Favism: Current Problems and Investigations

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Summary. Haemolytic favism is a severe, acute anaemia which occurs in glucose-6-phosphate dehydrogenase deficient individuals, usually following the ingestion of *Vicia faba* seeds. Current interest is focused on the nature of the active substances of *Vicia faba* and on the causes of the varying susceptibility among G6PD-deficient individuals to episodes of severe haemolysis.

The results of experiments *in vitro* favour the hypothesis that *Vicia faba* contains several active substances which may act in a synergistic way.

Red cell acid phosphatase and thalassaemia genes appear to play a remarkable role in conditioning the susceptibility to severe haemolysis in G6PD-deficient subjects.

In addition to erythrocyte enzymes and to enzymes which intervene in the absorption and metabolism of the active substances of *Vicia faba*, another field for future investigations may be that of plasma factors which influence the stability of reduced glutathione in the red cells.

Subjects with red blood cell (RBC) deficiency of G6PD may present episodes of acute haemolytic anaemia (haemolytic favism) after ingestion of *Vicia faba* beans. The current interest in this severe anaemia is concerned first with the nature of the active substances of *Vicia faba* responsible for the haemolysis and secondly, with the causes of the considerable inter-individual variability in response to the active substances of *Vicia faba* in the presence of G6PD deficiency.

Haemolysis in these subjects is preceded by a fall in the level of RBC-reduced glutathione (GSH) (Panizon and Pujatti, 1958); although not yet proven, this is usually regarded as a cause-effect relationship. Experiments carried out *in vitro* have shown that *Vicia faba* juice or aqueous extract induces a fall of GSH in G6PD-deficient RBC similar to that induced by various drugs with haemolytic action (Mela and Perona, 1959; Walker and Bowman, 1960; Contu *et al*, 1961; Zacchello, Panizon, and Zanesco, 1964). From studies on the action of vicine and convicine aglycons* (Lin and Ling, 1962a, b, and c; Mager *et al*,

1965) on GSH *in vitro*, it was then suggested that the 'haemolytic principle' of *Vicia faba* could be identified with some of these substances. However, the glycosides vicine and convicine, both of which are present in *Vicia faba*, are inactive on GSH (Mager *et al*, 1965) and it is not known whether the relevant aglycons are present in the extracts of broad beans tested *in vitro*. A similar action on GSH by β -(3-4-dihydroxyphenyl) L-alanine (L-dopa) observed in some studies (Kosower and Kosower, 1967) has not been confirmed by Razin *et al* (1968) who suggested a synergistic action of this substance with the aglycon of divicine. More recently (Beutler, 1970) it has been suggested that the active haemolytic principle is dopaquinone, produced from L-dopa through the action of tyrosinase. Appropriate experiments *in vivo* (Gaetani *et al*, 1970) and clinical observations (Braham and Sarova-Pinhas, 1971) have failed to provide any evidence in favour of the L-dopa hypothesis.

G6PD deficiency is the necessary condition for the occurrence of haemolytic episodes but it is not, by itself, the sufficient condition. It has been reported that the incidence of clinical favism in a group of enzymopenic subjects taken at random was less than 30% (Bernini *et al*, 1960). The action of

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* Vicine and convicine are β -glycosides occurring in broad beans. Their aglycon moieties are divicine and isouramil, respectively.

some other genetically determined factor has been suggested by Sartori in 1959 on the basis of his clinical findings. Siniscalco *et al* in 1961 observed that the presence of thalassaemia trait makes G6PD-deficient individuals less liable to acute haemolysis due to *Vicia faba*. In 1966, Stamatoyanopoulos *et al* reported data showing the existence of an autosomal gene which favours the haemolytic episodes in subjects deficient in G6PD. More recently Beutler (1970) has suggested a polymorphism of tyrosinase; this would influence the rate of production of dopaquinone from L-dopa and in turn the susceptibility to haemolysis.

In the present article some recent results obtained by our group (Bottini *et al*, 1970b and 1971) are briefly reviewed; these contribute to the elucidation of the problems discussed.

Materials and Methods

An extract of *Vicia faba* in an organic solvent (methanol-chloroform 1:1 by volume) was fractionated (1) by partition in the two phases of a mixture of chloroform-methanol-water (8:4:3) and (2) by paper ascending chromatography in a mixture of isopropanol (46.7%), ethanol (23.3%), formic acid (2.5%), and water (27.5%). The crude extract and its fractions were incubated in both aerobic and anaerobic conditions at 37° C either with a GSH solution, 0.005 M in phosphate buffer pH 7.4 (I=0.1) with 0.02M EDTA, or with a suspension of RBC from G6PD-deficient male subjects. GSH assay was carried out according to the method of Beutler, Duron, and Kelly (1963).

