Absorption by Rats of Iron in Wine.* (29494)

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Wine is a complex substance, containing approximately 100 chemical constituents, one of which is iron, chiefly in the form of ferrous sulfate(1). The amounts of iron vary in different wines and when large amounts are present they appear to be derived chiefly from the metal equipment of preparation or storage(2). U. S. wines presently have an average of 1.9 to 6.0 mg per liter(3) and French wines an average of 6.2 mg per liter (4). U. S. wines prepared prior to World War II had larger quantities, up to 35 mg per liter, and Rumanian wines had as much as 350 mg per liter(2). Wine has been advocated in treatment of anemia and chlorosis(5) but it is not known how well the iron in wine is absorbed. This is a study of the absorption by rats of iron in water and in wine, as well as in other alcoholic beverages.

Materials and methods. One hundred and forty-nine male inbred rats of the WR strain, 8-9 weeks of age, were used. They had been obtained from the Schmidt Laboratories, Madison, Wisc., at 4 weeks of age. The wines used were commercially available brands from California and France, of the red and white table and the "dessert" types such as muscatel, port, and sherry. These had iron contents of 0.6 to 6.7 mg/liter. The amounts of iron administered to rats were those present in the purchased wines except for addition of the radioactive iron. Control animals received the same amounts and concentrations of iron in the form of ferrous sulfate mixed with tap water and with commercially available U. S. beer, bourbon, gin, and whiskey. To water, wines, or other alcoholic beverages, ferrous sulfate Fe-59, obtained from Abbott Laboratories, Oak Ridge, Tenn., was added 3 hours prior to administration. It contained 0.0025 mg iron per ml and had a specific activity of 7.7 mc per mg of iron. Each animal received 0.25 μg or approximately 2 μc. Each was given a total volume of 1.0 ml; this was 0.9 ml of alcohol or water mixed with 0.1 ml of the radioactive iron solution.

Wine with radioactive iron derived from grapes made radioactive by injecting Fe-59 and Fe-55 ferrous sulfate into the trunks(6) of Cabernet Sauvignon vines prior to harvesting was also used. This was prepared by Drs. J. A. Cook, M. A. Amerine, and W. M. Kliewer, Department of Viticulture and Enology, University of California College of Agriculture, Davis, who will report full details of the method elsewhere. Briefly, 2 injections of 10 ml each of 2% ferrous sulfate solution of pH 4, each injection containing 0.25 mc Fe-55 and 0.25 mc Fe-59 were injected into the vine trunks midway between ground and foliage 41 and 26 days before the grapes were harvested. The final iron content of the wine was 2.5 mg per liter. At the time it was administered to rats, approximately 2 months after preparation, one ml gave an average of 309 net counts per 10 minutes in a Packard Armac model 410-A Gamma Spectrometer. Background counts averaged 140 per 10 minutes. Tap water with the same concentration of iron and radioactivity was prepared for control animals.

The iron content of all beverages and solutions was determined by a modification of the method of Kitzes *et al*(7). Five ml were evaporated to dryness, ashed, dissolved in acid, neutralized, and alpha, alpha bipyridine was added for the development of color, which was read in a Coleman spectrophotometer at 510 mμ.

All animals were housed in individual cages in a constant temperature and humidity room. They were fed Purina laboratory chow and tap water *ad libitum* until 3 hours prior to intubation, when they were allowed only 2% sucrose in water *ad libitum* until 4 hours after intubation. Solutions were ad-

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TABLE I. Absorption by Rats of Iron in Water, Wine and Other Alcoholic Beverages.

	% absorption					
	Water		Wine		Other alcoholic beverages	
Oral iron, γ/kg body wt	No. of rats	Avg ± S.E.	No. of rats	Avg ± S.E.	No. of rats	Avg ± S.E.
5	7	16.7 ± 5.1	6	23.1 ± 6.0	3	18.9 ± 1.2
15	7	12.1 ± 1.8	8	14.7 ± 1.5	9	12.7 ± 1.7
*15	8	14.0 ± 1.9	10	9.9 ± .6	—	— —
25	11	11.8 ± 1.1	23	14.1 ± 1.4	10	17.4 ± 2.2
30	4	10.9 ± 2.7	9	9.0 ± 1.1	10	14.5 ± 3.1

* Radioactive wine prepared from grapes containing Fe-59 that had been injected into trunks of growing vines. All other solutions contained Fe-59 that was added 3 hr prior to intragastric administration.

ministered between 10 and 11 A.M. by intragastric tube. The hematocrit of each animal was determined using tail blood and a microhematocrit technique(8); no rats used in this study had anemia.

