Neutron Activation of Iron Tablets to Evaluate the Effects of Glycine on Iron Absorption

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Abstract □ Neutron bombardment (neutron flux, 3×10^{12} neutrons/cm^2/s) of prepared iron tablets containing glycine-iron or iron alone was performed to prepare radioactive tablets to assess the effects of glycine on iron absorption from tableted formulations. No interfering isotopes of sufficient quantity were generated during neutron activation of the iron tablets. Cobalt-60 was the major trace mineral detected and accounted for only 1.3% of the total activity. There may have been trace amounts of zinc-65 or chromium-51 present, but they were not detectable above background radioactivity in the final tablet produced. Iron-59 represented >98% of the radioactivity present in the tablets used in the study. Glycine-containing iron tablets produced dramatically higher amounts of iron in blood and tissues of rabbits ($p < 0.05$) than did the same tablet formulations without glycine. The area under the iron blood concentration time curve over 4 h increased by 67% with glycine added to the formulation over control iron tablets. Iron concentrations in tissues 4 h after iron administration was in the order of blood > liver > heart > kidney > muscle.

Keyphrases □ Absorption—iron, effects of glycine, neutron activation of iron tablets □ Neutron activation—iron tablets, effects of glycine on iron absorption □ Formulations—tablets, neutron activation to evaluate the effects of glycine on iron absorption

Several investigators have noted that amino acids may increase iron absorption from the GI tract (1–7). It was proposed that amino acids facilitate increased iron absorption by (*a*) buffering the intestine and delaying the rise in pH toward neutralization at sites where iron is oxidized to insoluble salt forms, (*b*) forming iron-amine chelates that act to enhance iron absorption, and (*c*) stimulating iron transport systems within the animal (6).

In previous studies, it has been shown that glycine enhances iron absorption (6, 7). Also, it has been shown that administration of diets containing glycine compared with diets lacking glycine result in a greater amount of iron absorbed (8). It was the purpose of this study to determine whether glycine added to iron (at a ratio of 2:1, respectively) in a tablet dosage form could increase iron absorption.

To prepare radioactive iron tablets, neutron activation was tested to determine whether this technique offers a viable method of preparing intact tableted dosage forms that can conveniently be made radioactive after formulation. By preparing tableted dosage forms first and then making them radioactive by neutron activation, there is less of a potential for contamination of facilities and there are unique opportunities to use radiolabeled compounds to test some qualities of solid dosage forms.

Table I—Radionuclides Expected To Be Activated in Tablets from Neutron Flux [a]

Element Produced During Neutron Activation	Element Half-Life
^{24}Na	15 h
^{42}K	12.4 h
^{27}Mg	9.5 min
^{56}Mn	2.6 h
^{60}Co	5.26 years
^{51}Cr	27.8 d
^{38}Cl	37.3 min
^{65}Zn	243.6 d
^{59}Fe	45.1 d

[a] Neutron flux, 3×10^{12} neutrons/cm^2/s.

EXPERIMENTAL SECTION

Production of Tablets—Direct compression was used to produce tablets[1]. Two types of tablets were prepared. The first type contained glycine and ferrous chloride. The control tablets did not contain glycine.

Type 1 Tablet—The average weight of each tablet was 0.42 g. The formula for one tablet of type 1 is (in milligrams/percent): granulated sucrose[2] (296.6/71.4), magnesium stearate (29.7/7.1), glycine (39.5/9.5), and ferrous chloride (49.4/11.9).

Type 2 Tablet—The average weight of each tablet was 0.41 g. The formula for one tablet of type 2 is (in milligrams/percent): granulated sucrose[2] (328.1/81), magnesium stearate (28.9/7.1), and ferrous chloride (48.2/11.9).

Disintegration tests were done for both tablet types by using a USP tablet and disintegration tester[3] (9).

Neutron Activation of Tablets—Tablets were put into small scintillation bags[4]. Each bag was sealed by heating and placed in a polyvial[5]. The polyvials were then sealed by heating. Tablet samples were then put in the Oregon State University (OSU) Triga reactor in the Lazy Susan and activated with a neutron flux of 3×10^{12} neutrons/cm^2/s generated by 1 MW of power from the reactor. The irradiation time was 4 h. The tablets were allowed to stand for 1 week after irradiation to allow short-half-life radionuclides to decay.

Neutron Activation Equation—For neutron activation analysis, the following was used:

$$A = N\phi\sigma(1 - e^{-\lambda T})e^{-\lambda t} \qquad \text{(Eq. 1)}$$

where A is the activity at end of irradiation (disintegrations/s), N is the number of atoms, ϕ is the flux (neutrons/cm^2 s), σ is the cross-section (cm^2), λ is the decay constant (s^{-1}), T is the length of irradiation (s), and t is the time elapsed between activation and measurement.

