AND MED., 1953, v82, 593.
14. Nicelle, P., Jude, A., Diverneau, G., *Ann. Inst. Pasteur*, 1953, v84, 27.
15. Gaines, A., Landy, M., Edsall, G., Trapani, R., *Fed. Proc.*, 1956, v15, 588.
16. Nagington, J., *Brit. J. Exp. Path.*, 1956, v37, 397.
17. Stanziale, W. G., *J. Immunol.*, 1957, v78, 156.
18. Hahn, F. E., Schaechter, M., *Bact. Proc.*, 1957, p.138.

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Studies on *in vivo* Stability of an Iron Chelate. (23749)

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N - hydroxyethylethylenediamine triacetic acid is a chelating agent similar to ethylenediamine tetraacetic acid, but with one acetic acid group replaced by an ethanol group. This alters the chelating properties for iron and other heavy metals(1). This compound has been studied in hemochromatosis and found to be effective in the removal of iron from the tissues when given intravenously(2). Since the stability constant of the iron chelate formed *in vitro* is high, it might be expected to hold iron firmly in the body. However, experimental studies have shown that when ferric chelate is injected intramuscularly in iron-deficient mice, hemoglobin regeneration occurs, and degree of hemoglobin response is slightly greater than with the corresponding chelate of ethylenediamine tetraacetic acid.† The studies here reported were carried out in 2 iron deficient males. One received Fe^{59} tagged ferric disodium N-hydroxethylethylenediamine triacetate intramuscularly, and the second received the tagged chelate orally, and by duodenal tube.

Methods. The Fe^{59} tagged ferric chelate of N-hydroxyethylethylenediamine triacetic acid was prepared from the disodium salt of this chelating agent by simultaneous addition of a ferric chloride solution and dilute sodium hydroxide within a pH range of 6.0-7.5. This preparation was evaporated to a small volume and sterile filtered, resulting in a solution stable for 3 weeks. The first subject, T.R., a 71-year-old male, showed depletion of body iron stores with hypochromic anemia after repeated phlebotomies for study purposes. At the time of the study, fasting serum iron levels varied between 19 and 24 μg% with unsaturated iron binding capacity (UIBC) between 262 and 286. Hemoglobin values varied between 9.4 and 9.7 g, hematocrit between 32 and 35%, RBC between 4.14 and 4.33 million, reticulocytes between 0.1 and 1.6%, and indices showed MCV 75-85, MCH 22-23, MCHC 27-29. He was given 2.75 ml of iron chelate by intramuscular injection in the left hip, representing 50 mg elemental iron and 10 μc Fe^{59}. Surface counting was carried out over the injection site and other body areas at regular intervals with a scintillation detector. Blood and plasma samples were analyzed for radioactivity in a "well-type" scintillation detector. Fractional urine collections were obtained on the day of injection and at daily intervals thereafter. These were evaporated to a small volume before counting in three 4 ml aliquots. Feces collected over a 4 day period were passed directly into a Waring Blendor, blending was carried out for 20 minutes with a small amount of water, and three 4 ml aliquots were taken directly from this slurry to be counted in the same "well" counter. After 12 days, studies were undertaken to determine the effect of therapeutic doses of iron chelate without radioactive iron on the serum iron and UIBC levels within the hours immediately following injection. Serum iron and UIBC values were obtained by a modification of the method of Schade(3) using Nitroso R as the

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† Rubin, M., personal communication.

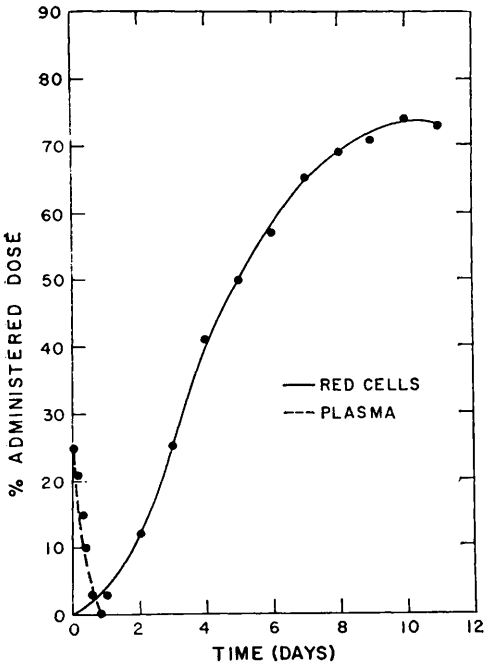

FIG. 1. Disappearance of Iron[59] from plasma and its incorporation into circulating red cells.

ferroin reagent. J.E., a 19-year-old male, also showed iron deficiency with hypochromic anemia after repeated phlebotomies for study purposes. One month prior to this study, his fasting serum iron level was 36 μg% with UIBC of 170. At the time of the study, the fasting serum iron was 22 μg% with UIBC of 280, the hemoglobin 8.6 g, hematocrit 28%, RBC 3.6 million, reticulocyte count 0.3%, and indices of MCV 77, MCH 24, and MCHC 30. While in a fasting state, he was given 25 ml of Fe^{59} tagged ferric chelate intraduodenally per Rehfuss tube, followed by 75 ml water. This dose was equivalent to 50 mg elemental iron and 10 μc Fe^{59}. On the 12th day, this subject was fasted again and given 50 ml of the Fe^{59} tagged ferric chelate orally, followed by 50 ml water and 50 ml tomato juice. This dose represented twice the iron content of the intraduodenal dose, or 100 mg elemental iron and 20 μc Fe^{59}. Hematologic values at the time of this study revealed a hemoglobin of 8.6 g, hematocrit 30%, RBC 3.66 million, and indices of MCV 81, MCH 23, and MCHC 28. Blood, serum, urine, and stool specimens were collected and counted in a manner similar to that for the first subject.

