ACTION OF *VICIA FABA* ON ERYTHROCYTES: POSSIBLE RELATIONSHIP TO FAVISM

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THE disease favism is a hæmolytic anæmia which results from the ingestion of the broad bean (*Vicia faba*) by some people. The disease has been recognized in most countries around the Mediterranean Sea for many years, and its clinical manifestations have been well documented[1]. Work both in Italy[2] and Israel[3] has shown that the disease only occurs in persons who have an inherited abnormality of the erythrocytes in that there is a deficiency of the enzyme glucose-6-phosphate dehydrogenase. This basic deficiency is reflected in a lowered level of the amount of reduced glutathione in the red blood cells and an instability of the glutathione in the presence of certain substances such as acetylphenylhydrazine, aniline, hydroxylamine, α- and β-naphthol and the anti-malarial drug primaquine[4]. Such agents cause both *in vitro* and *in vivo* breakdown of erythrocytic glutathione, whereas agents such as ascorbic acid and cysteine cause *in vitro* destruction only[5,6].

We recently reported[7] that extracts prepared from fresh beans of *Vicia faba* show a selective effect upon the glutathione of erythrocytes from sensitive subjects, that is, those possessing the genetic abnormality. This work has now been extended to show that the active agent of *Vicia faba* is present also in extracts of pollen and pistils, that the effect on glutathione can be demonstrated simply by incubating whole blood of sensitive subjects with whole beans and that the effect is not due to common reducing agents such as ascorbic acid and cysteine.

Table 1 shows the effect of incubating 1-ml. blood samples from both sensitive and non-sensitive subjects with 0·1 or 0·2 ml. amounts of extracts of pollen and pistils. Blood glutathione-levels were measured before and after incubation[6]. The pollen extracts were prepared by suspending 1 vol. of loose pollen in 10 vol. of saline at 4° C. for 1 hr. followed by centrifugation at 500 r.p.m. for 2 min. Pistil extracts were prepared in a similar manner using 5 vol. of saline. Fresh young beans (approx. 8 mm. in maximum diameter) were used in the whole-bean experiments. In all three cases, the incubations showed a significant *in vitro* effect on blood from sensitive subjects compared with non-sensitive subjects.

This effect cannot be due to cysteine because this would give a colour with the nitroprusside reaction used for estimating glutathione. Control experiments showed that the addition of extracts to blood followed by immediate precipitation of the proteins and glutathione estimation (with no incubation period) has virtually no effect upon the apparent blood glutathione-level. Hence the extracts do not contain significant amounts of cysteine. Further, the effect of the extracts is not due to the action of ascorbic acid. To demonstrate this, an extract of fresh young beans was prepared using ½ vol. of saline by the method previously described[7]. The action of 0·2 ml. of this extract (which contains not more than 3 μgm. of ascorbic acid in the 0·2 ml., an amount far below the concentrations reported to have an *in vitro* effect on erythrocytic glutathione[5]) was compared with the effect of incubating 1 ml. of blood with 2 mgm. of ascorbic acid in 0·2 ml. of saline. With an incubation time of 10 min. only the *Vicia faba* extract had a marked effect upon erythrocytic glutathione (Table 2).

Table 1. Effect of Extracts of Pollen and Pistils and of Whole Beans on Erythrocytic Glutathione

Source of activity	Vol. of extract used (ml.)	Subjects	No. of samples	Glutathione (mgm. per 100 ml. red blood cells) before incubation	Glutathione (mgm. per 100 ml. red blood cells) after incubation	*P* value on basis of '*t*' test
				Mean S.D.	Mean S.D.	
Pollen extract	0·2	Sensitive	10	33·2±5·0	21·3±3·2	0·01
		Non-sensitive	8	55·3±8·3	48·0±6·3	
Pistil extract	0·1	Sensitive	10	36·1±4·8	26·8±4·2	0·001
		Non-sensitive	10	56·5±6·0	52·6±7·0	
Whole bean	—	Sensitive	10	36·2±5·3	28·4±5·0	
		Non-sensitive	10	61·8±8·8	61·9±9·1	

These experiments show, therefore, that *Vicia faba* pollen and pistils, in addition to the bean, itself contain an active principle which has a marked effect on the erythrocytic glutathione of sensitive subjects. The action is not due to ascorbic acid or cysteine so that a specific effect of *Vicia faba* is still indicated. This direct action on the red blood cells *in vitro* suggests a way in which ingestion of the bean or inhalation of the pollen might result in *in vivo* hæmolysis.

