10,000 M NaF, we found it necessary to increase the fluoride concentration to 1-500 M. This 20-fold increase should be favorable to the binding of calcium by fluoride. The increase was necessary because the bones were grown on the surface of a clot. Between the bone and the clot, diffusion is the sole means of exchanging materials.

Summary. In embryonic bones growing in tissue culture the process of calcification of cartilage can be inhibited by NaF without influencing growth. To achieve this effect the concentration of fluoride had to be 20 times greater than that which will produce inhibition of calcification in test-tube experiments. The inhibition in the present experiments is probably due to at least two things: 1st, interference with the production of phospho-pyruvate as a substrate for bone phosphatase; 2nd, some degree of binding of calcium by fluoride.

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Use of a Chelating Agent for Accelerating Excretion of Radio-Iron (19432)

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(Introduced by J. H. Lawrence.)
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The ability of certain organic compounds to form strong non-ionic water-soluble combinations with metal ions suggests the possibility that these compounds might be useful for removing metal ions from the body. In this respect, Kety(1) has shown that use of citric acid may play a role in the removal of lead from bone by such a mechanism. Foreman and Hamilton(2) demonstrated that ethylenediamine tetra-acetic acid (EDTA) will chelate plutonium and yttrium *in vivo* and thereby greatly accelerate their excretion from the body.

This report deals with the application of this principle for hastening the excretion of radio-iron. Interest in accelerating excretion of iron is stimulated by the possibility of removing the excessive quantities of iron accumulated with hemochromatosis and transfusion hemosiderosis and for possible use as a tool for studying iron metabolism. "Fe-3 Specific"† is the compound chosen for this study. It has a very strong affinity for iron, particularly in the alkaline pH range(3). (It is used commonly for scavenging iron traces from solutions.) It was selected because it has, in addition, suitable characteristics for *in vivo* use, *i.e.,* it is relatively non-toxic, forms serum soluble chelates, is poorly catabolized, and is readily excreted through the kidney.

Methods. The effect of "Fe-3 Specific" on iron excretion was studied in a series of three experiments, using iron 59, *i.e.,* a) when iron 59 was given intravenously and "Fe-3 Specific administered intraperitoneally, b) when iron 59 was given intravenously and "Fe-3 Specific" by mouth, and c) when both iron 59 and "Fe-3 Specific" were given orally. In addition, the concentration of iron 59 in erythrocytes was studied. Adult male Slonaker

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† A commercial chelating agent manufactured by Bersworth Chemical Co., Framingham, Mass. Its structure is not revealed.

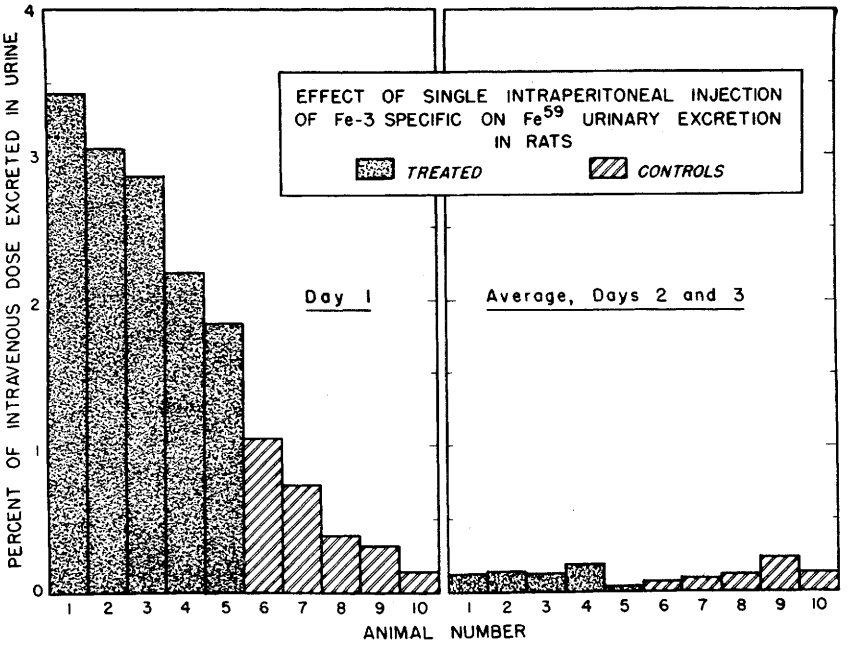

FIG. 1.

