protein molecules. This could be either because the binding sites are non specific or else because the several specific binding sites converge onto the same population of P450 molecules.

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Fate of intravenously injected iron compounds: ferric–fructose complex, iron–EDTA, ferric hydroxide and iron–albumin labeled with ^{59}Fe

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Several authors have already studied the mechanism of iron intestinal absorption, especially concerning the effect of sugars and polyols in that absorption[1, 2] and the chelation by EDTA.[3] In order to obtain a more detailed view of the iron fate, a comparative study of the body distribution of four different radioiron labeled compounds has been performed.

The ^{59}Fe-labeled compounds were prepared as follows

(a) *^{59}Fe-ferric hydroxide.* To a 0·01 M $FeCl_3$ solution containing the ^{59}Fe activity as ferric chloride, 0·01 N NaOH was added to bring the pH to 10. Immediately 1 ml of 1% dextrose and 0·1 ml of 6% gelatin solution were added. The colloidal ferric hydroxide solution formed was heated for 10 min in a boiling water-bath and after cooling it down to room temperature it was dialyzed against distilled water until no basic reaction appears in the water.

(b) *^{59}Fe-ferric-fructose complex.* To 0·1 ml of 0·1 M $FeCl_3$ (with the ^{59}Fe activity incorporated) 0·1 ml of concentrated HCl and 2 ml of 10% fructose were added. The mixture was boiled for a few

seconds and neutralized with 1 M $NaCO_3H$. After cooling it was passed through a Dowex 50-X1 (Na form) column and then filtered through filter paper.

(c) *^{59}Fe-EDTA.* 0·1 ml of 0·1 M $FeCl_3$ (with ^{59}Fe activity included) was precipitated as $Fe(OH)_3$ with an excess of NH_4OH. The centrifuged precipitate was washed 3 times with distilled water. Later, 5 mg of ethylenediaminetetraacetic acid disodium salt (in 10 ml of water) was added. After boiling for 15 min, it was neutralized with 1 M $NaCO_3H$ and filtered.

(d) *^{59}Fe-albumin.* The ^{59}Fe activity (as 0·06 mg of Fe in the ferric chloride form) and 0·1 ml of 0·2 M acetate buffer pH 5·1 were added to 100 mg of albumin dissolved in 2 ml of distilled water. After 1 hr of incubation at 37° it was dialyzed against distilled water until no activity was found in the water.

The radiochemical purity of these compounds was assayed in the following way

(a) *^{59}Fe-ferric hydroxide.* By centrifugation at 4000 rpm and counting of the radioactivity in the precipitate and in the supernatant.

(b) *^{59}Fe-ferric-fructose complex by electrophoresis on paper as described by Charley* et al.[3]

(c) *^{59}Fe-EDTA and ^{59}Fe-albumin by electrophoresis on paper using* 0·025 *M phosphate buffer pH* 6·8.

Concerning the lability of the ^{59}Fe tag in these labeled compounds the analysis performed at different times after the preparation, demonstrated a very good stability. The expected more unstable ^{59}Fe-albumin was assyaed by gel filtration on Sephadex G-25 fine and no change has been found even after a month of its preparation.

Groups of adult albino rats (200–250 g) were injected into the tail vein with 50 μC of colloidal ^{59}Fe-ferric hydroxide (0·2 mg), 50 μC of ^{59}Fe-ferric fructose (1·2 mg), 50 μC of ^{59}Fe-EDTA (1·0 mg) and ^{59}Fe-labeled albumin (2·3 mg). After different intervals groups of 3 animals were sacrificed and the activity of the different organs and tissues was counted with a well type scintillometer (Gamma-Guard Tracerlab). Table 1 shows the counting (as the mean value of 3 animals), after 1 day, 7 days and 14 days of the i.v. administration. Fig. 1 shows the whole body counting performed on 3 animals for the four iron compounds essayed. As it can be seen the complexes with fructose and EDTA present a similar behavior, which differs from that of the colloidal hydroxide and the albumin-complex.

In both Table and Fig. 1, the results indicate that, when the radioiron is bound as a complex (chelate), it is readily excreted and little activity is retained by liver, spleen and bone. A similar fast elimination has been observed in this laboratory using radioisotope scanning for ^{68}Ga and ^{51}Cr fructose and EDTA chelates. Contrarely, the ferric hydroxide and ^{59}Fe-labeled albumin remain in greater amounts in liver, spleen, lung and bone. As shown by the experimental results, the ^{59}Fe-albumin activity after the first 24 hr remains with a little change until the end of the experiment (14 days) in liver, lung, spleen and kidney. On the other hand, the ferric hydroxide is slowly eliminated in a significant amount. In bone (femur and tibia) in all the cases the activity decreases considerably after 1 day, but being always higher for ferric hydroxide and Fe-albumin.

The radioactivity incorporated into the blood cells increases with the time when Fe-fructose complex and Fe-albumin were injected. While the opposite occurs with ferric hydroxide and Fe-EDTA. This could be explained as they are not extensively fagocyted or are unable to provide ionic iron.

