2. The administration of ascorbate to vitamin A-deficient rats led to a lowered synthesis of ubiquinones in the livers compared with that in the livers of ascorbate-deprived vitamin A-deficient animals. The incorporation of [2-¹⁴C]mevalonate into the sterols was higher and that into the squalene and ubiquinone fractions lower in the ascorbate-supplemented vitamin A-deficient animals, when compared with those in the ascorbate-deprived vitamin A-deficient rats.

3. Liver preparations *in vitro* from ascorbate-supplemented vitamin A-deficient rats showed increased incorporation of [2-¹⁴C]mevalonate into the unsaponifiable materials, with greater radioactivity in the sterol fraction than in the squalene fraction, as compared with the ascorbate-deprived vitamin A-deficient rats. The addition of vitamin A alcohol or acid or ascorbate had no effect.

P.M. is a Lady Tata memorial scholar.

REFERENCES

- Axelrod, J., Udenfriend, S. & Brodie, B. B. (1954). *J. Pharmacol.* **111**, 176.
- Bentley, R., Ramsey, V. G., Springer, C. M., Dialameh, G. H. & Olson, R. E. (1961). *Biochem. biophys. Res. Commun.* **5**, 443.
- Bucher, N. L. R. & McGarrah, K. (1956). *J. biol. Chem.* **222**, 1.
- Cooper, J. R. (1961). *Ann. N.Y. Acad. Sci.* **92**, 208.
- Crane, F. D., Lester, R. L., Widmer, C. & Hatefi, Y. (1959). *Biochim. biophys. Acta*, **32**, 73.
- Gloor, U., Schindler, O. & Wiss, O. (1960). *Helv. chim. acta*, **43**, 2089.
- Gloor, U. & Wiss, O. (1958). *Experientia*, **24**, 410.
- Gloor, U. & Wiss, O. (1959a). *Biochem. biophys. Res. Commun.* **1**, 182.
- Gloor, U. & Wiss, O. (1959b). *Arch. Biochem. Biophys.* **83**, 216.
- Gornall, A. G., Bardawill, C. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
- Hofmann, H. & Staudinger, H. (1951). *Arzneim. Forsch.* **1**, 416.
- Johnson, B. C. & Wolf, G. (1960). *Vitam. & Horm.* **18**, 457.
- Joshi, V. C., Jayaraman, J. & Ramasarma, T. (1963). *Indian J. exp. Biol.* **1**, 113.
- Kahnt, F. W. & Wettstein, A. (1951). *Helv. chim. acta*, **34**, 1790.
- Kernsten, H., Leonhauser, S. & Staudinger, H. (1958). *Biochim. biophys. Acta*, **29**, 350.
- Knox, W. E. & Goswami, M. N. D. (1961). *Ann. N.Y. Acad. Sci.* **92**, 192.
- Levin, E. Y., Levenberg, B. & Kaufman, S. (1960). *J. biol. Chem.* **235**, 2080.
- Lowe, J. S., Morton, R. A., Cunningham, N. F. & Vernon, J. (1957). *Biochem. J.* **67**, 215.
- Lowe, J. S., Morton, R. A. & Harrison, R. G. (1953). *Nature, Lond.*, **172**, 716.
- Lynen, F. (1961). *Ciba Found. Symp.: Quinones in Electron Transport*, p. 233. Ed. by Wolstenholme, G. E. W. & O'Connor, C. M. London: J. and A. Churchill Ltd.
- Lynen, F., Eggerer, H., Henning, U. & Kessel, I. (1958). *Angew. Chem.* **70**, 738.
- Malathi, P. & Ganguly, J. (1964). *Biochem. J.* **92**, 521.
- Malathi, P., Seshadri Sastry, P. & Ganguly, J. (1961). *Nature, Lond.*, **189**, 660.
- Malathi, P., Subba Rao, K., Seshadri Sastry, P. & Ganguly, J. (1963). *Biochem. J.* **87**, 305.
- Morton, R. A. & Phillips, W. E. J. (1959). *Biochem. J.* **73**, 416.
- Olson, R. E., Dialameh, G. H. & Bentley, R. (1961). *Ciba Found. Symp.: Quinones in Electron Transport*, p. 284. Ed. by Wolstenholme, G. E. W. & O'Connor, C. M. London: J. and A. Churchill Ltd.
- Phillips, W. E. J. (1961). *Canad. J. Biochem. Physiol.* **39**, 855.
- Ramasarma, T. (1961). *J. sci. industr. Res.* **20A**, 492.
- Staudinger, H. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, vol. 4, p. 74.
- Staudinger, H., Krisch, K. & Leonhauser, S. (1961). *Ann. N.Y. Acad. Sci.* **92**, 195.
- Stoffel, W. & Martius, C. (1960). *Biochem. Z.* **333**, 440.
- Stone, N. & Meister, A. (1962). *Nature, Lond.*, **194**, 555.
- Weber, F., Gloor, U. & Wiss, O. (1960). *Helv. physiol. acta*, **18**, C97.
- Wiss, O. & Gloor, U. (1960). *Vitam. & Horm.* **18**, 485.