rats were used for all experiments except in Experiment 4, wherein Long-Evans rats were used. The animals were kept in metabolism cages allowing for collection of urine and feces separately. The feces samples were dry ashed at 650°C. The ash was dissolved in 10 cc of aqua regia. Two ml aliquots were withdrawn and counted directly using a scintillation counter which has been previously described (4). Urine samples were wet ashed using concentrated nitric acid and brought to 10 ml volume. Again, 2 ml aliquots were withdrawn for direct counting in the scintillation counter. *Exp. I.* Five control and 5 experimental animals were each given 2.5 x 10^5 c/m of iron 59, representing 5.3 μg of iron, by single intravenous tail injection ($FeCl_3$ buffered with sodium citrate). Five minutes before the iron 59 injection, the 5 experimental animals were each given 100 mg of "Fe-3 Specific" in a single intraperitoneal injection. The first excreta samples were collected 24 hours later. The results of the urinary excretion are presented in Fig. 1. Thereafter, collections were made of 2-, 3-, and 5-day periods as indicated in Fig. 2 and 3. On the 14th and 26th days following the iron 59 injection, "Fe-3 Specific" in 100 mg doses was again injected intraperitoneally into each of the experimental animals. Excretion was studied for a total of 90 days. *Exp. II.* A group of 6 rats was given a diet in which "Fe-3 Specific" was intimately mixed. The diet was prepared by mixing neutralized "Fe-3 Specific" solution with Purina Chow in such proportions that each animal received 400 mg, (*i.e.*, 20 cc of a 2.0% solution per animal daily). Five days following the initiation of the diet, the animals were each injected intravenously by tail vein with 5.3 μg of iron tagged with iron 59. The controls of Experiment I were used as controls for this experiment. Excreta collections and analyses were carried out during the next 16 days. A total of 9.6 g of "Fe-3 Specific" was fed to each animal (24 days) after which they were given the regular diet and were observed for one year. *Exp. III.* A group of 6 rats was fed in the same manner as in Experiment II. Three days after the initiation of the diet, tracer iron was added to the diet of the 6 animals receiving the "Fe-3 Specific" as well as to 6 others not receiving it. The iron intake was approximately 6.2 mg per day, and the tracer made up less than

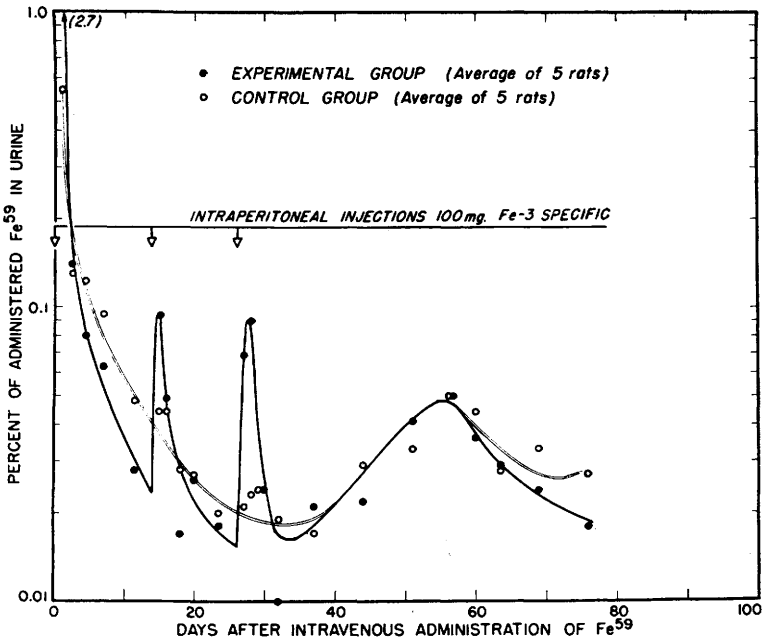

FIG. 2. Effect of single intraperitoneal injections (100 mg each) of "Fe-3 Specific" on urinary excretion rate of intravenously injected radio-iron.

