were counted in a counting chamber using a phase-contrast microscope.

Results. Table II shows results. The hypoprotein diet caused marked reduction of nitrogen content without conspicuous decrease in enzymatic activities of single mitochondria. Fasting caused a very distinct decrease of nitrogen content with simultaneous decrease of enzymatic activities of single mitochondria. However, since decrease in enzymatic activities was smaller than decrease in nitrogen content, activity/mg of mitochondrial nitrogen appears to have increased. The hyperprotein diet did not cause significant changes in mitochondrial nitrogen content. On the other hand, it caused marked increase of cytochrome-oxidase activity/mitochondrion and/mg of mitochondrial nitrogen. Other enzymatic activities studied did not change significantly.

Discussion. It is obvious that under suitable experimental conditions the nitrogen content of hepatic mitochondria may undergo conspicuous variations and that by means of suitable protein deficient diets it is possible to reduce mitochondrial nitrogen content without reducing activity of certain enzymes significantly.

The results of these experiments suggest that hepatic mitochondria contain 2 nitrogen fractions with different functional characteristics: an enzymatically inert labile fraction and an enzymatically active one. Protracted fasting causes greater decrease of mitochondrial nitrogen and a much more pronounced fall of enzymatic activities, suggesting that, in this condition, there is significant loss of enzymatically active nitrogen. This appears to be bound more firmly to the mitochondrial structure than the enzymatically inactive fraction, which can be reduced by protein deficient diet. The increased cytochrome-oxidase activity observed following the hyperproteic diet suggests that the portion of the enzyme which under normal conditions is enzymatically inactive, may become activated. Our results further suggest that in experimental conditions able to change the nitrogen content of mitochondria, the data concerning enzymatic activities should be reported in terms of single mitochondria, rather than in terms of mg of nitrogen, as it is usually done.

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Blood Thrombocyte Levels after Administration of Equine Estrogens. (25563)

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Intravenous estrogens in uncontrolled studies have been reported beneficial in cases of hemorrhage of various types including neonatal(1), uterine(2,3), post dental extraction (4), nasal and post adenoidectomy(2,5), as well as rectal, gastric, and subarachnoid bleeding. However, the mechanism of reported hemostatic effect has not been adequately explained. In dogs, Johnson(6) found elevation of prothrombin and Ac-globulin as well as decrease of antithrombin following intravenous Premarin.* Increased levels of prothrombin and prothrombin-conversion accelerators have been found in late pregnancy(7). Thrombocytosis following estrogen injection was reported by Menger(2) but not seen by Jacobson(8) or Whittington(4). A sharp elevation of thrombocyte counts occurs just

* "Premarin," available from Ayerst Lab., consisting of a mixture of equine conjugated estrogens.

before ovulation(9,10), coinciding with the peak in estrogenic hormone levels. We have compared platelet counts before and after single intravenous injections of 20 mg of Premarin.

Method and materials. Nine patients with lymphoma or chronic leukemia, who had thrombocytopenia either as manifestation of the disease or secondary to chemotherapy, and 5 patients with normal platelet counts were studied. The experimental period lasted 4 days. Each patient received 5 cc of physiological saline intravenously at 10 a.m. on first and fourth days. On second and third days, 20 mg (5 cc) of Premarin was given intravenously at 10 a.m. Venous blood was withdrawn for platelet counting immediately before, and 2 and 4 hours after, these injections. Patients ate lunch soon after the 2 hour sample was obtained. In a few instances, patients could not be studied over 4 day periods and one of the control days was omitted. Siliconized syringes, needles, and blood containers were used. Syringes were moistened with heparin solution for anticoagulation. Platelet counts were performed in duplicate within 1 hour of drawing blood samples by the same technician, using a modification of hemocytometer with phase microscopy method of Brecker, Scheiderman and Cronkite(11). Reproducibility of this method was more satisfactory than with indirect methods for counting platelets. Hematocrit and WBC determinations were performed on all samples as additional check of technic.

Results. Patients with initial platelet counts under 100,000/cmm (ranging from 12,700 to 80,000/cmm) and those with counts over 100,000/cmm (ranging from 182,000 to 360,000/cmm) were grouped separately. Reproducibility of counts was satisfactory. There were no consistent variations between the 2 control days or the 2 treatment days. Percentage deviations from pre-injection values after 2 hr and 4 hr were calculated. Mean changes and their standard errors, for both groups of patients, are shown in Fig. 1. It is evident that there was no significant difference between effects of saline and Premarin.

No significant variations in white blood cell counts or hematocrit were seen.

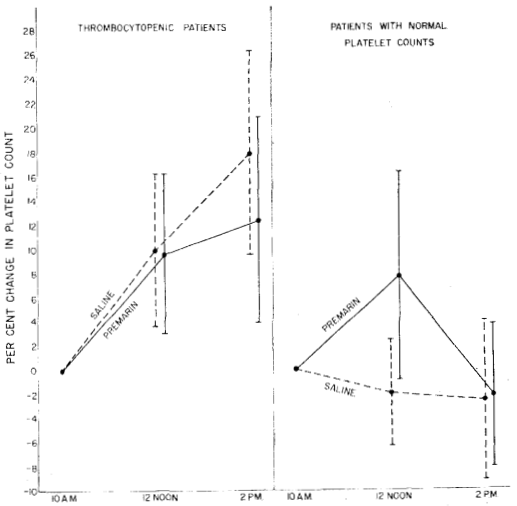

FIG. 1. Response of thrombocytopenic patients and patients with normal platelet counts to intrav. normal saline and Premarin inj. Vertical bars represent stand. errors of percentage changes.

On control days of saline injections, a slight increase of platelet counts occurred in the thrombocytopenic group, but no change was noted in the group with normal platelet counts. On treatment days, platelet counts increased slightly during first 2 hours after Premarin injection. This elevation was maintained for the 4 hour study period in the thrombocytopenic group but returned to resting values in patients with normal counts.

Change of platelet counts from 10 a.m. to 2 p.m. after saline injection may reflect part of a diurnal variation, since such variations of thrombocytes have been observed. Matis and Gross(12) found a significant increase in platelet counts during the late afternoon in subjects on normal routines. On the other hand, Ivanitzky and Klimova(13) observed a decrease in numbers of circulating platelets under these conditions, with disappearance of this variation during a 24 hour fast. The variations in the present study appear to parallel those described by Matis and Gross; however, the changes in Fig. 1 are not statistically significant at the 5% level of probability. Although diurnal variation is suggested, our study revealed little or no change in thrombocyte counts from day to day. Since the life span of platelets is between 3 and 10 days (14), a day to day increase in thrombocyte

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.

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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