Biochem. J. (1964), **92**, 531

The Distribution of ⁵⁹Fe in Albino Rats after Intravenous Administration in the Ionic or Chelated Form

By B. NAGARAJAN, V. M. SIVARAMAKRISHNAN AND S. BRAHMANANDAM*
Isotope Division, Cancer Institute, Madras 20, India

(Received 28 January 1964)

Our previous investigations have shown that the simultaneous administration of chelating agents can profoundly alter the tissue distribution of ⁶⁰Co

* Present address: The Chemical Testing and Analytical Laboratory, Madras 32, India.

in albino rats (Sivaramakrishnan, Sreenivasan, Sekhara Varma & Brahmanandam, 1962a). In an attempt to explain the changes produced we have studied the exact state in which the cobalt chelate exists in the blood after intravenous administra-

tion (Sivaramakrishnan, Sekhara Varma & Lakshmanan, 1961) and, also, through time-distribution studies, the movement of the tracer among the various tissues after it leaves the vascular compartment (Sivaramakrishnan, Sekhara Varma, Brahmanandam & Nagarajan, 1962*b*). We have carried out similar investigations with ^{65}Zn and ^{59}Fe . In this paper we present our results on the tissue distribution of ^{59}Fe at various times after intravenous administration and the effects of some chelating agents and of inert carrier iron on such distribution. Because of its biological importance, the distribution and metabolism of iron in laboratory animals and in man have been fairly extensively studied, notably by Hahn and Whipple (reviewed by Hahn, 1948), Huff & Judd (1956), Lamerton, Belcher & Harris (1959) and Beutler (1961); but there are few reports on the tissue distribution of iron chelates in laboratory animals. Rubin & Princiotto (1960) have observed that ^{59}Fe , administered as the chelate with ethylenediaminetetra-acetic acid, its hydroxy analogue or diethylenetriaminepenta-acetic acid, is largely excreted through urine in a few hours. Details of the movement of these chelates in the body are not available.

MATERIALS AND METHODS

Radioactive iron (^{59}Fe), obtained from the Oak Ridge National Laboratory, U.S.A., was a processed preparation of high specific activity (over 11.5 c/g.). It was as a solution of FeCl_3 in 1.2*N*-HCl, but when suitably diluted with physiological salt solution, so that 0.5 ml. of the solution used for each injection contained about 5 μC , at pH 4–4.6 it was well tolerated by the rat. The dilution was usually carried out just before administration to a series of rats. No citrate was used, as citrate by itself is a complex-forming agent. The actual amount of iron injected was less than 1 $\mu\text{g.}/\text{rat}$.

The tissue distribution of ^{59}Fe at various times after intravenous administration was studied. Adult male Wistar rats, weighing 200 ± 20 g., divided into groups of five, were used. They were about 5–6 months old but not litter mates. The procedure followed was more or less the same as for ^{60}Co (Sivaramakrishnan *et al.* 1962*a, b*), except that before the various organs were dissected out the blood was completely removed by viviperfusion with Krebs–Ringer solution. Perfusion of the animals was necessary because blood contained a high percentage of the injected activity and contamination of any other tissue with blood could lead to erroneous results. Perfusion was accomplished by anaesthetizing the animal with ether and slowly injecting Krebs–Ringer solution through one of the tail veins while simultaneously withdrawing blood from the heart with a hypodermic syringe at about the same rate until the perfusate was nearly colourless. After separating the various tissues and digesting them with HNO_3 , radioassays were carried out as before with a well-type scintillation counter. As the scaler and the counter used (both from Ekco Electronics Ltd., England) had a resolution time of less than 1 $\mu\text{sec.}$ and as sufficient counts were taken for each

sample the error involved in the counting was less than 2%. Recoveries varied from 90 to 110%.