The erythrocyte acid phosphatase* phenotype of 173 G6PD-deficient male subjects with a past history of haemolytic favism was determined by starch gel electrophoresis (Hopkinson *et al*, 1963). Sixty-nine of these subjects were collected from the population of Rome and 104 from that of Oristano district of Sardinia. The mean ages at the first haemolytic episode were 4 and 17 years respectively. Control groups of individuals from the same populations were also examined or were available from the literature.

Results

The experiments carried out with our extract and its fractions have shown the existence in *Vicia faba*

* Acid phosphatase is an SH-dependent erythrocyte enzyme with an electrophoretic polymorphism determined, in Caucasian populations, by three common codominant alleles (P^A, P^B, P^C) at an autosomal locus (Hopkinson, Spencer, and Harris, 1963). Correspondingly there are six phenotypes: A, B, C, BA, CA and CB. In previous *in-vitro* investigations (Bottini and Modiano, 1964 and 1966; Bottini *et al*, 1967), we observed that under the action of oxidized glutathione or acetylphenylhydrazine, this enzyme undergoes some typical changes of its electrophoretic pattern, associated with a striking decrease of its enzymatic activity. Moreover the various isozymatic fractions were found to have a different resistance to inactivation induced by those substances. Such findings *in vitro* prompted us to search for an association between the phenotype of RBC acid phosphatase and oxidative haemolysis.

FIG. 1. The action of two fractions of a *Vicia faba* extract on the GSH of G6PD-deficient RBC.

The two phases obtained from a mixture of chloroform-methanol-water in which a certain amount of highly concentrated extract of *Vicia faba* had been dissolved, were divided into various fractions and dried at reduced pressure and low temperature.

The dry extract was dissolved with 0.5 ml of ACD solution and 0.6 ml of whole blood from a G6PD-deficient subject was added. After gentle mixing, this suspension was incubated at 37° C in air. At the same time another sample was prepared in the same manner and after shaking in CO current for nearly 5 min, was incubated in CO atmosphere. The controls consisted of mixtures of blood and ACD solution incubated in air or in CO. After three hours of incubation, GSH was assayed in the RBC suspension. Four experiments, indicated in the figure by different symbols, were carried out. Each experiment included incubation of RBC in CO (white symbols) and in air (black symbols) with separate extracts from the aqueous and from the chloroformic phases. In each experiment two other samples of the same blood with ACD were incubated with APH either in the presence of air or of CO. In all cases CO almost totally suppressed the APH effect on RBC GSH.

In the ordinate GSH disappeared during incubation is expressed as a percentage of the value in controls incubated for the same period of time without extract. Both fractions are active on the GSH of G6PD-deficient RBC: inhibition by CO is strong in the chloroformic phase activity but is much weaker in the aqueous phase activity.

The original data concerning these experiments are reported in Bottini *et al* (1970b).

of two substances (or classes of substances) which induce an *in-vitro* fall of GSH in aqueous solution and in G6PD-deficient intact red blood cells. The effect is very slight or absent on normal RBC (Bottini *et al*, 1970b). The results of a series of experiments carried out with fractions obtained by partition in the chloroform-methanol-water mixture are summarized in Fig. 1. One fraction acts upon the GSH of G6PD-deficient RBC better, or only, in aerobic conditions whereas the second shows the same activity in the presence and absence of oxygen.

Although less intense, activity on GSH similar to that of *Vicia faba* has also been observed in extracts obtained from *Pisum sativum* and some other vegetables.

In two groups of G6PD-deficient male subjects

TABLE I
FREQUENCY OF P^B ALLELE OF ERYTHROCYTE
ACID PHOSPHATASE IN G6PD-DEFICIENT MALE
SUBJECTS WITH A POSITIVE HISTORY OF
HAEMOLYTIC FAVISM

Subjects	P ^B Allele Proportion	Total Number of P Alleles	p
Normal Romans (Modiano <i>et al.</i> , 1967)	0.658	834	< 0.01
Roman males with favism	0.536	138	
Sardinian control group	0.727	528	< 0.01
Sardinian males with favism	0.606	208	
Sardinian G6PD- deficient males without favism	0.759	58	< 0.05

TABLE II
PROPORTIONS OF ERYTHROCYTE ACID
PHOSPHATASE PHENOTYPES IN G6PD-DEFICIENT
MALE SUBJECTS WITH A POSITIVE HISTORY OF
HAEMOLYTIC FAVISM. INCIDENCE OF HAEMOLYSIS
CALCULATED FOR ACID PHOSPHATASE
PHENOTYPES

