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An Exploratory Study of Fe^{59}-Labeled Ferrocene As a Carrier in Tracer Techniques[1]

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INTRODUCTION

The number of isotopic compounds generally useful in the field of nuclear medicine is severely limited, particularly those of the organic type. The discovery of ferrocene in 1951 opened up a whole new field of organometallic chemistry (1-5). A large number of compounds involving many different metals has been described. Some classes of these compounds may have potential applications in nuclear medicine. Those of the ferrocene configuration, in particular, present a number of remarkable chemical properties as well as great structural diversity. Accordingly, we have undertaken an investigation of the prototype compound of this class, ferrocene, di-π-cyclopentadienyl iron. Ferrocene was chosen because of its ready availability, its known chemical stability, its low toxicity, and the simplicity of preparing the radioactively labeled compound by neutron activation (6). By a study of the biological distribution of Fe^{59}-labeled ferrocene in rats, we have established that compounds of this type have potential applications in the field of nuclear medicine.

Ferrocene [$(\pi$-$C_5H_5)_2$ Fe] is a crystalline orange solid and has the properties of a typical covalent compound. It is soluble in non-polar organic solvents, may be steam distilled, and can be readily sublimed. Ferrocene is extremely stable and very resistant to chemical attack. In acid media it can be oxidized to a blue, water-soluble ferricinium ion $([\pi\text{-}C_5H_5)_2Fe]^+$. This ion can be reduced by various agents, even by alkali alone. Ferrocene has an aromatic character similar to that of benzene, and will undergo typical substitution reactions yielding various derivatives (1-5). In general, these derivatives undergo most of the reactions of their benzene analogs.

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METHOD

A two gram sample of ferrocene was irradiated for two weeks in the Oak Ridge reactor at a flux of 9×10^{11} n/cm^2/sec. Using the solvent extraction, chromatographic and oxidative techniques described by Sutin and Dodson (6) for the purification of irradiated samples, we obtained a dark blue ferricinium silicotungstate precipitate. Reduction to ferrocene was accomplished by suspending this precipitate in 25 milliliters of water, slowly adding 10 grams of sodium bicarbonate, and then steam distilling at a relatively slow rate. Ferrocene was recovered from the distillate by hexane extraction. The hexane solution was dried with alumina and evaporated *in vacuo*. The yields of ferrocene were 60 to 65 per cent. The specific activity was approximately 14 microcuries per gram two weeks after removal from the reactor.

The labeled ferrocene, dissolved in warm soya bean oil, was administered by stomach tube to thirteen 240-400 gram pentobarbital-anesthetized rats. Twelve of the rats received 1 ml. aliquots of oil solution containing 48 to 55 milligrams of ferrocene. Rat Two received 68 mgm in 2 ml of oil. Due to decay, the iron-59 content of these aliquots varied from 0.3 to 0.9 μc. The animals were kept in glass metabolism cases from one to twelve days, and the feces and urine were collected. Prior to sacrifice, the rats were anesthetized and scanned with a linear scanner, functionally similar to that described by Kyker *et al* (7), in order to locate all major sites of activity. A blood sample was taken from the vena cava, and complete organs and other tissue samples were excised. These were weighed and digested with hot concentrated nitric acid. Intestinal contents and cage feces were similarly treated. Some of the digests contained considerable amounts of fat which was extracted with a one to one hexane-acetone mixture. After diluting the digests and hexane-acetone extracts to known volumes, 5 ml aliquots were prepared and counted. The summation of the total digest counts and the extract counts represented the total counts present in the sample.

The counting system included a 3×3 inch sodium iodide well crystal heavily shielded in all directions, and a pulse height analyzer whose 30 per cent window was centered across the two Fe^{59} peaks. With 5 ml samples the sensitivity of this system was 3×10^5 cts/min per μc of Fe^{59}, and the background averaged 18 counts per minute. Most of the samples were counted for a maximum of fifteen minutes, and although this counting time was too short for the precise Fe^{59} assay of some samples, it was sufficient to establish the relative orders of magnitude.

RESULTS

The total amounts of Fe^{59} in rat tissues and excreta following the gastric administration of ferrocene are tabulated in Table I. Over half of the administered Fe^{59} was recovered in urine; most of this excretion occurred within six days. This activity was not present as ferrocene since it was water-soluble, and was not extractable with non-polar solvents from acid or alkaline urine. About one-third of the activity was readily extractable with diethyl ether.