The activity at the time of measurement of radiation depends on the half-life of the isotope of interest and the time that has elapsed between activation and measurements (10, 11). Activation analysis is subject to a variety of errors. These include errors due to flux gradients, self-shielding, and interfering nuclear reactions (10).

The errors due to neutron flux gradients within the reactor can be minimized by rotating or spinning the sample during irradiation (12). The OSU Triga reactor is a circulator reactor, and the samples were rotated around the reactor core during irradiation in the Lazy Susan. For other reactors, the use of an internal standard to determine the flux and to ensure that future samples have the exact orientation and position of irradiation as those of previous samples reduces the necessity to vary flux gradients (12, 13).

The effects of self-shielding can be shown to be a function of the sample mass and length (12). More self-shielding occurs when each of the parameters is larger. With samples of <4 g in mass, very little self-shielding occurs (12, 13). Also, self-shielding is reduced when the sample is homogeneously spread in an inert material (*e.g.*, sucrose) (12). Tablets produced for this study were small (0.41–0.42 g), and the major ingredient in 71–81% of the tablets is granulated sucrose. Both of these factors minimize self-shielding.

Interference in counting iron-59 may occur from cobalt-60 and zinc-65 when counting with a sodium iodide detector in a solid scintillation counting chamber[6]. The 1.099- and 1.292-MeV gamma rays of iron-59 with the 1.173-

[1] Single-punch tablet machine, model TPK-12; Chemical and Pharmaceutical Co., Inc.

[2] Dipac, prepared by co-crystallization of 97% sucrose and 3% dextrin; Armerstone.

[3] New England Nuclear Corp.

[4] Olympic Plastic Co., Los Angeles, Calif.

[5] Tracer Northern TN-1705 MCA 40 cc NAI (T1) detector.

[6] Model No. Gc19ED detector, with Ortec 572 spectroscopy amplifier, Nuclear-Data 600 multichannel analyzer; Princeton Gamma Tech., Princeton, N.J.

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Table II—Sources of Radioactivity in Tablets Used in Animal Study 1 Week after Activation

Sample	^{60}Co, ppm ± 16	^{51}Cr, ppm ± 16	^{65}Zn Upper Limit, %[a]	^{59}Fe, cpm	^{60}Co, cpm
Tablet	0.72 ± 0.04	ND[b]	>0.013	30,176	392
$FeCl_3$	6.8 ± 0.1	11.0 ± 3.0	ND	287,750[c]	3700[c]
Glycine	ND	1.1 ± 0.2	ND	ND	ND
Magnesium stearate	ND	ND	ND	ND	ND
Granulated sucrose[2]	ND	ND	ND	ND	ND

[a] Value represents the upper limit plus 3 standard deviations of the percentage of zinc activity that may be contained in the tablet that was being hidden by background counts. [b] Not detectable. [c] Ratio of count of ^{60}Co-^{59}Fe was equivalent to that of the tablet.

and 1.332-MeV gamma rays of cobalt-60 and the 1.115-MeV gamma rays of zinc-65 would appear as a single peak in a sodium iodide detector. Other radionuclides that produce gamma rays in this range, *i.e.*, nickel, aluminum, copper, manganese, *etc.*, are either not generated to a sufficient extent on bombardment by slow thermal neutrons due to their low cross sections or have half-lives too short to be active at the end of 1 week of storage.

To ensure that the tablets contained only trace amounts of cobalt-60 after activation, all components of the tablet, the powders used for tablet formulations, and the tablets were activated and counted for 100 min in a germanium-lithium [Ge(Li)] detector[7] with a multichannel analyzer. This detector was calibrated at 1 KeV per channel, with the screen having 2048 channels. The samples were analyzed for radioactivity having gamma energys in the range of 80 keV-2.04 MeV, to make certain that the samples to be collected later in the animal study could be reliably counted by the NaI detector as iron-59 counts.

Procedure with Rabbits—The iron-glycine activated tablets, as well as the control activated tablets, were each tested on groups of four rabbits. The tablets were administered to each rabbit before 2 weeks had elapsed from the neutron activation of the tablets. Female rabbits were used (weight, 2.0–3.0 kg each). The ears of each rabbit were shaved with an electric shaver and commercial ointment[8]. The rabbit was then put in a rabbit restrainer with its head out and given orally four tablets with a total activity of 1.1 μCi.

At time periods of 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after tablet administration, 100-μL blood samples were collected from the ears with capillary tubes. Bleeding was initiated by pricking the vein of the ears with a 20-gauge needle. Procaine was used as a local anesthetic. Blood samples were placed into counting vials. The 1.099- and 1.292-MeV gamma rays of 44.6-d iron-59 of each sample were counted for 4000 s in a solid scintillation counter chamber.

