

# On the Pathogenesis of Favism

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In some Mediterranean areas—mainly the Balearic Islands, Sardinia, Sicily, Greece, Cyprus, and Israel—where the cultivation and consumption of broad beans (*Vicia faba*) are extensive, favism is rather common especially in children. It occurs after the inhalation of some, as yet unidentified constituent(s) of the plant and/or more often after eating the beans, especially when they are fresh. The clinical and epidemiological behaviour of favism presents several apparent inconsistencies with what is known of its pathogenesis. In fact, while some patients present with only mild complaints, such as a mild headache, uneasiness, and weakness or also nausea, vomiting, and dizziness, others are severely ill, the prominent features being sudden severe anaemia, subicterus, and haemoglobinuria (or urobilinuria).

Both these clinical states and all the possible intermediate forms may be unique or may occur more than once without any rule in the same individual. Furthermore, it is well known, though often forgotten, that there are many subjects, who tolerate *Vicia faba* well for their whole lives even though they have G6PD deficiency which is always associated with haemolytic anaemia induced by *Vicia faba* or by a series of drugs. For instance a series of 50 subjects with severe favism was tested in north Sardinia. Among the male relatives of the propositi there were 61 fathers and brothers (aged over 10) who were all severely enzyme deficient. Of these only 15 were found to have had severe favism at one time though they all ate broad beans regularly, and even though 2 of the fathers without favism had previously had severe quinine haemoglobinuria.

Mild or 'minor' favism has been studied rarely and almost all the published reports deal with the severe form, also called 'haemoglobinuric' or 'haemolytic' favism. I believe that favism should not be identified *a priori* with drug-induced haemolysis nor should it be studied only in its severe form. Therefore, I would like to present the results of

observations, not previously published, made between 1955 and 1961 at the Paediatric Clinic of the University of Sassari (Sardinia) together with other cases from the literature, which will allow some of the puzzling peculiarities of favism to be clarified. However, the most important factors in the pathogenesis of favism will first be considered.

## *Vicia faba*

There is good evidence that the degree and rate of the ripening are more important for the pathogenesis of favism than are the particular variety or some disease of the plant. Marcolongo (1951) found that favism was more frequent and severe during a sunny, dry spring, and it can be shown (Fig. 1) that latitude

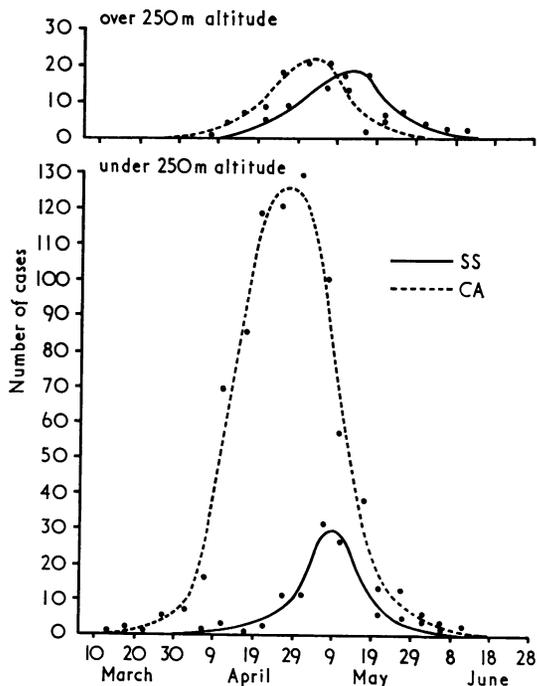


FIG. 1. Influence of latitude and altitude on the chronological incidence of severe favism. There is a difference in latitude of  $1^{\circ}21'$  between Sassari (SS) in the north and Cagliari (CA) in the south.

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and altitude influence the chronological occurrence of favism in proportion to the ripening of the beans. Furthermore, the oxidizing action of the juice of fresh broad beans on the reduced glutathione of sensitive erythrocytes—tested by the method of Mela and Perona (1959)—is greater when the beans are gathered after sunshine than after rain (Table I; E. Sartori and C. Mela, unpublished observations).