In the twelve day animals, 7 to 13 per cent of the Fe^{59} was recovered in

TABLE I

THE TOTAL Fe^{59} CONTENT OF RAT TISSUES AND EXCRETA FOLLOWING GASTRIC ADMINISTRATION OF LABELED FERROCENE

Days After Fe^{59} Adm.	Rat No.*	Per Cent of Administered Fe^{59}												
		Cage Urine†	Cage Feces†	Intestinal Contents	Adipose Tissue (Est.)‡	Intestinal Mesentery◊	G. I. Tract	Liver	Kidney	Lung	Muscle (Est.)‡	Whole Blood (Est.)‡	Σ Five Tissues●	Total
1	5	5.1	0.2	5.4	49.1	6.9	2.2	7.5	0.5	0.1	9.9	0.4	0.3	87.6
	8	8.4	1.9	11.3	39.2	5.3	3.4	10.2	0.6	0.1	7.2	0.3	0.4	88.3
2	7	30.7	3.4	5.7	21.4	2.0	2.1	21.7	0.6	0.1	2.9	0.6	0.3	91.5
	9	16.7	1.9	2.6	39.5	3.6	1.7	18.2	0.9	0.2	3.7	0.6	0.3	89.9
3	2	43.3	4.3	2.4	10.6	0.3	1.3	20.3	0.6	0.2	4.8	0.3	0.2	88.6
	3	38.4	4.0	1.0	15.5	1.5	1.8	19.9	0.6	0.3	3.1	···	0.2	86.3
	4	47.5	3.3	1.0	11.8	1.0	1.4	24.7	0.5	0.2	2.7	0.8	0.2	95.1
6	6	60.4	6.3	0.1	0.1	0.1	0.3	19.6	0.3	0.2	0.7	1.5	0.1	89.7
	10	53.3	6.2	0.5	3.8	0.3	0.4	23.4	0.4	0.2	1.2	1.3	0.2	91.2
	11	54.6	7.8	0.8	0.6	0.1	0.2	24.6	0.5	0.2	0.5	1.3	0.2	91.4
12	12	52.4	13.0	0.1	0.3	0.1	0.2	21.8	0.4	0.2	0.7	2.9	0.2	92.3
	14	54.7	7.3	0.1	···	0.1	0.2	19.3	0.4	0.2	1.4	2.7	0.2	86.6
	15	51.4	8.8	0.1	0.2	0.1	0.2	19.4	0.5	0.3	0.7	2.5	0.2	84.4

* Rats 1 and 13 - anaesthetic deaths.

† Cumulative amount excreted in experimental time period.

‡ Estimated amounts calculated from known concentrations and assumed tissue weights of 9, 40 and 6 per cent body weight, respectively, for adipose tissue, muscle, and whole blood.

◊ Includes pancreas and mesenteric fat.

● Spleen, long bones, cardiac muscle, brain and testes.

feces. Most of the activity was excreted in the first six days. Small but significant amounts (1-3%) were excreted in the final six days and activity was also present in the intestinal contents at autopsy.

The relative concentrations of Fe^{59} in various tissues are shown in Table II. Initially, the highest values were observed in adipose tissue; these rapidly decreased to levels of less than one per cent of those originally seen. Some variations in the data can be attributed to differences in fat content, particularly in rats Seven and Ten which were obese. The total amount of Fe^{59} in adipose tissue is estimated in Table I. The presence of ferrocene in fat was qualitatively demonstrated in Rats Five, Eight, and Nine; the samples turned blue when nitric acid was added.

Throughout the experimental period the concentrations of Fe^{59} in the intestinal mesenteric fraction were similar to those of adipose tissue. In gastrointestinal tissue, the concentration and total content of Fe^{59} also decreased with time, but at a much lower level and slower rate. Probably not more than one-tenth of this gastrointestinal tract activity can be attributed to contamination by residual amounts of feces.

In liver, the Fe^{59} concentration increased with time to levels higher than the initial values of fat. After the first day, the total amount of Fe^{59} in liver was relatively constant (Table I), averaging 21 $\pm$ 0.7 per cent of the administered dose. The concentrations of Fe^{59} in liver (Table II) are much more variable due to differences in liver weights. Both the total content and concentration of Fe^{59} in liver are considerably greater than those of other tissues throughout most of the experimental period.

The observed concentrations in leg muscle decreased in the experimental period. Assuming that muscle in the rats amounts to 40 per cent of body weight, these concentrations would represent about 9 per cent of the administered activity at one day and about 1 per cent at twelve days.

Both kidney and lung contained appreciable concentrations of Fe^{59}; however, the total content was quite low (Table I). Although kidney values tended to decrease and those of lung to increase with time, these differences are not statistically significant.