At the end of 4 h, the animals were sacrificed by ether administration, and liver, kidney, muscle, and heart samples were taken from the animals, weighed, and then counted as reported previously (7).

RESULTS AND DISCUSSION

When samples are exposed to neutron activation, all elements with atomic numbers greater than 10 are activated. The abundance of the different isotopes of the elements that are to be generated depends on the neutron flux to which they are exposed and whether the isotope of the element has a high or low cross section to the neutron flux to which it is exposed. For example, iron that is subjected to a neutron flux of 3×10^{12} neutrons/cm^2/s produces only iron-59. The other isotopes of iron are not produced to any detectable level because of their low cross sections when exposed to the neutron flux employed.

After the tablets and powders were activated by the OSU Triga reactor in the Lazy Susan, there was too much radioactivity emanating from them to be easily quantitated. The tablets and powders were thus sealed in a lead storage area and kept for 1 week. In Table I is a list of elements that were expected to be generated and suspected to be the cause of the initially high radioactive emissions of the tablets and powders. All elements except iron-59, cobalt-60, zinc-65, and chromium-51 have very short half-lives and would become nonradioactive after 1 week of storage. The tablets and powders were counted in the Ge(Li) detector after 1 week of storage (Table II). Iron-59 accounted for >98% of the radioactivity counted. Cobalt-60 accounted for ~1.3% of the radioactivity present. Only a trace amount of zinc-65 was observed, and no detectable levels of chromium-51 were present in the tablets to be used in the animal study. The ratio of counts of cobalt-60 in the tablet was obtained as:

$$\text{Ratio} = \frac{^{60}\text{Co}_{(\text{cpm})} \times 100}{^{59}\text{Fe}_{(\text{cpm})} + {}^{60}\text{Co}_{(\text{cpm})}} \qquad \text{(Eq. 2)}$$

The majority of the radioactivity contained in the tablets (>98%) originated from one isotope, iron-59. For this reason, the NaI detector could be used to count the samples obtained from the animal study.

The tablets prepared by neutron activation for use in this study were administered to the rabbits before 2 weeks had elapsed from initial activation. Allowing the tablets to stand for longer periods would have caused the cobalt-60/iron-59 ratio to change in the tablets. After 2 months, the cobalt-60 would contribute to >3% of the tablet radioactivity instead of the 1.3% of tablet radioactivity that was measured. The percentage of radioactivity in the tablets due to cobalt-60 would increase the longer the tablets were stored, since iron-59 has a half-life much shorter than that of cobalt-60.

Ferrous chloride was used rather than the more commonly prescribed ferrous sulfate because the tablets were to be activated, and the less radioactive the tablets, the more convenient they are for handling. Sulfur-35, with a half-life of 88 d, is produced when ferrous sulfate is activated. Chlorine-38, with a half-life of 37.3 min, is produced when ferrous chloride is activated (14). Since, chlorine-38 decayed very quickly after the end of bombardment, it was more convenient to use to count the 45-d iron-59.

After the tablets were prepared and before neutron activation, disintegration tests were conducted by using a tablet disintegration apparatus[3]. Tablet hardness averaged 15 kg/in^2 when tested with a tablet hardness tester[9]. Eighteen tablets of each type were tested. Seventeen glycine-iron tablets disintegrated within 18 min, whereas the 18th tablet disintegrated in 23 min. On the other hand, 16 control tablets disintegrated within 15 min, whereas the two others disintegrated within 19 min. Thus, any difference in iron absorption should not be due to differences in tablet disintegration after administration. Dissolution studies, which often correlate more closely with bioavailability, were not conducted.

In Figs. 1 and 2 are shown, respectively, the iron concentration in blood and different tissues following oral administration of glycine-iron and iron control neutron-activated tablets to rabbits. It is evident from these experiments that iron appears more quickly and in greater amounts in blood when the tablets contained glycine. The area under the iron concentration curve for the time during which data were collected increased by 67% when the tablets contained glycine (Fig. 1). From an assessment of the total amount of iron absorbed, the iron-glycine tablets showed a statistically significant increase in the amount of iron absorbed during the first 4 h postdose ($p \leq 0.05$) based on the