TABLE I  
GLUTATHIONE LOSS IN  
6 SAMPLES OF G6PD-  
DEFICIENT  
ERYTHROCYTES  
INCUBATED WITH FRESH  
JUICE OF *VICIA FABA*  
GATHERED FROM THE  
SAME FIELD AFTER  
SUNSHINE AND AFTER  
RAIN

Sample	Glutathione Loss (mg%/ml)	
	After Sunshine	After Rain
1	9	2
2	10	3
3	16	7
4	24	8
5	27	12
6	24	6
Mean	18.3	6.3
t = 17.799; p < 0.001		

But the differences in concentration of active substances in the plant which are connected with the ripening, and also with the withering as claimed by Marcolongo (1959), cannot account for all the inconsistencies mentioned above; nor can they explain the results of Biddau's classic experiment. Biddau (1930) divided several broad beans in half and gave one portion to a subject who then suffered severely, he himself ate the rest without experiencing any discomfort.

**The Hereditary Background**

There is now good evidence that the obvious hereditary background of favism, already postulated by Montano (1894), is really composite. In 1959, this author pointed out that: 'two different diatheses, the favic and the haemolytic, are encountered in Sardinia, frequently associated in the same individual' (Sartori, 1959).

**The Autosomal or Favic Predisposition.**

The uncertain hereditary behaviour of favism was studied in some detail and depth many years ago (Sartori, 1957a, 1957b, and 1958). Analysing a first group of 100 pedigrees it was stated that the

strong prevalence of males among patients admitted to hospital was a result of the greater severity of favism in boys, and that the genetic predisposition exhibited was consistent with a recessive autosomal condition. But these hypotheses were neglected for several years after Sansone and Segni (1956, 1957, and 1958) recognized the strict biochemical analogy of the erythrocytes in favism and in primaquine sensitivity (Tarlov *et al*, 1962) in respect of reduced glutathione content and stability and G6PD deficiency.

Then in a study of the familial predisposition to favism in Greece, Stamatoyannopoulos *et al* (1966) concluded that 'an autosomal gene in the heterozygous state enhances the susceptibility to favism of G6PD-deficient individuals'. But Stamatoyannopoulos and his co-workers dealt only with 'haemoglobinuric' favism and failed to distinguish it from the drug-induced haemoglobinuria, and *a priori* required G6PD deficiency for the diagnosis of favism. More recently, but with the same bias, Kattamis, Chaidas, and Chaidas (1969) found considerable differences in the susceptibility of individuals sensitive to *Vicia faba* in Rhodes.

In the present paper the autosomal nature of the favic predisposition, without which neither mild nor severe favism occurs, can be confirmed by the following results.

(1) In a random sample of the inhabitants of two villages in north Sardinia (Bessude and Seneghe), the frequency of favism, regardless of severity, was found to be the same in both sexes (Table II).

TABLE II  
SEX DISTRIBUTION OF FAVISM  
REGARDLESS OF SEVERITY AMONG  
RANDOMLY-SELECTED INHABITANTS  
OF TWO NORTH SARDINIAN  
VILLAGES

	Favism			
	Absent		Present	
	Obs.	Exp.*	Obs.	Exp.*
Males	369	366.6	23	25.4
Females	396	398.4	30	27.6
Total	765		53	
$\chi^2 = 0.466; p > 0.40$				

\* Numbers expected if favism is assumed not to be sex-linked.

(2) This was also true in another north Sardinian village (Lodè) where Siniscalco and his team (Bernini *et al*, 1960) had previously investigated G6PD deficiency. Dr G. P. Perona (unpublished

TABLE III  
SEX DISTRIBUTION OF MILD AND SEVERE FAVISM IN RANDOMLY-SELECTED INHABITANTS OF LODÈ

	Favism							
	Absent		Present		Mild		Severe	
	Obs.	Exp.*	Obs.	Exp.*	Obs.	Exp.*	Obs.	Exp.*
Males (n = 270)	235	237.5	35	32.4	27	30.1	8	4.9
Females (n = 204)	182	179.5	22	24.5	22	18.9	0	3.1
Total (n = 474)	417		57		49		8	
	$\chi^2 = 0.508; p > 0.40$				$\chi^2 = 0.889; p < 0.025$			

\* Numbers expected if favism is assumed not to be sex-linked.

observations) questioned many inhabitants and particularly analysed the complaints and symptoms attributable to the inhalation and ingestion of the components of either fresh or dry broad beans. Assuming that favism is not sex-linked these data show that the differences between the observed and expected values are not significant for the disease itself; however, the differences are significant in severe favism (Table III).

(3) In Lunamatrona, a small south Sardinian village, where clinical favism is very frequent, among 19 males who had had favism we found no less than 8 in whom repeated Motulsky tests were normal; therefore they were not G6PD deficient although they had favism.

