counts would have been expected if Premarin mediated an acute release of new platelets from bone marrow.

Summary. Administration of mixed natural estrogens (Premarin) intravenously to 5 patients with normal platelet counts and to 9 patients with thrombocytopenia did not produce a significant change in thrombocyte counts of peripheral blood, as compared to saline injections in the same patients. Premarin appears to be of no value in controlling bleeding due to thrombocytopenia.

1. Hirst, J. C., *Am. J. Obst. & Gyn.*, 1933, v26, 127.

2. Menger, H. C., *J.A.M.A.*, 1955, v159, 546.

3. Greenblatt, R. B., Barfield, W. E., *J. Clin. Endocrin.*, 1951, v11, 821.

4. Whittington, P. B., Jr., *J. Am. Dent. Assn.*, 1956, v53, 595.

5. Jacobson, P., *West J. Surg. Obst. & Gyn.*, 1955, v63, 711.

6. Johnson, J. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 92.

7. Olwin, J. H., Allen, E., *Am. J. Obst. & Gyn.*, 1950, v60, 1363.

8. Jacobson, P., *Arch. Otolaryngol.*, 1954, v59, 523.

9. Pepper, H., Lindsay, S., *Science*, 1956, v124, 180.

10. Pepper, H., Liebowitz, D., Lindsay, S., *AMA Arch. Path.*, 1956, v61, 1.

11. Brecker, G., Schneiderman, M., Cronkite, E. P., *Am. J. Clin. Path.*, 1953, v23, 15.

12. Matis, P., Gross, R., *Schweiz. Med. Wochensch.*, 1951, v81, 1043.

13. Ivanitzky, V., Klimova, M. S., *Arch. Int. de Pharm. et de Ther.*, 1937, v55, 365.

14. Cronkite, E. P., Jackson, D. P., Editor, L. M. Tocantins, *Progress in Hematology*, Vol. 2, p. 243, Grune and Stratton, N. Y., 1959.

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In vitro Effect of *Vicia faba* Extracts upon Reduced Glutathione of Erythrocytes. (25564)

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Glutathione (GSH) stability test of erythrocytes and other technics indicated that the basic abnormality in persons sensitive to primaquine, other drugs, and to fava bean (*Vicia faba*) is due to the same genetically determined erythrocytic defect(1). Destruction of GSH both *in vitro* and *in vivo* is caused by primaquine, phenylhydrazine, aniline, hydroxylamine, nitrofurantoin, α and β naphthol, α and β naphthoquinone, and certain Vit. K derivatives(1). Although extracts of *Vicia faba* have been reported as not destroying red cell GSH *in vitro*(2), in the present study certain effects of *Vicia faba* extracts on GSH of erythrocytes have been observed.

Materials and methods. Preparation of *Vicia faba* extract (VFE) was based upon the technic of Dunsford and Bowley(3) for plant hemagglutinins. Fresh beans were soaked in excess of water at 18 to 20°C for 12 to 18 hours. Remaining water was then drained off. The beans were macerated in Waring blendor for about 1 minute. Two volumes of 0.9% sodium chloride solution were added to 1 volume of macerated material. After mixing and standing 1 hour, the mixture was filtered through several layers of fine mesh gauze. The filtrate, which still contained a small amount of sediment, was collected into convenient lots and stored at –20°C until required. Full activity remained for at least 3 months. Three volumes of blood were added to 1 volume of standard ACD solution. The hematocrit of these suspensions was measured. For preliminary experiments, blood from persons of known response to GSH stability test was used. Survey bloods were taken from random selection of members of the Mamassani tribe of Southwestern Iran. Reduced glutathione (GSH) measurements and GSH stability test with acetylphenylhydrazine (APH) were performed as described by Beutler(4). For testing effect of VFE, 0.5 ml of the extract was added to 1

TABLE I. Effect of APH and VFE on 113 Blood Samples from Members of the Mamassani Tribe.