The influence of the chelating agents sodium diethyldithiocarbamate, 1-nitroso-2-naphthol and EDTA, and also of inert carrier iron administered at various concentrations, was investigated by a similar procedure. In all these experiments each rat was injected with the same amount of the tracer solution (0.5 ml.) plus a known volume of the chelating agent (0.3–1.0 ml., 10 mM solution) or the carrier solution (100–600 $\mu\text{g.}$ of iron as partially neutralized FeCl_3 solution). The rats were killed 15 min. after injection and the tissues analysed as before. More than 600 $\mu\text{g.}$ of iron was found to be toxic.

The tissue distribution of ^{59}Fe administered with 500 $\mu\text{g.}$ of inert carrier iron at various times after administration has been investigated. The procedure followed is similar to that for studying the time-distribution of the tracer alone and consists, in brief, of administering a mixture of 0.5 ml. of ^{59}Fe solution and 0.5 ml. of carrier iron (partially-neutralized FeCl_3 solution, containing 500 $\mu\text{g.}$ of iron) to each rat, killing the various groups of rats at specified times and analysing the various tissues for radioactivity. By a similar procedure, with 0.5 ml. of 10 mM solution of diethyldithiocarbamate or EDTA instead of the carrier iron solution, the distribution of ^{59}Fe administered as the diethyldithiocarbamate or EDTA chelate was studied.

As blood contained a large percentage of the injected activity, the distribution of radioactivity in the blood between the plasma and the cells at various times was studied after administering to different groups of rats the tracer alone or with inert carrier iron or with diethyldithiocarbamate. At specific times, from 5 min. to 10 days, about 1 ml. of blood was withdrawn from each rat by cardiac puncture into a heparinized syringe. The plasma and the cells were separated by centrifugation at 1500 rev./min. for 15 min. and the radioactivity in the two fractions was determined as usual. As cardiac removal of blood has been reported to stimulate erythropoiesis (Lamerton *et al.* 1959) blood was withdrawn from any particular rat only once. The stability of the binding of the tracer in the cells was investigated through dialysis. For this purpose, cells obtained from 5 ml. of blood by the above procedure were washed twice with 0.9% NaCl, suspended in about 3 ml. of 0.9% NaCl and transferred into a previously prepared cellulose dialysis tubing (supplied by Arthur H. Thomas and Co., U.S.A.). It was then dialysed against 100 ml. of 0.9% NaCl in a tall beaker in the cold (0–5°) for 72 hr., the saline being changed every 24 hr. The radioactivity in the diffusate and the final residue were determined as usual.

RESULTS

Data for the distribution of ^{59}Fe with time are presented in Table 1. It will be observed that during the first few minutes most of the radioactivity is present in the blood, but as time progresses the activity in the blood decreases and reaches a minimum between the fifth and tenth hours. Simultaneously, there is a rapid build-up of radioactivity in the bones, which attains a maximum of over 60% between the third and fifth hours. After this time the activity in the bones falls off whereas that of the blood increases progressively, so that by the

third day the activities in the blood and the bones return approximately to their original values at 10 min. after injection. It is thus obvious that there is a rapid passage of ^{59}Fe from the blood into the bones initially and a subsequent gradual return to the blood. This transfer involves over 60 % of the administered isotope, so that this process seems to be important in iron metabolism. At all times studied the blood and the bones account for most of the radioactivity. The changes observed in the radioactivity in the spleen and the muscles with time are similar to those with the bones, but at considerably lower values. The activity in the liver remains fairly constant throughout the whole period, except for a small rise towards the end. The excretion is negligible initially, but becomes appreciable after 24 hr. and, by the eighth day, about one-fifth of the injected activity has been excreted.