Thus, favism itself is not sex-linked and may occur without haemolysis.

On the other hand Panizon and Vullo (1961) stated that the same G6PD-deficient  $^{51}\text{Cr}$ -labelled erythrocytes were rapidly destroyed after eating fresh beans only when given to subjects who had suffered from severe favism and not when given to subjects whose personal and familial history were negative for favism. It may be also the case that some G6PD-normal subjects who have had only mild favism will react like the former group and, conversely that some enzyme-deficient subjects who tolerate the beans well will react like the latter. Unfortunately, there has been no opportunity to test this hypothesis nor to utilize this procedure to detect an autosomal favic predisposition among G6PD normal subjects. This would obviously be easier to test should this result in some way in the isolation of some substance related to a 'dosable' metabolite of the active substance, eg, dopaquinone as suggested by Beutler (1970).

This substance, as yet unidentified, may be influenced by age because the age incidence of the disease is exactly the same in both sexes, while G6PD deficiency is 4 times more frequent in males (Fig. 2). Its absorption may decrease or its degra-

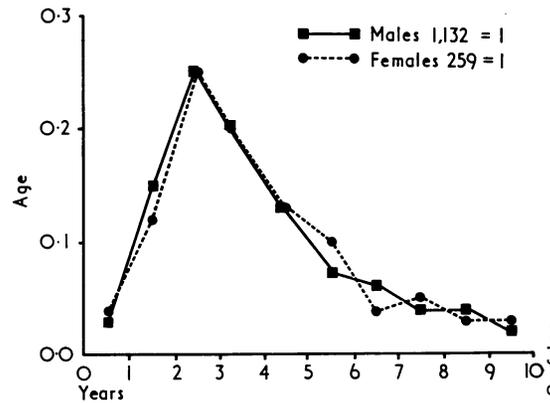


FIG. 2. Age dependence of severe favism among children admitted to hospitals in Sardinia and in Greece. There is no difference between the sexes.

ation accelerate from birth to puberty reaching a peak during the third year of life resulting from the opposite influence of the factors that generally inhibit poisoning in infancy. Finally, at times, it seems possible that some acquired condition may mimic this hereditary autosomal predisposition.

**The X-linked or Haemolytic Predisposition.** G6PD deficiencies are X-linked. The deficient enzyme involved in favism is a variant called 'Mediterranean', whereas that involved in the previously discovered primaquine sensitivity of the American negroes is a variant now called 'A-'. Erythrocyte enzyme activity is always about  $\frac{1}{10}$  of normal in the 'A-' variant and below  $\frac{1}{20}$  in the 'Mediterranean' type in hemi- and homozygotes. Thus the enzyme deficiency involved in favism is more severe.

While, as has just been seen, favism can also occur in the absence of any G6PD deficiency, severe favism is never encountered without a strong deficiency. In my experience, there was one partial exception; a boy with acute haemoglobinuria who

for some months afterwards had a decolorization time of less than 4 hours (Motulsky's method). The American negroes, whose enzyme deficiency is milder, are not known to suffer from favism. Some of them failed to experience favism when fed suitable amounts of fresh beans from a batch proven to have induced severe favism in Sardinia; the beans had been sent by air mail within 48 hours to Dr G. L. Brewer in Chicago (unpublished observations).

While G6PD<sup>Med</sup> deficiency—like the A<sup>-</sup> form—is much milder in almost all females, those who have suffered from severe favism have a very low enzyme activity; the result of the Motulsky test was delayed over 4 hours in all cases. Thus, there is a high correlation between the severity of G6PD deficiency and the severity of favism in both sexes. As severe G6PD deficiency is much more frequent in males it follows that many more males are hospitalized. The erroneous opinion which identifies G6PD deficiency with a predisposition to favism arises from this indirect connexion between the enzyme deficiency and the number of patients admitted to hospital. In reality, strong G6PD deficiency only causes the haemolytic course of the disease among individuals bearing the autosomal favic predisposition or its phenocopy. Thus severe favism is always less frequent than mild favism.

Marcolongo (1951) found 33 severe cases among 132 in the area around Ogliastra (south Sardinia) and in Lodè we found 8 out of 57. Severe favism is self limited (Balsamo, 1956) as is drug-induced haemolytic anaemia, whereas patients with mild favism may relapse every day. Thus G6PD<sup>Med</sup> deficiency really predisposes only to a drug sensitivity more severe than primaquine sensitivity correlated with G6PD<sup>A-</sup> deficiency.