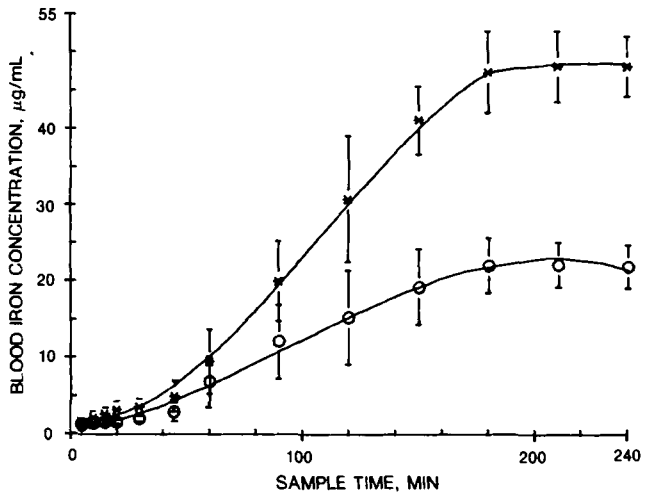

Figure 1—*Blood iron concentration (±SD) versus time curve in rabbits following oral administration of four tablets containing 192.92 mg of ferrous chloride (○) and four tablets containing 197.64 mg of ferrous chloride and 158.12 mg of glycine (*).*

[7] Nair.
[8] Vanderkamp; Van-Kel Industries, Edison, N.J.
[9] Strong Cobb Corp. Inc., Cleveland, Ohio.

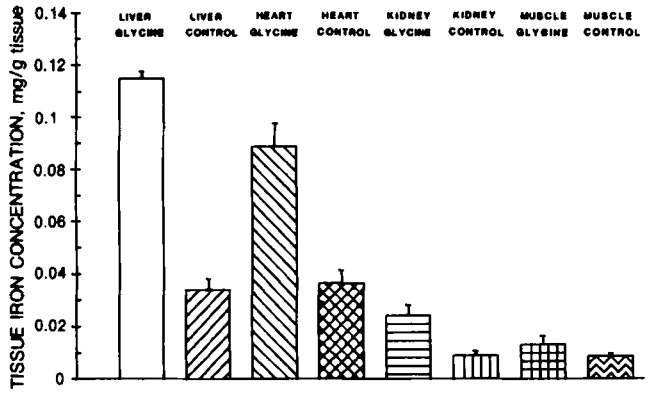

Figure 2—*Iron concentration (±SD) in body tissues 4 h after oral administration of four tablets containing 192.92 mg of ferrous chloride (control) and four tablets containing 197.12 mg of ferrous chloride and 158.12 mg of glycine.*

assumption that the area under the iron concentration–time curve (0–4 h) is an indicator of absorption.

The amount of iron in the different tissues was also consistent with iron levels in the blood. Iron concentration was highest in the tissues of the rabbits that received the glycine–iron tablets (Fig. 2). Iron–glycine tablets generated iron concentrations in tissue that were statistically significantly different from those of control iron tablets ($p < 0.001$ for the tissues of the liver, heart, and kidney; $p < 0.05$ for muscle).

All data were consistent with those from a previous report (7) from our laboratory indicating that glycine increases iron absorption into both blood and tissues. Neutron activation analysis is known to be very useful in analytical problems in which high sensitivity is required (sensitivity can reach 10^{-9} g). It is safe, economical, fast, practical in elemental analysis, and nondestructive (15). Also, thermal neutrons do not activate isotopes with atomic numbers of less than 10; therefore, carbon, hydrogen, oxygen, and nitrogen are not activated.

The data show that neutron activation analysis of final tableted dosage forms of iron can be utilized to investigate the effects of variable formulation factors on the absorption of iron. Further work is needed in the area utilizing multiple dosing, mixed protein hydrolysate, and pure defined amino acid mixtures to optimize tablet formulations for iron absorption. The technique of neutron-activation analysis can be employed for other minerals such as zinc or magnesium and may be used for comparing the rate and extent of absorption of minerals from commercially available vitamin-mineral mixtures (tablets or capsules). This application is currently under investigation in our laboratories.

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Potential Inhibitors of Tyrosine Hydroxylase and Dopamine-β-Hydroxylase

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Abstract □ A series of methyl-substituted 1,2,3,4-tetrahydrocarbazoles was synthesized and screened for *in vitro* activity against tyrosine hydroxylase and dopamine-β-hydroxylase. The most potent compounds were evaluated for inhibition of norepinephrine biosynthesis in rats. The results indicated no significant decrease in norepinephrine levels at three dosage levels.

Keyphrases □ Tyrosine hydroxylase—*in vitro* activity, effect of methyl-substituted 1,2,3,4-tetrahydrocarbazoles □ Dopamine-β-hydroxylase—*in vitro* activity, effect of methyl-substituted 1,2,3,4-tetrahydrocarbazoles □ Methyl-substituted 1,2,3,4-tetrahydrocarbazoles—effect on tyrosine hydroxylase and dopamine-β-hydroxylase, *in vitro*

It has been shown that the 1,2,3,4-tetrahydrocarbazole nucleus is an active pharmacophore. Previous studies have shown that the various carbazoles exhibit anti-inflammatory (1–6), antidepressant (7–12), hypoglycemic (13), analgetic (14), cardiotonic (15), anti-infective (16), and anticancer activities (17). It was of interest to extend the initial work of

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