Results. Surface counting at selected sites after intramuscular injection of 10 μc Fe^{59} as the iron chelate showed that disappearance from the injection site (left hip) was rapid, with little radioactivity detectable 15 minutes later. Counting at this site after 2 to 4 hours was stable and paralleled that over the liver. On the 7th day, counts over both hips were similar, further suggesting that there had been complete uptake from the injection site.

Fig. 1 shows radioactivity of the plasma and incorporation into the RBC of this subject. Maximal uptake by plasma occurred prior to the first collection, drawn 2 hours after injection. There was a gradual loss to the tissues and marrow, and, at 12 to 24 hours the uptake of Fe^{59} by circulating RBC became apparent. Uptake by the RBC was rapid until the 4th to 5th day and reached maximal incorporation at about the 11th to 12th day. Based on the known hematocrit and a blood volume estimated 74 ml/kg, about 73% was present in the circulating RBC after 11 days.

Table I summarizes urinary and stool output of radioactivity in this subject. Little activity was found in the stool over a 4-day period. Most of the excreted Fe^{59} was found in the urine within 5 hours, and this approximated the total amount excreted by both routes over 4 days, or 9% of the injected dose. This amount plus the 73% found in circulating RBC accounted for 82% of the injected dose. The remainder probably was retained in body tissues.

TABLE I. Summary of Urinary and Fecal Output of Radioactivity in Patient T. R. following Intramusc. Injection.

Period, hr	Counts/min.	% inj. dose
	Urine	
0– 5	602,400	8.1
5–12	53,404	.7
12–24	1,125	
24–48	818	
48–72	275	
72–96	627	
Total 4 days	658,649	8.9
	Feces	
4 "	13,016	.1
Total urine & feces	671,665	9.0

TABLE II. Serum Iron and UIBC Levels following Intramuscular Injection of 40 mg Iron in Chelated Form.

Time following inj., hr	Serum Fe, μg %	UIBC, μg %
0	22	248
½	71	228
1	92	182
2	146	112
4	294	0
8	267	0

Since the uptake of this iron chelate was rapid from the intramuscular site, it was of considerable interest to determine the effect on the serum iron levels and UIBC. Table II records these results at various intervals after injection of 40 mg iron as the ferric chelate. Iron determinations by the method employed did not determine iron present in the serum in the chelated form.

Intraduodenal administration of the Fe^{59} tagged chelate in the second subject resulted in little uptake by plasma and RBC. Following administration of 10 μc minimal radioactivity was detected in plasma only at 8 hours, and incorporation into RBC of the small amount absorbed from the duodenum was not obvious until the 6th day. Seventy-two percent of the dose was detected in the 4-day stool collection while only 0.1% was found in the urine over 2 days. From the hematocrit determination and a blood volume estimated at 69 ml/kg, it can be calculated that 2.4% was present in the circulating RBC after 12 days. Twenty-five percent of the administered dose was unaccounted for, but may have remained in the gastrointestinal tract beyond 4 days. Oral administration of a tagged dose twice as large, representing 100 mg iron and 20 μc Fe^{59}, produced similar results. On the 9th day, RBC radioactivity represented 3.4% of the administered dose. Seventy-two percent again was found in the 4-day stool collection and 0.1% in the urine over 3 days.

Discussion. Uptake of the iron chelate from the injection site was rapid, indicating a rapid diffusion into circulating fluids. In the first subject, *in vivo* counting over both buttocks yielded similar results after 7 days, suggesting a complete uptake by that time. Unchelated iron was found in the serum ½ hour after injection and completely saturated the iron binding protein in serum by 4 hours, indicating that chelate breakdown was rapid after its entrance into the circulation. The clinical significance is that iron was available to the marrow within minutes after injection. Incorporation into circulating RBC, undoubtedly reticulocytes, first became obvious at 24 hours, indicating that the available iron was quickly utilized in hemoglobin formation.

Administration of the chelate by oral and intraduodenal routes was not effective. The incorporation of Fe^{59} into circulating RBC indicated an uptake from the gastrointestinal tract similar to that found with administration of ferrous sulfate in normal and iron deficient patients(4), suggesting that in each event the chelate was broken down in the gastrointestinal tract to form an inorganic iron salt. Our subject did not show color changes in his feces, but this might not be expected with the small dose given.

If the intramuscular use of a specific iron chelate is a means of introducing iron into the body, this method may have application in parenteral iron therapy. The intramuscular use of a chelate may also be applicable in other trace metal deficiency states and in the administration of radioactive elements for diagnostic and therapeutic purposes.

Summary. The Fe^{59} tagged ferric chelate of disodium N-hydroxyethylethylenediamine triacetate was administered to 2 subjects made iron-deficient by repeated phlebotomies. Intramuscular injection in one subject resulted in a rapid uptake from the injection site, early incorporation into circulating RBC, and effective utilization of Fe^{59} by the RBC. Oral and intraduodenal administration of the tagged chelate to the second subject showed that iron in this form was not readily absorbed from the gastrointestinal tract.

1. Chaberek, S., Jr., Martell, A. E., *J. Am. Chem. Soc.*, 1955, v77, 1477.

2. Seven, M. J., Gottlieb, H., Israel, H. L., Reinhold, J. G., Rubin, M., *Am. J. Med. Sc.*, 1954, v228, 646.

3. Schade, A. L., Oyama, J., Reinhart, R. W., Miller, J. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 443.

4. Will, J. J., Vilter, R. W., *J. Lab. and Clin. Med.*, 1954, v44, 499.

Received October 22, 1957. P.S.E.B.M., 1958, v97.