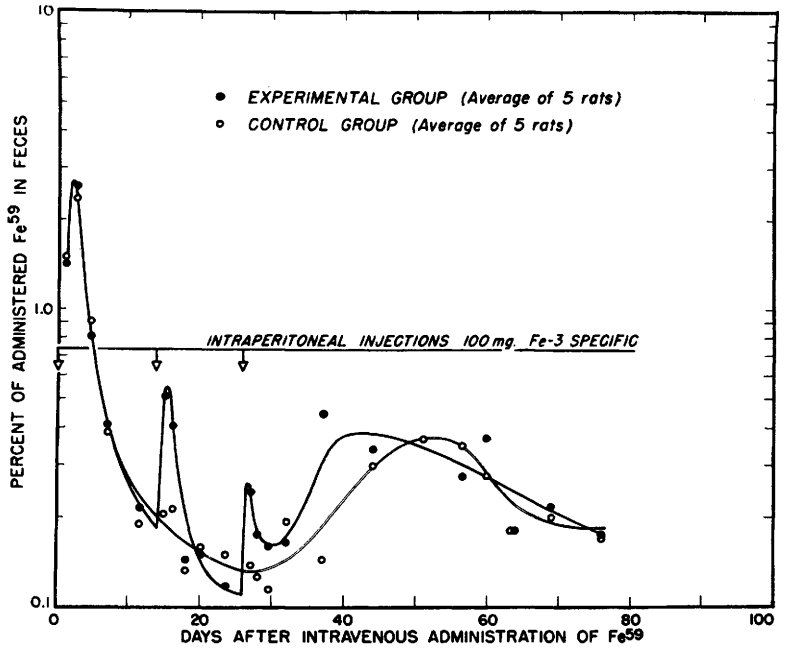

FIG. 3. Effect of single intraperitoneal injections (100 mg each) of "Fe-3 Specific" on the fecal excretion rate of intravenously injected radio-iron.

1% of this. It is not certain that the dietary iron was uniformly tagged. The iron was added as an aqueous solution of ferric chloride, intimately mixed with the feed. Excreta collections and analyses were carried out for 9 days following the addition of the tracer to the diet. *Exp. IV.* Five adult male Long-Evans rats were given a single intravenous

injection of iron 59 tagged ferric chloride solution buffered with sodium citrate. Nine blood samples were taken from each of the 5 animals on the 2nd, 5th, 12th, 38th, 49th, 66th, 74th, 80th, and 87th days after the iron 59 injection. The samples were collected from an incised tail vein in dried heparinized calibrated capillary tubes. Sample size was from 58 to 80 microliters. The capillary was sealed and centrifuged at 3000 rpm. The packed cell volume was recorded and the sample analyzed for radioactivity in the apparatus described above.

Results and discussion. Fig. 1, 2, and 3 show the marked effect on the excretion of iron 59 as a result of single intraperitoneal injections of 100 mg "Fe-3 Specific". The effect is manifest in urinary excretion shortly after the injection of iron 59 and occurred each time after the "Fe-3 Specific" was administered. Approximately a 4-fold increase over the control levels was obtained. Fecal excretion was enhanced only by the injections on the 14th and 26th days and not with the original "Fe-3 Specific" injection. This, of course, indicates that the iron must undergo some transformation before it is susceptible to enhance fecal excretion by "Fe-3 Specific". The results suggest that "Fe-3 Specific" accelerates iron 59 excretion through depletion of plasma iron but since equilibrium exists between this and tissue iron, repeated prolonged administration of "Fe-3 Specific" might eventually affect tissue iron levels. Fig. 2 and 3 show an additional gradual but more

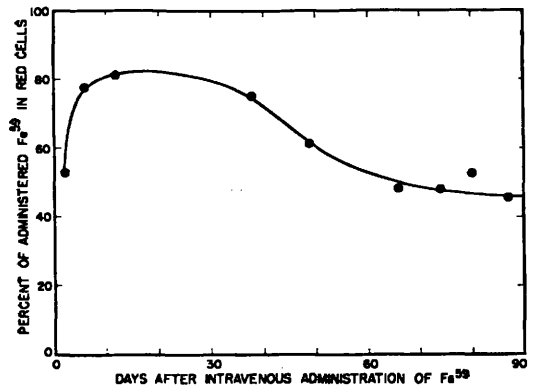

FIG. 4. Intravenously administered radio-iron present in erythrocytes as a function of time.

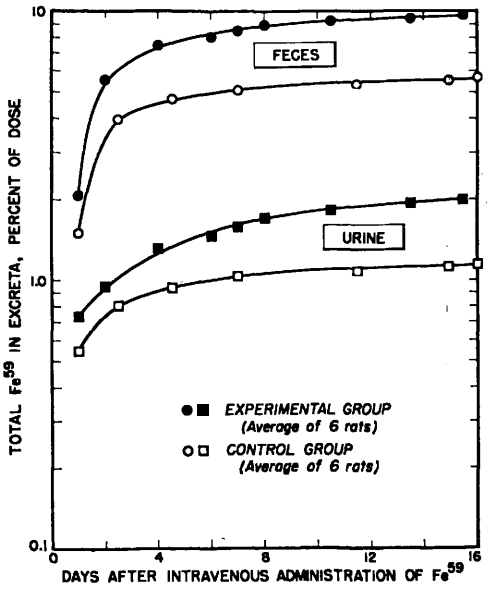

FIG. 5. Percent of a single intravenous dose of radio-iron in feces or urine of untreated rats and of rats given 400 mg "Fe-3 Specific" daily by mouth.

