Iranian J. Publ. Hlth.
Atumn, 1979, Vol. 8, No. 3

EFFECT OF FAVA BEAN EXTRACTS ON THE ERYTHROCYTE POTASSIUM TO HEMOGLOBIN RATIO IN NORMAL SUBJECTS AND THOSE WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT ERYTHROCYTES*

M. Rahmanian,
M. Sadre, G. Donoso**
H. Ghassemi and
O. Mickelsen

ABSTRACT

1. A procedure for the *in vitro* evaluation of the toxicity of fava beans has been applied to 17 varieties of the bean and one batch of beans purchased on the open market; the latter consisted of unknown varieties. The procedure involves the incubation of a saline extract of the beans with red cells from normal individuals and with red cells from G6PD deficient patients.

2. Normal red cells under those circumstances absorb potassium from the fava bean extract whereas the cells from G6PD deficient patients lose potassium.

---

* Institute of Nutrition Sciences & Food Technology,
P.O. Box 3234, Tehran, Iran

**Present address: WHO Regional Office South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi - 110002, India.

3. This work suggests a relation between the toxicity of the fava beans and the loss of potassium by the red cells. On the basis of this assay, it appears that two of the 17 varieties may be non-toxic.

From the nutritional, agricultural and economic aspects, fava beans provide an excellent source of food in many parts of the world. fava beans are among the lowest priced pulses and are produced in large quantities in many of the countries along the Eastern Mediterranean and throughout much of southern Asia. From the nutritional standpoint, they offer many advantages in that they are rich in protein and low in fat. However, their use as a food or as a supplement incorporated into foods is limited by an acute, hemolytic disease that develops in many individuals who eat anything containing fava beans. The susceptibility to fava beans appears to be a genetic trait which is common to many Asiatics and the people who reside in. the countries around the Mediterranean.

The disease associated with the ingestion of fava beans is quite common in Iran where it is called Baghalzaleh-favism (Donoso , Hedayat and Khayatian, 1969). The basic biochemical disturbance associated with the disease is not clearly understood. It has been linked with a deficiency of the enzyme, glucose-6-phosphate dehydrogenase (G6PD) (ECl.1.1.49) in the red cells of susceptible individuals. However, that alteration in erythrocytes is not specific for favism since other conditions unrelated to favism are also associated with a deficiency of that enzyme in the red cells (Micheksen, Yang and Goodhart, 1973).

The active substance(s) in the bean which induces the acute hemolytic episode in susceptible individuals has not been completely identified. Recently, Engel and Wijnands (1968) proposed an empirical method for identifying the varieties of fava beans which are likely to bring on the acute hemolytic disease in susceptible individuals. This method is based on the earlier observation that when red cells from primaquine sensitive individuals are incubated with this anti-malarial compound the potassium to hemoglobin ratio is reduced before any hemolysis becomes apparent (Weed, Eber and Rothstein, 1961; Weed, 1961).

This report describes the changes in potassium to hemoglobin concentrations in red cells incubated with saline extracts of 17 varieties of fava beans from Iran. The red cells were secured from a normal individual and from pediatric patients whose blood cells showed no detectable levels of the enzyme G6PD.

METHODS AND MATERIALS

Most of the samples of fava beans (Vicia faba) were secured through Dr. Walter G. Kaiser. These beans came from the Regional Pulse Improvement Project in Iran. They had been picked when they were at the proper stage for human consumption and then were air dried. A few samples were purchased in local markets; these fresh beans were kept frozen until used in the tests.

To prepare the fava bean extracts, 2 g of the dried bean and 19 ml of 0.85% NaCl solution were shaken for one-half hour, or 7 g of fresh beans and 18 ml of the saline solution were ground in a mortar with a pestle until a uniform suspension was formed. The suspension from both extracts were transferred to large test tubes which were then put into a boiling water bath for 5 minutes to destroy any hemolytic agent(s) that might be present in the bean. After cooling, the suspensions were centrifuged for 10 minutes at 3500 rpm. The supernate was used in the subsequent work.

Blood for the control assays was secured from a normal man who volunteered 10 ml of venous blood whenever it was needed. That, as well as the blood from the patients, was secured from the antecubital vein. In all cases, heparin was the anticoagulant. The red blood cells deficient in G6PD were secured from pediatric patients at two of the local hospitals.* In all cases, the red cells were used in the assay the same day the blood was collected.

