in the Japanese and Swiss families having this enzymatic defect. It conforms to a transmission by an autosomal gene with incomplete dominance. It is not certain, however, whether the enzymatic defects in the subjects belonging to the three different ethnic groups — Japanese, Swiss, Jewish — are identical, since quantitative differences in the catalase activity of the red blood cells of the respective homozygotes appear to exist. Takahara, Hamilton, Neel, Kobara, Ogura and Nishimura (1960), in their study on the Japanese families, reported absence of catalase activity ($K_{cat} = 0$) in the homozygous individuals. Nishimura, Kobara, Takahara, Hamilton and Madden (1961) were also unable to demonstrate any catalase by an immunologic method using antihuman catalase rabbit serum. It should be pointed out, however, that there is some inconsistency in the data on these Japanese families, since in the latter report, contrary to the previous communications, the " acatalasemic " subjects appear to have a residual catalase activity of

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up to 4 per cent of normal. In the Swiss families residual catalase activity of about one half per cent of normal was found (Aebi, Heiniger, Bütler and Hassig, 1961), while in our presumed homozygous subject the catalase activity was about 8 per cent of normal. Electrophoretic studies by the Swiss authors proved that the residual enzymatic activity in their "acatalasemic" subjects was indeed due to catalase and not to other red cell components (Blumberg, Masti, Jeunet and Aebi, 1962). Similar proof was obtained in our severely hypocatalasemic propositus (IV, 3).

In view of the residual catalase activity found both in the Swiss subjects and in our propositus, we prefer to designate these subjects as *severely hypocatalasemic* (Pinkhas, Djaldetti, Joshua, Resnick and de Vries, 1963), and not as acatalasemic.

The genetic study on the presently described Iranian-Jewish family indicates that the hypocatalasemia is independent of the G-6-PD deficiency. In this family there are several members with intermediate catalase deficiency and normal G-6-PD activity. The findings are consistent with the hypothesis that the severe catalase deficiency in the propositus is a reflection of a homozygous condition. It seems improbable that the propositus is heterozygous for catalase deficiency and that there is an additional suppression of catalase activity resulting from the G-6-PD deficiency. Such a mechanism is inconsistent with the observation that the propositus' son (V, 6) and daughter (V, 9) have fully expressed G-6-PD deficiency and yet the catalase activity in their erythrocytes is intermediate and not different from that found in other members of this family who are presumably heterozygous for catalase deficiency and have normal G-6-PD activity.

Summary

A family of Iranian-Jewish origin with two hereditary enzymatic defects in the red blood cells, catalase deficiency and glucose-6-phosphate dehydrogenase deficiency, is described. The two enzymatic defects were inherited independently of each other. The findings are compatible with a transmission of the catalase deficiency by an autosomal gene of incomplete dominance, the homozygous state resulting in severe catalase deficiency and the heterozygous state in intermediate catalase deficiency.

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RIASSUNTO

Una famiglia ebraica, d'origine Persiana, presenta due difetti enzimatici ereditari dei globuli rossi: insufficienza della catalasi e della glucoso - 6 - fosfato - deidrogenasi.

I due difetti enzimatici sono stati ereditati indipendentemente l'uno dall'altro. I dati riguardanti la deficienza della catalasi ci suggeriscono una trasmissione per mezzo di geni autosomici a dominanza incompleta; la condizione omozigotica si manifesta in una grave deficienza, mentre la condizione eterozigotica si manifesta in una deficienza moderata.

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RÉSUMÉ

Une famille juive d'origine Iranienne présente deux défauts enzymatiques héréditaires des hématies: insuffisance de la catalase et de la glucose-6-phosphat déshydrogénase. Les deux défauts enzymatiques ont été hérités indépendemment. Les données concernant la déficience en catalase sont compatibles avec una transmission par gênes autosomiques à dominance incomplète, l'état homozygotique se manifestant par une déficience sévère et l'état hétérozygotique - par une déficience modérée.

ZUSAMMENFASSUNG

Es wird eine jüdische Familie aus den Iran stammend beschrieben mit zwei erblichen enzymatischen Defekten in den roten Blutkörperchen, Katalase-Mangel, Glukose-6-Phospat-Dehydrogenase Mangel.

Die zwei enzymatischen Defekte wurden unabhängig von einander vererbt. Die Ergebnisse stimmen mit der Überthagung des Katalase Mangels durch einen autosomalen Gen von unvollständiger Dominanz überein. Der homozygote Zustand führt zu einen schweren Katalase-Mangel, der heterozygote Zustand einem mittelmässigen Katalase Mangel.

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