	No. of subjects	Treatment	Mean GSH*
Nonsensitive males	55	None	61.2 ± 10.7
		APH	60.2 ± 12.1
		VFE	51.5 ± 12.5
Sensitive males	14	None	42.0 ± 7.4
		APH	6.9 ± 3.8
		VFE	14.1 ± 2.7
Nonsensitive females	41	None	72.8 ± 16.8
		APH	66.0 ± 16.7
		VFE	56.9 ± 12.5
Sensitive females	3	None	63.2 ± 12.3
		APH	16.8 ± 4.9
		VFE	26.6 ± 6.3

* GSH in mg/100 ml red blood cells ± stand. dev.

ml of oxygenated blood and incubated at 37°C in water bath for desired time with occasional shaking. Since volume of extract used was not in excess of 1 ml, no attempt was made to change volumes of reagents required for hemolysis and protein precipitation from those normally used in GSH stability test. However, calculations were corrected for increase in volume after protein precipitation.

Results. Preliminary experiments indicated that when blood from sensitive persons was incubated with VFE there was a fall of GSH in red blood cells. A lesser fall in GSH was observed with blood from nonsensitive individuals. Virtually all GSH decrease occurred within 10 minutes. No hemolysis was noted. Dilution of VFE diminished its effect on GSH so that, with 1:10 dilution, distinction between sensitive and nonsensitive bloods became negligible. *In vitro* effect was further shown by performing parallel incubations with APH for 2 hours and VFE for 30 minutes on 113 blood samples (Table I). VFE had a lesser effect on GSH of erythrocytes of sensitive persons than APH and a greater effect than APH on nonsensitive erythrocytes. Using less than 30 mg GSH/100 ml rbc as a criterion of sensitivity(5), the 2 agents gave anomalous results in 3 cases. Two males, nonsensitive with APH (post-incubation levels of 42.1 and 42.7 mg GSH/100 ml rbc), gave values after incubation with VFE of 28.3 and 28.8 mg GSH/100 ml rbc respectively. One female, sensitive with APH (post-incubation level of 22.4 mg GSH/100 ml rbc), had 33.7 mg GSH/100 ml rbc after incubation with VFE. Finally, extracts of beans boiled for 1½ hours were prepared as for fresh beans. There was no significant effect on GSH with these extracts.

Discussion. The mechanism of hemolysis on exposure to fava bean still is not explained (2). This bean is widely grown and commonly eaten in Iran. We have shown(6) that approximately 8% of Moslems in Iran are sensitive with the GSH stability test; yet fewer cases of favism are seen than might be predicted from this figure. Many of our sensitive subjects eat the bean regularly without noticeable effect. Furthermore, persons with symptoms of favism gave histories of previous ingestion without illness(2). Since favism usually follows ingestion of fresh beans(7), their preparation may influence the nature and severity of hemolysis. Although we showed a direct *in vitro* effect of VFE on erythrocytes GSH, we can not exclude evidence for allergy in addition to the genetic erythrocytic defect(2). Determination of the chemical nature and properties of the agent in our extract may clarify the pathogenesis of favism.

Summary. A heat labile extract of *Vicia faba* was prepared which, like APH, lowered the reduced glutathione of erythrocytes of sensitive individuals.

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1. Beutler, E., *Blood,* 1959, v14, 103.
2. Szeinberg, A., Sheba, C., *Israel Med. J.,* 1958, v17, 158.
3. Dunsford, I., Bowley, C. C., *Techniques in Blood Grouping,* 1st. Ed., Oliver & Boyd Ltd., Edinburgh, 1955, p154.
4. Beutler, E., *J. Lab. & Clin. Med.,* 1957, v49, 84.
5. Szeinberg, A., Asher, Y., Sheba, C., *Blood,* 1958, v13, 348.
6. Walker, D. G., Bowman, J. E., *Nature,* 1959, v184, 1325.
7. Luisada, A., *Medicine,* 1941, v20, 229.

Received November 2, 1959. P.S.E.B.M., 1960, v103.