To prepare the red cells for the assay, 2 ml of blood was added to 10 ml of 0.85% NaCl solution in a 15 ml graduated centrifuge tube. The latter was gently inverted five or six times and then centrifuged for 5 min at 2000 rpm. The supernatant solution was discarded. The red cells were treated in the same manner with another 10 ml of the NaCl solution, and again the supernate was discarded. The red cells were diluted to 4 ml with the saline solution and used as such in the assays.

For the assays, 2 ml of the washed red cells suspended in saline were added to 1 ml of the boiled supernate from the broad or fava beans. Each batch of fava bean extract was incubated with the red cells from both a normal subject and from a G6PD deficient patient. As a check on the spontaneous hemolysis of the red cells, each time an assay was run, the red cells from both the normal subject and the G6PD deficient patients were incubated with saline. All tubes involved in the assay were shaken for four hours in a water bath maintained at 37^{o}. As soon as the incubation was completed, 5 ml of saline was added to each tube. The suspensions were then centrifuged for 5 minutes at 2000 rpm. After discarding the super-

nate, the red cells were washed with 10 ml of saline and again centrifuged. After the supernate was again discarded, 5 ml of distilled water was added to the red cells with stirring. This mixture was frozen overnight to ensure complete hemolysis of the red cells.

The following day, 1.5 ml of hemolysate was added to 5 ml of 15% trichloroascetic acid to precipitate the protein. After filtration, the clear filtrate was assayed for potassium by means of a flame photometer. The concentration of potassium in ppm as given by the flame photometer was multiplied by 4.33 which gave the mg of potassium per liter of hemolysate. The hemoglobin in the hemolyzed suspension was determined by means of the cyanmethemoglobin procedure using a standard curve prepared from Hycel solution.* The hemoglobin concentration of potassium in mg per liter of hemolysate was divided by the hemoglobin concentration in grams per liter; this ratio for the red cells incubated with the boiled fava bean extract was divided by the comparable ratio secured when the same kind of red cells were incubated with saline. The quotient thus obtained was multiplied by 100.

RESULTS AND DISCUSSION

The red cells from the normal individual, in all but one instance, absorbed potassium when they were incubated with the boiled fava bean extract (Table 1). In contrast to that, the G6PD deficient cells, when incubated with the boiled fava bean extract, lost potassium. (In all cases, these results were compensated for the reaction of the red cells with the saline solution since the value secured with the boiled fava bean extract was divided by the value secured when red cells from the same person were incubated with only the saline solution).

These findings suggest that, by and large, when red cells were incubated with the boiled fava bean extract, there was a marked difference in the behavior of the cells from normal individuals and those from patients whose red cells were deficient in the enzyme G6PD. In the latter case, there was no detectable G6PD enzyme activity in the cells. If the red cells did show any enzyme activity, they responded to the boiled fava bean extract in the same way as the cells from the normal individual, i.e. by absorbing rather than losing potassium to the fava bean extract. For a number of fava bean extracts, the same extract was incubated with red cells from two different patients whose red cells enzyme activity was undetectable. In those cases, the results for the potassium lost from the red cells differed by no more than 10 percentage points. Where this was done,

the values in Table 1 are the means of the two results.

For none of the fava beans was there any relation between the magnitude of the potassium lost from the cells deficient in the enzyme and the uptake of this cation by the normal cells (Table 1). However, there was a direct relation between the uptake of potassium by the normal cells and the concentration of this cation in the boiled fava bean extract (Fig. 1). For the latter, the correlation coefficient was 0.73 ($P < 0.01$). For the red cells deficient in the enzyme, there was no relation between the concentration of potassium in the fava bean extract and the loss of this cation from the red cells. These findings suggest that there are two separate mechanisms involved in the potassium exchanges in normal red cells and those which show no G6PD activity. There must be one mechanism involved in the uptake of potassium by the red cells from a normal individual. This may involve primarily a diffusion process since the uptake of potassium by the normal red cells was highly correlated with the concentration of this cation in the fava bean extract which the cells were incubated. If the process consists primarily of diffusion, then the potassium present in the cells must not be in an ionic form, since if it were, they should diffuse out into the media. That is based on the report (Altman, 1961) that red cells contain 437 mg of potassium per 100 ml. Since red cells contain 63% water, the potassium concentration would be 694 mg per 100 ml of cell water. That is considerably higher than the approximately 200 mg of potassium per 100 ml of fava bean extract (Fig. 1).