To test this statement the frequency of the favic phenotype was calculated from the totals of Table III and that of the haemolytic phenotype was equalized with the phenotypic frequency of G6PD-deficient males found by Bernini *et al* (1960)

TABLE IV

CASES OF SEVERE FAVISM IN LODÈ, OBSERVED AND EXPECTED FROM THE PHENOTYPIC FREQUENCIES FOR FAVISM AND FOR G6PD DEFICIENCY

Phenotypic Frequency		Severe Favism			
Favism	G6PD Deficiency	Present		Absent	
		Obs.	Exp.	Obs.	Exp.
0.12	0.29	8	9.4	262	260.6
$\chi^2 = 0.216; p > 0.60$					

(Table IV). The product of these two frequencies, obtained independently in Lodè, permits the number of males in this village who are expected to have favism with the more severe haemolytic course to be calculated. The number of cases of severe favism corresponds closely to the expected numbers (Table IV). Conversely the frequency of G6PD deficiency is not as strictly connected with the frequency of favism. For instance, a difference ranging between 0.198 and 0.290 for G6PD deficiency corresponds to a difference ranging between as little as 0.107 and 0.290 for favism in Seneghe and Lodè respectively.

### 'Mithridatization'

There are some factors which may account for the irregular occurrence of favism. One, probably the least important, is the variable toxicity of the beans.

Another may be the variable exposure to broad beans, which are generally avoided after acute episodes but for different lengths of time and with varying stringency. This abstinence is the major source of bias when the segregation of clinical favism is studied in families where one or both parents have suffered from the disease; as a rule they are careful to keep the beans out of the reach of their children.

A third important factor may be that by coming into frequent contact with the growing and ripening plant, individuals become proof against its poison by being submitted to gradually increasing doses (mithridatization). Since self limitation and dose dependency are proved also for the haemolysis observed in G6PD<sup>Med</sup>-deficient subjects (Panizon and Zacchello, 1965), the lack of responsiveness, which is well known to occur for a few months after a severe haemolytic episode may occur more often after repeated mild episodes which escape detection but destroy the more sensitive older erythrocytes in small 'instalments' without any appreciable discomfort.

There is good evidence, both indirect (Perona, 1966) and direct (Panizon *et al*, 1970), for this gradual modification of the blood. This is obviously less likely to occur when the plant ripens faster as during a sunny, dry spring which—as stated above—can account for the outbreak of severe favism. It may be that the relative rarity of episodes of severe drug-induced haemolysis in Sardinian and Greece also results—at least in part—from this slow reduction of the mean age of the erythrocytes. It should be noted that several cases of haemolytic favism in its mild form in severely G6PD-deficient subjects have been observed.

### Sex Ratio

The apparently chaotic sex ratios observed among various groups of patients from not greatly different populations has certainly been one of the greatest puzzles of favism for many years. Very recently (Sartori, 1970), I suggested a possible solution.

Assuming that severe favism depends on the aggravating influence of strong G6PD<sup>Med</sup> deficiency on the autosomal favic predisposition, the simplest hypothesis which follows—that only deficient hemizygotes and homozygotes are prone to *Vicia faba*-induced haemolysis—does not fit. If this were true, the frequency of severe favism in the female should be equal to the square of the frequency found in the male. Actually, severe favism is always more frequent in the female than would be expected from the phenotypic frequency of the hemizygotes. Analysing the data reported in Table V, where the proportional values were calcu-

TABLE V

FREQUENCIES OF OBSERVED PHENOTYPES AND OF POSTULATED G6PD GENOTYPES IN THREE MEDITERRANEAN AREAS

	Cagliari	Sassari	Athens
Period	1951-60	1951-60	1955-66
Age of subjects (yr)	0-10	0-10	0-10
Morbidity rate for severe favism	120	47	6
Frequency of G6PD-deficient males	0.20	0.10	0.06
<i>Observed values</i>			
Male	546	183	403*
Female	156	36	67*
Sex ratio (m:f)	3.5	5.1	6.0
<i>Proportional values</i>			
Deficient homozygotes	109.2	18.3	24.2
Heterozygotes	873.6	329.4	757.6
Heterozygous patients†	46.8 (47.8)	17.7 (18.0)	42.8 (41.5)
<i>Estimated percentages</i>			
Heterozygotes prone to severe favism	5.36	5.37	5.65
$\chi^2 = 0.070$ ; $p > 0.95$			

\* From Kattamis *et al* (1969).