The prolonged presence of the ferric hydroxide and the Fe-albumin resembles the results already obtained when chromic hydroxide,[4] chromic phosphate[5] and ^{51}Cr-albumin[6] were injected in the same way. Several authors have also reported[7–10] on a similar behavior of various low molecular weight colloids. They found that, when there are very large particles, those which disappear very rapidly from the blood stream ($T_{1/2} \cong 2$ min) are deposited mainly in the liver and spleen. The colloids of smaller particle size are deposited primarily in the bone marrow and spleen, secondarily in the liver.[8] The steady activity observed in the intestine agrees with the already observed excretion of colloidal material through the biliary duct.[5] The little change observed in the iron pool of the organism can be explained by the insolubility or the complex stability of these tested compounds as well as the absence of ionic iron in the tissues which make a significant isotopic exchange unexpected.

The experimental fact that the Fe-albumin remains much longer in the organs of primary incorporation indicates that the iron-protein bond has a great stability or that the complex is split giving an insoluble ferric hydroxide which remains *in situ* without suministrate ionic iron to the body's iron pool.

TABLE 1. DISTRIBUTION OF THE RADIOACTIVITY AT DIFFERENT TIMES (AS PERCENT OF THE INJECTED DOSE)

	1 day				7 days				14 days			
	A	B	C	D	A	B	C	D	A	B	C	D
Lung	2·79	0·14	0·17	2·49	1·36	0·12	0·06	2·01	0·63	0·16	0·02	1·92
Heart	0·36	0·05	0·05	0·40	0·24	0·05	0·01	0·36	0·11	0·06	0·01	0·69
Liver	24·90	2·47	2·96	33·98	15·60	2·03	2·38	32·04	6·06	1·41	2·48	30·31
Stomach	0·65	0·11	0·10	0·73	0·12	0·02	0·01	0·41	0·07	0·03	0·01	0·31
Spleen	10·95	0·91	0·61	6·83	8·06	0·66	0·50	6·36	5·22	0·40	0·51	6·98
Pancreas	0·42	0·06	0·07	0·20	0·34	0·03	0·03	0·38	0·09	0·03	0·01	0·42
Kidney	0·62	0·30	1·54	5·52	0·48	0·21	0·81	6·24	0·35	0·17	0·59	4·27
Intestine	3·10	0·81	1·38	4·55	1·20	0·62	0·42	1·26	0·41	0·50	0·18	1·46
Brain	0·30	0·01	0·02	0·08	0·09	0·03	0·01	0·08	0·03	0·07	0·01	0·09
Testis	0·22	0·04	0·03	0·78	0·26	0·02	0·02	0·81	0·20	0·05	0·02	0·99
Muscles*	0·03	0·01	0·04	0·02	0·02	0·01	0·01	0·01	0·02	<0·01	<0·01	0·04
Bone*	1·21	0·29	0·43	0·87	0·20	0·09	0·06	0·64	0·16	<0·01	<0·01	0·14
Plasma*	0·12	0·01	0·03	0·20	0·06	0·01	0·01	0·09	0·04	<0·01	<0·01	0·04
Blood cells*	4·55	0·46	0·41	2·11	3·69	0·59	0·38	x 3·26	2·88	0·93	0·35	4·21

A: ^{59}Fe-Ferric hydroxide; B: ^{59}Fe-Iron-fructose; C: ^{59}Fe-Iron-EDTA; D: ^{59}Fe-Ferric-albumin.
* As percent of the injected dose/g.

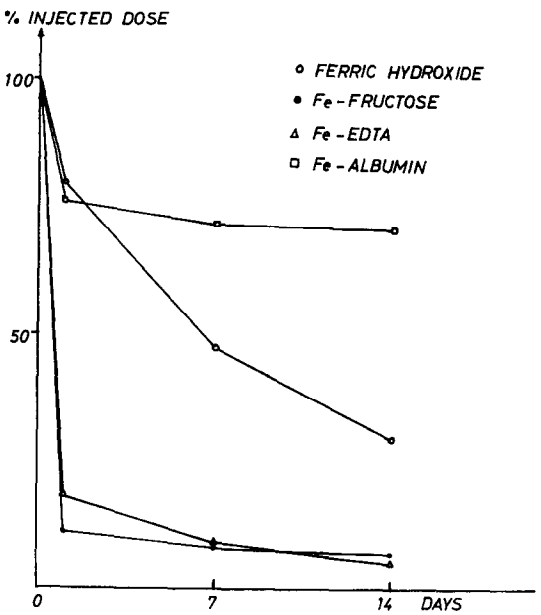

FIG. 1. Whole body radioactivity at different times after the i.v. injection of different ^{59}Fe-labelled compounds.

SUMMARY

The distribution in various organs and tissues of the rat at different times, of i.v. injected ferric hydroxide, Fe-EDTA, Fe-fructose complex and Fe-albumin labeled with ^{59}Fe has been studied.

Fe-EDTA and Fe-fructose complex are readily excreted. On the contrary ferric hydroxide and Fe-albumin remain in liver, spleen, bone and blood cells. After 24 hr, the Fe-albumin radioactivity in the organism has a little change throughout the whole experiment.

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