There was considerable variation in the extent to which the G6PD deficient cells lost potassium when incubated with the extracts from different varieties of fava bean. The biological importance of the loss of potassium from the red cells of individuals deficient in the G6PD enzyme cannot be evaluated from these data. However, there may be some relation since the only two varieties of fava bean for which there is an indication of human tolerance are Bam (No. 116) and Mazandaran (No. 205). Both of these are supposed to be relatively innocuous even to those individuals who are reported to react to the ingestion of fava beans with symptoms of distress. Both of these varieties of fava beans had no effect on the loss of potassium when their extracts were incubated with G6PD deficient red cells and only slightly increased potassium uptake by normal red cells (Table 1).

There is a possibility that the release of potassium by red cells deficient in G6PD, suspended in a fava bean extract, may not be due to the same factor that causes favism. This suggestion is based on reports of a decade ago. According to Businco, Spenati, Filipi,

Capotorti and Bottini (1967), fava beans from which the lipids were removed by extraction with an organic solvent markedly lowered the reduced glutathione content of G6PD deficient red cells during incubation. There was no such reduction when normal red cells were incubated with the solvent extracted fava beans. That the factor in fava beans studied by Businco and coworkers (1967) may not be the same as the assayed in the present study is suggested by the observation of Walker and Bowman (1960). The latter reported that the factor in fava beans responsible for the reduction in glutathione activity was destroyed by boiling. The substance studied in the present work was stable to five minutes of boiling and thus more nearly approaches the fava bean toxic factor which is stable to cooking (Donoso, Hedayat and Khayatian, 1969).

Additional work is needed to determine whether the loss of potassium by G6PD deficient red cells is a reliable indicator of the toxicity of different varieties of fava beans for sensitive human subjects. The present work suggests that such an exercise might prove profitable.

FOOTNOTES

1. 16, p. 3

* These blood samples were secured from pediatric patients at the Shohada and Khomeini Hospitals in Tehran. All children from whom blood samples were secured had no detectable G6PD enzyme in their red cells. They had been admitted to the hospital with severe icterus associated with favism.

1. 2, p. 5

* The standardized hemoglobin solution was purchased from Hycel Inc., P.O. Box 36329, Houston, Texas 77036.

Table 1. Influence of fava bean extract on the uptake or loss of potassium from red blood cells of normal individuals and glucose-6-phosphate dehydrogenase deficient patients.

Source	Sample No.	K loss or gain* from Normals†	K loss or gain* from Red cells of deficient†
Varamin	112	102	66
Mazandaran	213	112	78
Mazandaran	203	123	83
Shahi	33	125	89
Dezful	152	131	89
Shahi	110	100	90
Shoushtar	209	124	91
Varamin	119	112	91
Mazandaran	212	118	93
Mazandaran	100	133	94
Mazandaran	215	103	95
Shoushtar	211	103	97
Dr. Balard (#121)	108	95	98
Bam	116	105	102
Mazandaran	205	113	102
Zarazi	151	99	--
Shoushtar	111	--	87
Tehran (fresh		--	92

* See text for derivation of these values. Numbers above 100 indicate that the red cells absorbed potassium when they were incuabated with boiled fava bean extract; values below 100 indicate that the red cells lost potassium under the same conditions.

† Normal and deficient indicate that the red cells were from the blood of a normal individual or from a patient whose cells contained no detectable amount of glucose-6-phosphate dehydrogenase.