† Figures in parentheses are the expected values if there is no difference among the three localities.

lated from the observed ones according to the Hardy-Weinberg law, it can be seen that not only homozygous but also heterozygous females must suffer the disease. There is a highly suggestive lack of difference between the percentage of heterozygotes estimated to be at risk, despite the very different observed enzyme-deficiency frequencies, morbidity rates, and sex ratios (notwithstanding the total independence between the data obtained in Sardinia and those which Kattamis, Kyriazakon, and Chaidas obtained in Greece in 1969).

To test the reliability of this procedure, Table VI compares the observed values for some parameters obtained independently in north Sardinia with the respective values expected from the results for Sassari (see Table V). None of the differences between the observed and expected values is significant.

TABLE VI

SOME PHENOTYPES IN NORTH SARDINIA OBSERVED AND EXPECTED FROM THE POSTULATED GENOTYPES

	Observed	Expected	$\chi^2$	p
Females with severe favism				
Heterozygous (n = 17)	8	8.4	0.323	> 0.50
Less anaemic (n = 36)	18	17.7	0.183	> 0.60
Mothers of males with severe favism; not in turn affected (n = 254)	229	227.6	2.187	> 0.10

On the other hand, the hypothesis that among patients with severe favism the number of females may exceed the square of the number of males because more deficient homozygotes suffer the disease than do deficient hemizygotes, should be rejected because, on average, the severity of haemolytic anaemia is never greater in the female; on the contrary it is less. Thus, the conclusion to be drawn is that really  $\frac{1}{2}$  of the heterozygotes behave as the deficient hemizygotes and homozygotes in aggravating the *favic* predisposition. This is easily explained by the Lyon theory as was confirmed by Sartori, Panizon, and Zacchello (1968), and is also the case for enzyme deficiencies of the Mediterranean type. According to this hypothesis either the paternal or the maternal X is inactivated in the cell.

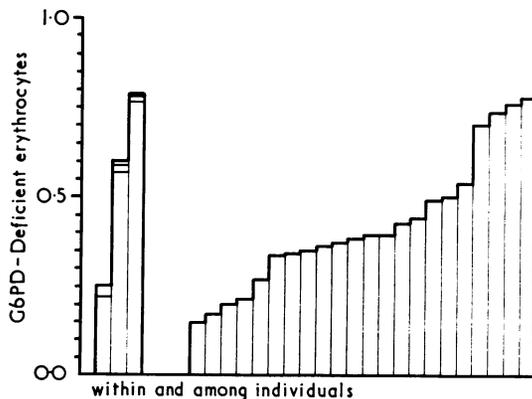


FIG. 3. Variations in the percentage of deficient erythrocytes within 3 and among 22 women proven to be heterozygous for G6PD<sup>Med</sup> by pedigree studies in male relatives only.

Therefore the blood of heterozygous females behaves as a mosaic of normal and enzyme-deficient erythrocytes. Only heterozygotes whose mosaic contains a sufficiently high proportion of deficient enzymes, estimated to be about  $\frac{2}{3}$ , will be predisposed to severe favism. Fig. 3 shows that among 22 heterozygotes the proportion of deficient enzymes is nearly as high as this in only one case.

### Summary

Evidence is provided that the pathogenesis of favism is complex. Apart from the inhalation or ingestion of *Vicia faba* (especially when fresh and rapidly ripened), a favic predisposition is always necessary. This predisposition—as yet not fully identified—behaves as an autosomal recessive trait and seems to be age-influenced. But a second haemolytic predisposition, identified with strong G6PD<sup>Med</sup> deficiency, is responsible for aggravating favism to the severe haemolytic syndrome, which prevails in boys and generally requires hospital admission and blood transfusion. This enzyme deficiency alone is not enough, and its aggravating action on the autosomal predisposition may be neutralized by a kind of spontaneous mithridatization. Severe favism may occur in mutant hemi- and homozygotes, and in 5 out of 100 heterozygotes who have at least  $\frac{2}{3}$  deficient erythrocytes in their blood mosaic.