Subjects	Acid Phosphatase Phenotype Proportions			Total Number of Subjects
	A, CA, and C	BA and CB	B	
<i>Romans</i>				
Normal (Modiano <i>et al.</i> , 1967)	0.122	0.439	0.439	417
Males with favism	0.203	0.522	0.275	69
Incidence of haemolysis*	0.499	0.357	0.188	
<i>Sardinians</i>				
Control group	0.080	0.386	0.534	264
Males with favism	0.192	0.404	0.404	104
Incidence of haemolysis*	0.720	0.314	0.227	

* Assuming a mean incidence of 0.30 for favism among G6PD-deficient males and assuming that the distribution of acid phosphatase phenotypes in the whole group of G6PD-deficient males (favic and non-favic) is equal to that observed in the general population (Bottini *et al.*, 1971), the incidence (I) of favism in a given acid phosphatase phenotype can be calculated according to the following formula:

$$I = \frac{P_f}{P_g} \times 0.30$$

where P_f and P_g are the proportions of the acid phosphatase phenotype in favic subjects and in the general population respectively.

The carriers of P^B in a single dose (BA and CB phenotypes) show an incidence of favism intermediate between carriers of P^B in double dose (B phenotype) and subjects carrying only P^A or P^C or both (A, C, and CA phenotypes).

with a past history of severe haemolytic favism a marked deficiency in P^B, corresponding to an increase in the proportions of both P^A and P^C alleles, was observed as compared to controls from the same population (Bottini *et al.*, 1971; see Table I).

Assuming a mean incidence for favism of 30% among G6PD-deficient males, one can calculate that the incidence of haemolytic crisis is ~60% for subjects of A and CA phenotype, ~35% for BA and

CB subjects, and ~20% for G6PD-deficient subjects of phenotype B (Table II).

Discussion

The findings reported above suggest that the factors which determine the extreme variability in the occurrence of severe episodes of haemolytic favism in G6PD-deficient subjects, may indeed be manifold.

The existence of several active substances in *Vicia faba* suggests a possible synergistic action in determining the haemolytic event. A variability in the concentration of the various active substances could possibly depend both upon the variety of *Vicia faba* and upon seasonal conditions. The interaction of the substances with the genotypic characteristics and the physiological status of G6PD-deficient individuals could explain the considerable variability in the response to *Vicia faba* observed both among different individuals and in the same individual on different occasions. It is also interesting that an action on GSH, although less intense, has been observed in extracts from other vegetables currently used in human alimentation. The observation brings to mind the definition of 'vegetable disease' given to favism more than a century ago (Minà la Grua, 1856). Haemolytic crises following the ingestion of vegetables other than *Vicia faba* are not exceptional in the experience of the author.

Two alleles (P^A and P^C) of a gene coding for an intra-erythrocytic enzyme with electrophoretic polymorphism appear to play a fairly remarkable role in bringing about severe haemolytic favism, whereas thalassaemia seems to have a protective role. It is known that several other erythrocyte enzymes show genetic polymorphism and it is conceivable that the variability of the metabolic activity of RBC may be connected with these polymorphisms. There is therefore the possibility of associations between haemolysis and the phenotypes of several RBC proteins.

This, of course, does not exclude the possibility that extra-erythrocytic factors may have an important role in conditioning susceptibility to haemolytic favism. In addition to enzymes concerned in the absorption and metabolism of the active substances of *Vicia faba*, plasma factors which influence the stability of GSH in RBC may have some importance. It is known that such factors do exist (Jocelyn, 1960; Bottini *et al.*, 1970a) but up to now little attention has been paid to their possible role in physiological and pathological conditions.

In 1971, Sartori reconsidered his theory on the genetics of favism. He investigated a general form

of 'favism' which apart from 'haemolytic favism' also includes a form 'without haemolysis'. The symptoms of 'favism without haemolysis' are those referred to as 'favism minor' in the old medical literature (mild headache, uneasiness, weakness, vomiting, and dizziness). According to Sartori's theory, the susceptibility to these symptoms (which follow the ingestion of *Vicia faba* beans or the inhalation of pollen) is determined by an autosomal recessive factor. The additional occurrence of G6PD deficiency (X-linked factor) would predispose to severe haemolytic crises ('haemolytic favism').

In our clinic we have only been able to study patients with haemolytic favism. The investigation of any possible association between 'favism minor' and the phenotype of erythrocyte acid phosphatase (and possibly of other proteins) would be of great interest. However, clear and workable definitions of the set of clinical and laboratory parameters which represent the necessary and sufficient conditions for the confirmation of the diagnosis of 'favism minor', are important preliminary requisites for any further investigation. Moreover, 'favism without haemolysis' is not observed in other Italian populations who eat large quantities of broad beans, but do not show G6PD deficiency. Therefore if one accepts Professor Sartori's hypothesis of a 'favism' independent from G6PD deficiency which is determined by a recessive autosomal factor, one must assume that this is a factor much more common in Sardinians than in other Italian populations.

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