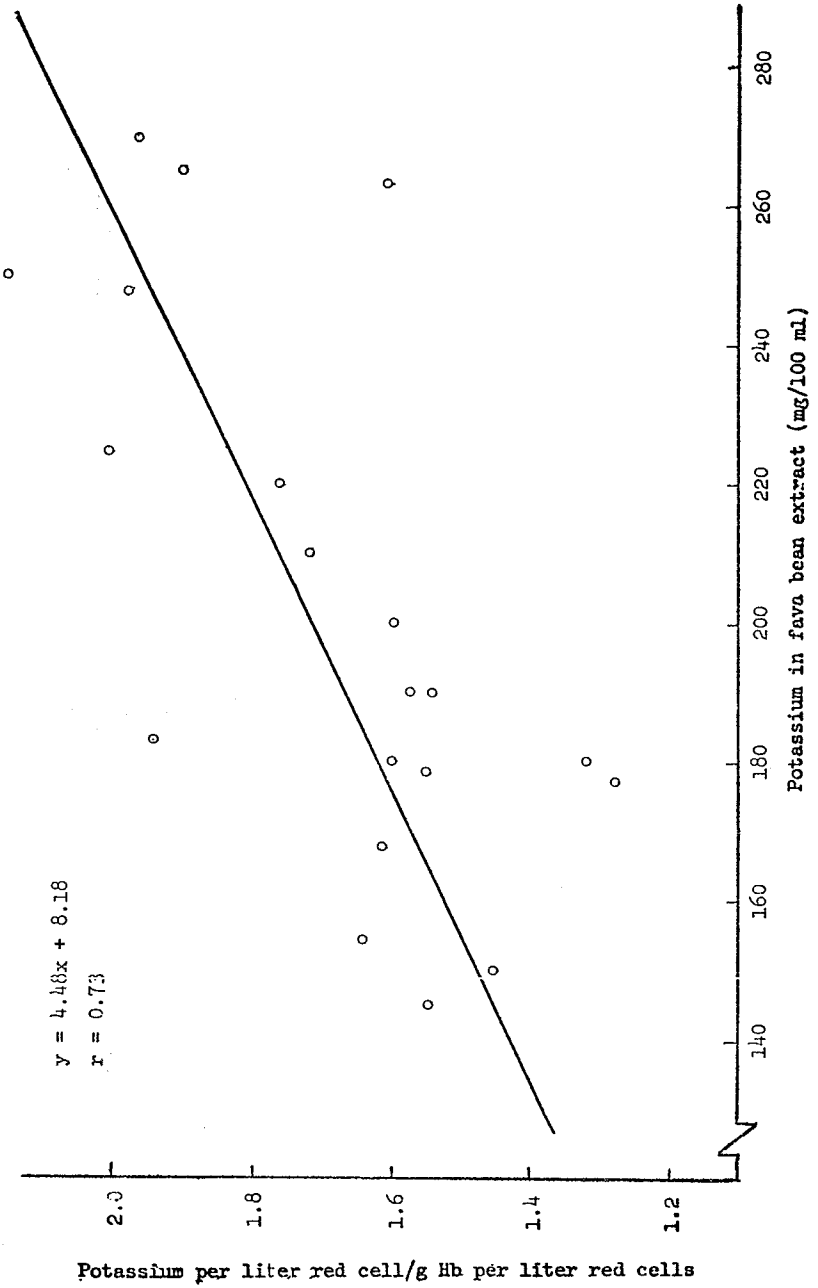

Fig.1. Relation between concentration of potassium in fava bean extract and uptake of this cation by red cells from a normal individual

REFERENCES

Altman, P.L. (1961). *Blood and Other Body Fluids*, p. 22. Washington, DC: Fedn Am. Socs. exp. Biol.

Businco, L. Spenati, G.F., Filipi, G., Capotorti, L. & Bottini, E. (1967). *Minerva pediat.* 19, 608. Quoted by Donoso, Hedayat & Khayatian (1969).

Donoso, G., Hedayat, H. & Khayatian, H. (1969). *WHO Bull.* 40, 513.

Engel, A.B. & Wijnands, W.C. (1968). *FAO Rep.* no. R2765.

Mickelsen, O., Yang, M.G. & Goodhart, R.S. (1973). In *Modern Nutrition in Health and Disease*, p. 421 (R.S. Goodhart and M. E. Shils, editors). Philadelphia: Lea and Febiger.

Walker, D.G. & Bowman, J.E. (1960). *Proc. Soc. exp. Biol. Med.* 103, 476.

Weed, R.I. (1961). *J. Clin. Invest.* 40,140.

Weed, R. I., Eber, J. & Rothstein, A. (1961). *J. Clin Invest.* 40, 130.