### REFERENCES

- Balsamo, V. (1956). Contributo alla conoscenza del favismo. *Clinica Pediatrica*, **38**, 507–536.
- Bernini, L., Carcassi, U., Latte, B., Motulsky, A. G., Romei, L., and Siniscalco, M. (1960). Indagini genetiche sulla predisposizione al favismo. III. Distribuzione delle frequenze geniche per il locus Gd in Sardegna. Interazione con la malaria e la talassemia al livello popolazionistico. *Rendiconti delle Sedute della Accademia Nazionale dei Lincei*, **29**, 115–125.
- Beutler, E. (1970). Glucose-6-phosphate dehydrogenase deficiency. *British Journal of Haematology*, **18**, 117–121.
- Biddau, I. (1930). Considerazioni sulla patogenesi e sulla terapia del favismé. *Terapia*, **20**, 106–118.
- Kattamis, C. A., Chaidas, A., and Chaidas, S. (1969). G6PD deficiency and favism in the Island of Rhodes (Greece). *Journal of Medical Genetics*, **6**, 286–291.
- Kattamis, C. A., Kyriazakou, M., and Chaidas, S. (1969). Favism: clinical and biochemical data. *Journal of Medical Genetics*, **6**, 34–41.
- Marcolongo, F. (1951). Favism. Fattori geografici e razziali nella patogenesi della malattia con particolare riguardo al favismo in Sardegna. *Rassegna Medica Sarda*, **53**, 89–131.
- Mela, C. and Perona, G. P. (1959). Sulla resistenza *in vitro* del glutatione ridotto eritrocitario al succo di fave fresche. *Bollettino della Società Italiana di Biologia Sperimentale*, **35**, 146–148.
- Montano, G. (1894). Del favismo o intossicazione fabacca. Cited in Biddau (1930).
- Panizon, F. and Vullo, C. (1961). The mechanism of haemolysis in favism. Researches on the role of non-corpuscular factors. *Acta Haematologica*, **26**, 337–343.
- Panizon, F. and Zucchello, F. (1965). The mechanism of haemolysis in favism. Some analogy in the activity of primaquine and fava juice. *Acta Haematologica*, **33**, 129–138.
- Panizon, F., Zucchello, F., Sartori, E., and Addis, S. (1970). The ratio between normal and sensitive erythrocytes in heterozygous glucose-6-phosphate dehydrogenase deficient women. *Acta Haematologica*, **43**, 291–295.
- Perona, G. P. (1966). L'emolisi subclinica da fave nei soggetti con difetto di G6PD. *Acta Paediatrica Latina*, **19**, 539–544.
- Sansone, G. and Segni, G. (1956). Prime determinazioni del glutatione (GSH) ematico nel favismo. *Bollettino della Società Italiana di Biologia Sperimentale*, **32**, 456–458.
- Sansone, G. and Segni, G. (1957). L'instabilità del glutatione ematico (GSH) nel favismo. Utilizzazione di un test selettivo. Introduzione al problema genetico. *Bollettino della Società Italiana di Biologia Sperimentale*, **33**, 1057–1060.
- Sansone, G. and Segni, G. (1958). Nuovi aspetti dell'alterato biochimismo degli eritrociti di favici: assenza pressoché completa della glucoso-6-P deidrogenasi. *Bollettino della Società Italiana di Biologia Sperimentale*, **34**, 327–329.
- Sartori, E. (1957a). Elementi per la genetica del favismo. *Studi Sarsaresi*, **35**, 1–6.
- Sartori, E. (1957b). Elementi per una teoria genetica del favismo. *Acta Paediatrica Latina*, **10**, 505–517.
- Sartori, E. (1958). Zum Fabismus als Erbleiden. *Experimentia*, **14**, 32–33.
- Sartori, E. (1959). Teoria genetica della diatesi fabica e della diatesi idiosincrasico-iperemolitica. *Studi Sarsaresi*, **37**, 11–18.
- Sartori, E. (1970). L'incidenza del favismo emolitico nel sesso femminile. *Acta Paediatrica Latina*, **23**, 1–15.
- Sartori, E., Panizon, F., and Zucchello, F. (1966). Biomodal distribution of erythrocytes in heterozygotes for strong Mediterranean glucose-6-phosphate dehydrogenase deficiency. *Journal of Medical Genetics*, **3**, 42–46.
- Stamatoyannopoulos, G., Fraser, G. R., Motulsky, A. G., Fessas, P., Akrivakis, A., and Papayannopoulou, T. (1966). On the familial predisposition to favism. *American Journal of Human Genetics*, **18**, 253–263.
- Tarlov, A. R., Brewer, G. J., Carson, P. E., and Alving, A. S. (1962). Primaquine sensitivity. Glucose-6-phosphate dehydrogenase deficiency: an inborn error of metabolism of medical and biological significance. *Archives of Internal Medicine*, **109**, 209–234.