It remains to demonstrate that the effects shown in this article are specific for *Vicia faba* and are not given by other vegetables at least to the same extent. Whole fresh peas, incubated as in the whole-bean experiments, showed no effect. Certain other vegetables tried, for example, runner beans, caused massive agglutination of the erythrocytes. When this occurs, false low glutathione-levels are obtained, probably due to lack of complete hæmolysis which is necessary for accurate estimation of the whole

Table 2. Effects of *Vicia faba* Extract and of Ascorbic Acid on Glutathione of Three Different Samples of 'Sensitive' Erythrocytes

Before incubation	After incubation with ascorbic acid	After incubation with *Vicia faba* extract
42*	43	29
34	35	24
44	44	28

* All values are in mgm. glutathione per 100 ml. red blood cells. The incubation time was 10 min.; for details see text.

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blood glutathione-level. With *Vicia faba* extracts, we have noted non-specific falls in glutathione if added to non-sensitive blood containing insufficient ACD. In these instances coarse clumps of blood were observed. This effect is abolished with the addition of further anticoagulant. Only those results obtained in the absence of agglutination are therefore valid for the purposes of comparison. In our experience, *Vicia faba* extracts are the only ones so far tested which give a statistically verified selective *in vitro* action on erythrocytic glutathione of sensitive subjects.

Though these results in no way prove that favism is caused by a direct action of an agent in *Vicia faba* on erythrocytes by initiating hæmolysis, they strongly suggest that such a possibility should be tested *in vivo*. Such a direct action appears to offer an attractive alternative hypothesis as to how sensitive subjects may develop a hæmolytic anæmia following ingestion of the broad bean, although the evidence for allergy in addition to the genetic erythrocytic defect cannot be excluded[8].

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A GENE DETERMINING PRESENCE OR ABSENCE OF ε-N-METHYL-LYSINE IN *SALMONELLA* FLAGELLAR PROTEIN

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ACCORDING to current views on the gene control of protein structure[1], the amino-acid which is to constitute a particular link in the polypeptide chain of a given protein is specified by a group of bases in a corresponding region of the deoxyribonucleic acid (DNA) molecule of a gene which determines the structure of this protein. Only twenty amino-acids, all in the L configuration if optically active, are common constituents of proteins, and it has been suggested that only twenty different base-groups occur in DNA which specify amino-acids. This hypothesis predicates: (*a*) that the presence of an uncommon amino-acid in a protein always results from some cause other than its specification by a group of bases in the DNA of the gene determining the amino-acid sequence of the protein (for example, from conversion of a common amino-acid into an uncommon one after its incorporation in a polypeptide chain); and (*b*) that heritable variation in respect of the presence or absence of an uncommon amino-acid in a particular protein must result from the action of a genetic determinant other than the gene determining the amino-acid sequence of this protein. An experimental test of predicate (*b*) has now been made in respect of the uncommon amino-acid ε-N-methyl-lysine, which Ambler and Rees[2] reported as a constituent of the flagellar protein ('flagellin') of some bacteria of the genus *Salmonella*; in the material they examined lysine and N-methyl-lysine were about equally abundant. We have now found that N-methyl-lysine is absent from some *Salmonella* flagellins; and by genetic analysis, using phage-mediated transduction[3], we have located a gene determining the presence or absence of N-methyl-lysine in flagellar protein.

Flagellins from many *Salmonella* strains of various species (serotypes) were tested for N-methyl-lysine by paper electrophoresis of acid-hydrolysed flagella with results to be reported in detail. In the case of diphasic strains (that is, strains able to produce flagella of either of two distinct antigenic types) flagella were collected from cultures in phase 1 and from cultures in phase 2. In each of the ten diphasic strains tested, the two flagellins obtained were alike in respect of presence or absence of N-methyl-lysine. Seven strains of *S. typhimurium* (phase 1 antigen *i*; phase 2 antigen 1,2,3) were all positive for N-methyl-lysine; four strains of *S. paratyphi B* (antigens *b* and 1,2) were all negative for N-methyl-lysine. But of thirteen strains of *S. derby* (phase 1 antigen *f,g*; no second phase), four were negative for N-methyl-lysine, the rest being positive.

We next tested flagellins from a series of motile derivatives of *SW* 543, a non-flagellated monophasic strain of *S. paratyphi B* with the (latent) phase 1 flagellar antigen *b*. The flagellar protein of a flagellated mutant of this strain lacked N-methyl-lysine, like the flagellins of other strains of *S. paratyphi B* tested. Strain *SW* 543 lacks flagella because of mutation at a *fla* locus close to the locus which regulates the phase 1 flagellar antigen, and motile clones obtained from it by transduction of the *fla*$^+$ gene from a motile donor strain, are of two sorts: one sort show flagellar antigen *b*, and are attributed to replacement of only the *fla*$^-$ gene of strain *SW* 543 by its wild-type allele from the donor strain; the other sort have the phase 1 flagellar antigen of the donor strain, and are attributed to replacement of both the *fla*$^-$ gene and the linked gene determining the phase 1 flagellar antigen *b* of strain *SW* 543 by their alleles from the donor strain[4]. Of nine motile transductant clones of the latter type, with phase 1 flagellar antigens derived from various donor strains, eight were positive for N-methyl-lysine; the donor