In spite of variations due to relatively large counting errors, the data indicate that the concentrations of Fe^{59} in plasma were consistently low (Table II). The whole blood concentrations of Fe^{59}, however, increased consistently throughout the experiment. Thus, the increased whole blood uptake must have occurred in the red cell fraction. This was confirmed by the radioassay of washed red cells taken from the twelve day rats; the cells contained 98 per cent (range 96-101) of the corresponding whole blood activity. Assuming the blood volume to be 6 per cent body weight, the total Fe^{59} content of blood was estimated (Table I).

The maximum observed plasma value represented one-fourth of the corresponding whole blood activity. Since relatively large amounts of Fe^{59} must be transported to and from the various tissues by blood, the plasma concentrations may appear to be surprisingly low. However, if this transport is a function of some small fraction of plasma, then the Fe^{59} concentration in this fraction could

TABLE II

THE RELATIVE CONCENTRATION OF Fe^{59} IN RAT TISSUES FOLLOWING THE GASTRIC ADMINISTRATION OF LABELED FERROCENE

Days After Fe^{59} Adm.	Rat No.	Cts per min/gram wet weight/10^4 cts per min administered†								
		Adipose Tissue	Intestinal Mesentery‡	G. I. Tract	Liver	Kidney	Lung	Leg Muscle	Whole Blood	Plasma
1	5	176	126	27	81	26	7	8.0	2.0	...
	8	148	105	39	104	34	7	6.1	1.6	0.8
2	7	68	35	22	176	32	9	2.1	3.0	<0.3
	9	141	85	20	185	42	11	3.0	3.0	0.6
3	2	49	11	12	185	28	9	5.1	1.9	0.4
	3	51	31	16	165	27	16	2.3	...	...
	4	38	20	11	238	25	10	1.9	3.8	...
6	6	1	2	3	280	17	10	0.6	8.7	<0.3
	10	12	4	4	207	18	10	0.8	6.0	<0.3
	11	2	1	2	247	20	11	0.3	5.6	<0.3
12	12	1	2	2	166	20	14	0.5	14.3	<0.3
	14	...	6	2	295	27	15	1.5	19.7	<0.3
	15	1	2	2	191	20	19	0.5	12.1	0.4

† The standard deviations of the sample counting rates averaged ± 0.13 concentration units for plasma, and ± 0.7 or less for the remaining one digit values; for all other values the standard deviations did not exceed ± 5 per cent.

‡ Includes pancreas and mesenteric fat.

be very high. For example, if the concentration units in Table II were expressed as activity per gram of lipid instead of activity per gram of tissue, the plasma values would be greater than those of adipose tissue.

The distribution of Fe^{59} was determined in five additional tissues. In the long bones, the concentration of Fe^{59} decreased with time from a maximum of 9 to a minimum of 3 cts per min/gram wet weight/10^4 cts per min administered. The total Fe^{59} content of these bones was quite small, varying from 0.04 to 0.16 per cent of the administered activity. The average concentrations observed in spleen, cardiac muscle, brain and testes were, respectively, 6, 3, 2 and 2 relative units; the individual values did not vary significantly with time. The total Fe^{59} content of these tissues ranged from 0.01 to 0.10 per cent of the administered dose. A summation of the contents of the five tissues is shown in Table I.

The totals of the known or estimated amounts of Fe^{59} in tissues and excreta are compiled in Table I. These totals account for 84 to 95 per cent of the administered Fe^{59}. This range would not be changed significantly by the use of other reasonable estimates of blood and muscle pool size. Different estimates for the size of the adipose tissue pool, however, would significantly change the totals for the early time periods. For example, if the adipose tissue pool were 6 per cent of body weight, the total Fe^{59} values for the first two days would range from 71 to 84 per cent; for a fat content of 12 per cent body weight, these one and two day totals would vary from 99 to 104 per cent of the administered activity. By estimating the fat pool at 9 per cent body weight, reasonably constant total Fe^{59} values were obtained throughout the time course of the experiment (Table I). This 9 per cent pool does not include fat in the other tissues selected for assay. The intestinal mesentery fraction, for example, included the mesenteric fat which, in many rats amounts to at least 1 per cent body weight.