sustained increase in excretion of iron 59 from about the 40th to 70th day after injection. This is thought to be related to the entrance into the excretory pool of a relatively large amount of radio-iron. Fig. 4, showing the marked drop in specific activity of the largest pool of iron 59, the circulating erythrocytes, is indicative of a source of the radio-iron for the positive excretory waves in urine and feces. The drop in specific activity in the red cell is a function of erythrocyte longevity and iron reutilization. The definite excretory maximum at 55 days affords an easy way of determining mean cell longevity without a model and without difficult if not impossible integration.

Exp. II, (Fig. 5), in which the animals were fed 400 mg "Fe-3 Specific" daily and were given a single intravenous injection of iron 59, demonstrated almost 10% of the tracer excreted in the feces and 2% in the urine in 16 days, in contrast to 5.7% and 1.2% in the controls. The animals of this experiment were observed for one year after receiving a total of 9.6 g of "Fe-3 Specific". No abnormalities were noted. Hematocrits remained constant during the period of "Fe-3 Specific" feeding.

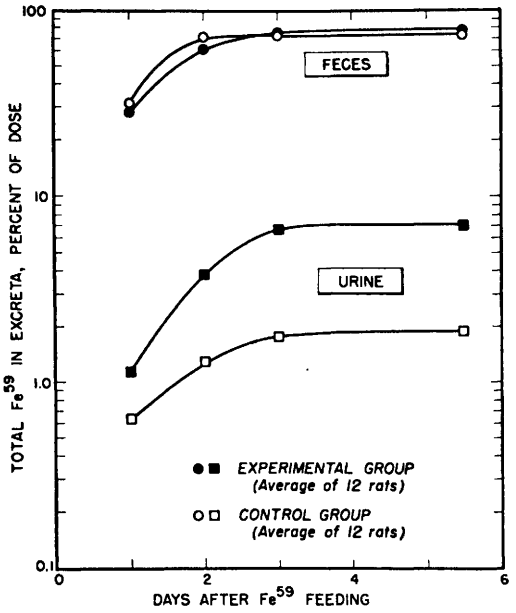

FIG. 6. Effect of oral administration of "Fe-3 Specific" (400 mg/day) on excretion of iron-59 given in a single day's diet.

In Exp. III, in which both the "Fe-3 Specific" and the iron 59 were administered in the diet, the difference in urinary excretion (Fig. 6) was as marked as in the 2 previous experiments. However, there was no great difference between fecal excretion of the control and treated animals. Approximately 7% of the administered iron was excreted in the urine of the experimental animals. Most of this occurred during the first 3 days. The urinary excretion of controls during the same period was 1.9%. The net body retention of iron 59 at the end of 7 days, was 16% and 24% in the experimental and control animals, respectively. If the diet (6.2 mg iron per day) were uniformly tagged with iron 59, then approximately 1 and 1.5 mg of iron was gained by the experimental and control animals respectively. It seems unlikely that such a great absorption could be occurring in adult rats. This would indicate that approximately 0.1 of the rat's total body iron is exchanged each day or that an even greater portion of a smaller pool is exchanged each day.

Summary. 1. Excretion of parenterally administered radio-iron is approximately 5 to 10 times as great in feces as in urine. The rate of excretion varies with the location of the tracer within the various iron pools in the body, (for example, cells, liver, reticulo-endothelial system, etc.). This is illustrated by high plasma levels of radio-iron, associated with high excretory rates in feces and urine. Fifty-five days after injection of tracer iron there is a second maximum in excretory rate which can be shown to correspond with a loss of radio-iron from the largest pool, the circulating erythrocytes. When "Fe-3 Specific" is administered intraperitoneally to animals which have been given iron 59, there is a prompt and marked increase in both the urinary and fecal excretory rates by a factor of 2 to 2.5. 2. The addition of "Fe-3 Specific" to the diet of rats causes an increase in the fecal and urinary excretion of intravenously administered radio-iron by approximately a factor of 2. 3. When labelled iron and "Fe-3 Specific" are fed simultaneously, a marked increase in urinary excretion of the tracer occurs. However, the amount found in feces is nearly the same as in controls. Thus the decrease in net retention resulted primarily because of increased urinary excretion. 4. Feeding as much as 9.6 g of "Fe-3 Specific" to animals at a rate of 400 mg per day, was not associated with any change in hematocrit nor any grossly observable abnormality in the animals.

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