Twenty minutes after intragastric instillation the radioactivity present in an entire animal was determined in the Packard Gamma Spectrometer. Each count was made for 3 minutes. Background counts were made frequently and underwent no change. Positioning of the animals in cardboard containers in the spectrometer well was standardized and checked for uniformity and reproducibility of counts. Corrections were made for decay of the radioactive iron, using standards prepared from each lot of iron or the wine that was used. The initial determination of the amount of radioactivity in an animal was used as a base line and subsequent daily counts were made for a total of 8 days. The radioactivity of each animal was constant by 48 to 72 hours; counts made at 72 hours were therefore used to calculate the percentage of original radioactivity above base line remaining in the body and assumed to represent absorbed iron.

Results. Iron absorption using different wines and other alcoholic beverages with the same iron content were similar, so that the findings are presented by type of beverage without further identification.

In preliminary experiments it was found that iron absorption was not affected by the length of the period between the preparation of a solution of iron in water and its administration to the animal, nor by the length of time before administration when Fe-59 was added to a solution of iron in water.

Iron absorption from wine, water, and other alcoholic beverages. There was no significant difference (P greater than 0.05) in absorption of the same doses of iron from water, wine, or other alcoholic beverages. This was the case when Fe-59 was added to the wine or when the wine was obtained from grapes containing Fe-59 (Table I).

Comment. The absorption of iron from wine by rats was similar to absorption from the same amounts mixed with water and alcoholic beverages. Thus there was no apparent interference or enhancement of iron absorption by the many constituents present in wine.

Summary. Absorption of iron from water, wine, and other alcoholic beverages administered by gastric tube to fasting rats was studied using Fe-59 and whole body counting. Wine was prepared from grapes grown to contain ferrous sulfate Fe-59 and Fe-55, and ferrous sulfate Fe-59 was added to commercially obtained wines. Absorption of iron was similar from tap water, wine and other alcoholic beverages.

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Effects of Oral Ovulation Inhibitors on Serum Protein-Bound Iodine and Thyroxine Binding Proteins.* (29495)

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Most oral ovulation inhibitors in clinical use are compounded of a progestational agent and an estrogen. Estrogens, even in low dosage, are known to elevate circulating levels of thyroxine binding globulin (TBG)(1) and the PBI(2,3). However, the clinical effect of ovulation inhibitors depends primarily upon the progestational agent and may range from strongly estrogenic, as with norethynodrel preparations(4) to mildly androgenic, as has been claimed for norethindrone(5). Steroids structurally related to norethindrone's progestagen have been shown to depress both PBI and TBG(6). The effects of oral ovulation inhibitors upon PBI and thyroxine binding proteins thus are not predictable *a priori*. In view of the large number of patients taking these drugs a study of their effect upon commonly used criteria of thyroid function seemed desirable.

Hollander(7) reported recently that norethynodrel caused an elevation of the PBI and a depression of the erythrocyte triiodothyronine uptake test. Medroxyprogesterone acetate did not affect either test in Hollander's study. We have extended his study to cover the effect of 4 commonly used ovulation inhibitors and have measured their effects on PBI and the 2 principal thyroxine binding proteins.

Materials and methods. The drugs studied were: 1. Norethynodrel, 17-α-ethinyl-5(10)-estrenolone, marketed as "Enovid" by G. B. Searle & Co. Tablets contain either 5 mg norethynodrel plus 0.075 mg ethinyl estradiol-3-methyl ether (mestranol) or 2.5 mg norethynodrel plus 0.10 mg mestranol.

2. Medroxyprogesterone acetate, 6-α-methyl-17-α-hydroxyprogesterone acetate, supplied as "Provest" by Upjohn Co., containing 5 mg medroxyprogesterone acetate plus 0.10 mg ethinyl estradiol.

3. Norethindrone, 17-α-ethinyl-19-nortestosterone, marketed as Orthonovum by Ortho Pharmaceutical Co., containing 10 mg norethindrone plus 0.06 mg mestranol.

4. Chlormadinone, 6-chloro-Δ6-dehydro-17-α-acetoxyprogesterone, supplied by Eli Lilly & Co., containing 2 mg chlormadinone plus 0.06 mg mestranol.

Sera from healthy women of child-bearing age who had been on the appropriate drug regimen for at least 3 months were obtained through the kindness of Dr. E. T. Tyler of Los Angeles and Dr. C. Gassner of Long Beach. Sera from 4 of these patients were available before initiation of therapy. In a further case we obtained repeated serum specimens during a 2-year period of norethynodrel therapy followed by 2 months off the drug and during a subsequent pregnancy.

PBI's were measured by a modification of Bodansky's wet ashing technique(8) which has given a normal range of 4.5 to 7.5 μg per 100 ml in our hands in several thousand determinations.

Serum thyroxine binding capacities, both pre-albumin (TBPA) and TBG were determined by Beierwaltes' modification(9) of Robbins' reverse flow technique. The thyroxine concentration in sera enriched with thyroxine-I^{131} (Abbott Laboratories) and stable thyroxine (California Corp. for Biochemical Research) was determined by Posner's butanol-extractable iodine method(10) after suitable dilution of the butanol extracts.

Results. As summarized in Table I, the subjects who had been treated with the 4

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