DISCUSSION

Although it would be of general interest to have more knowledge of the state of Fe^{59} following absorption of ferrocene, the relative lack of such information does not, of course, affect its utility as a tracer. The stability of ferrocene in hydrochloric acid or in alkaline media, its relatively rapid absorption from the gastrointestinal tract, and the qualitative and quantitative observations in fat suggest that ferrocene is absorbed intact, probably as a result of its fat solubility. The observed distribution of this Fe^{59} is qiute different from that observed after the administration of inorganic iron-59, even when due allowance is made for the fact that the distribution of inorganic iron is known to vary widely in various physiological and disease states (8, 9, 10). In these animals, the distribution pattern of ferrocene Fe^{59} was remarkably consistent throughout the time course of the experiment, indicating that all of the rats were either in comparable, presumably normal, physiological states; or, if differences existed, these had little or no effect upon the ultimate distribution pattern of the tracer. The urinary excretion of large amounts of activity, following the administration of ferrocene Fe^{59} is totally different from that observed following inorganic Fe^{59} administra-

tion where only traces of activity can be detected in urine. The very low uptakes of activity by spleen, bone and red cells indicate that Fe^{59} was not readily available to the hemopoetic organs, although it is apparent that for several days following the administration of ferrocene, considerable amounts of activity were transported to and from the various tissues. Thus, it appears quite probable that during this transport process the bonding between the iron atom and at least one of the cyclopentadienyl rings in ferrocene remains intact, and that little, if any, inorganic iron is liberated. It is conceivable, however, that the slow but very consistent uptake by red cells may represent some liberation of Fe^{59} from its parent molecule. The obvious source of such activity is the liver, which, after the first few days, contains the only appreciable amount of Fe^{59} remaining in the animals. It is apparent, however, that if any considerable quantity of Fe^{59} is liberated from the cyclopentadienyl rings, it must be retained by the liver in a non-labile form, and, therefore, is not readily available for isotopic exchange with hemopoetic iron.

The urinary excretion of Fe^{59} in a water-soluble form indicates that ferrocene is transformed to other compounds in the rat. Possible transformations that are consistent with this excretion would include oxidation of ferrocene to a ferricinium ion, or metabolic transformations similar to that of its analog, benzene (*i.e.*, oxidation to phenol and subsequent conjugation with sulfuric or glucuronic acids). Some active excretion of ferrocene Fe^{59} into the gastrointestinal tract apparently also occurs. It is highly improbable that the radioactivity found in the feces as late as the twelfth day could represent ferrocene that had not been absorbed.

The ultimate concentration of 21 per cent of the administered ferrocene Fe^{59} activity in liver with little activity remaining in the other tissues is highly suggestive of potential application for compounds of this type in scanning and in other areas of medicine. For example, chemically similar, stable dicyclopentadienyl compounds can be prepared in high specific activity from the iron homologs, ruthenium and osmium, incorporating osmium-191, ruthenium-103, or even the 70 hour half-life ruthenium-97. Diindenyl cobalt-57 is another possibility. These isotopes have gamma ray spectra suitable for scanning. Further studies of the distribution of ferrocene Fe^{59} in liver, fat and urine should be undertaken to determine if these vary significantly in various pathological conditions. It is quite possible that such studies may lead to diagnostic procedures involving a simple radioassay of urinary Fe^{59} activity. Potential applications of ferrocene derivatives, which are analogs of known metabolites, should not be ignored. For example, if it could be shown that monocarboxy ferrocene is conjugated and excreted in a manner similar to its analog, benzoic acid, it might provide the basis of a liver function test. The use of an Fe^{59} label would overcome many objectionable variables (11) in the classic benzoic acid liver function test.

Essentially all of the transitional metals form organometallic bonds similar to those of ferrocene. Other metals in the periodic table form less stable metal-carbon bonds. Since many of these metals have useful isotopes, some of these organometallic compounds may serve as potential carriers of tracer activity.

The biological response to these compounds is unknown. It is hoped that this exploratory attempt to evaluate the potential application of one such compound will stimulate the interest of other investigators in the field of nuclear medicine.

SUMMARY

The distribution of Fe^{59} in the rat following gastric administration of labeled ferrocene has been studied. Within twelve days, over half of the administered activity was recovered in urine, and about 10 per cent in feces. Fat initially contained the highest concentrations of Fe^{59}, but these rapidly decreased to levels of less than one per cent of those originally seen. Liver activity increased for two days to a concentration higher than that observed in fat; thereafter, liver contained a relatively constant 21 per cent of the administered activity. The Fe^{59} concentration in whole blood increased throughout the experimental period, while that of the plasma rapidly decreased to essentially zero values. However, at the end of twelve days the total Fe^{59} content of whole blood was still less than 3 per cent of the administered activity.

These data suggest that labeled compounds of the ferrocene type have potential applications in the field of nuclear medicine.

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