In Table 2 are presented results on the effects of the simultaneous administration of different amounts of carrier iron along with the tracer on the distribution of ^{59}Fe 15 min. later. The main changes observed are a large rise in the amount of radioactivity in the liver and a fall in the activity in the blood, the changes being more pronounced during the initial stages of increasing the metal concentration. The excess of iron administered is thus deposit-

ed from the blood in the liver. This is even more clearly brought out from the results given in Table 3. It will be observed that when ^{59}Fe is administered along with 500 μg . of inert carrier iron there is a large transfer of radioactivity from the blood to the liver initially. The maximum deposition in the liver occurs within 24 hr. after the injection, with a corresponding minimum value in the blood. The activity in the liver then falls slowly while that of blood increases gradually. This could imply an initial storage of excess of iron in the liver followed by a gradual mobilization later. Even at the end of 10 days the liver retains about 40 % of the injected activity. The activity in the bones ranges between 10 and 15 % and there is comparatively very little fluctuation between 10 min. and 6 days. This is in sharp contrast with the behaviour with the tracer alone, when the activity in the bones reaches a maximum of over 60 % in a few hours, followed by a gradual fall.

The distribution of ^{59}Fe is considerably altered by the chelating agents 1-nitroso-2-naphthol, diethyldithiocarbamate and EDTA, the changes being more pronounced at the higher concentrations tested (Table 4). Diethyldithiocarbamate causes a large deposition of radioactivity in the liver, the activity in the blood being correspondingly decreased. 1-Nitroso-2-naphthol has a similar

Table 1. *Distribution of ^{59}Fe at various times after its intravenous administration.*

Each value, expressed as a percentage of injected activity, is the mean for five rats. Standard deviations for the values of blood and bones are also given.

	5 min.	10 min.	15 min.	30 min.	1 hr.	3 hr.	5 hr.	8 hr.	10 hr.	16 hr.	1 day	3 days	8 days
Blood	77.8 ± 3.6	62.8 ± 5.8	58.1 ± 3.4	49.5 ± 3.3	45.1 ± 4.1	19.8 ± 1.6	15.8 ± 1.3	23.3 ± 2.7	17.3 ± 2.1	35.0 ± 1.9	43.4 ± 2.9	58.4 ± 3.1	60.9 ± 4.2
Liver	4.9	6.8	5.6	4.8	6.0	5.6	6.1	6.4	7.9	5.9	7.5	7.2	8.9
Bones	15.1 ± 2.0	18.2 ± 1.2	21.1 ± 1.3	31.3 ± 2.2	37.8 ± 3.0	61.6 ± 4.2	56.4 ± 3.2	51.0 ± 3.7	47.2 ± 3.4	43.7 ± 2.9	32.0 ± 4.1	16.2 ± 2.8	9.6 ± 2.3
Spleen	0.4	0.8	0.7	1.5	1.2	3.0	2.5	6.2	4.8	2.9	3.3	1.7	1.3
Muscles	0.0	0.0	0.3	1.8	2.0	7.3	5.6	4.8	4.0	3.0	2.5	0.1	1.3
Skin	4.2	3.7	3.9	3.6	3.6	5.7	5.0	4.0	4.4	4.4	5.5	6.0	5.9
Gastrointestinal tract	1.1	2.0	2.0	2.1	3.3	5.5	5.1	4.9	6.4	6.2	9.4	2.3	1.8
Kidneys	0.9	1.9	1.5	1.1	1.0	0.8	0.8	0.7	1.2	0.8	1.3	1.1	1.3
Lungs	1.7	2.1	2.1	2.1	1.8	1.5	1.6	0.6	0.9	0.6	1.4	0.9	0.6
Excretion	0.0	0.0	0.3	0.2	0.3	0.5	1.2	0.9	0.8	3.0	3.0	8.7	22.9

Table 2. *Influence of inert carrier iron on the distribution of ^{59}Fe after 15 min.*

Values are expressed as percentages of injected activity. Each value is the mean for five rats. Standard deviations for the values of blood, bones and liver are also given.

Tissue	Control (tracer only)	Tracer + 100 μg . of carrier	Tracer + 200 μg . of carrier	Tracer + 400 μg . of carrier	Tracer + 600 μg . of carrier
Blood	65.1 $\pm$ 4.9	45.5 $\pm$ 5.1	29.5 $\pm$ 1.7	34.4 $\pm$ 3.8	42.3 $\pm$ 2.7
Liver	5.1 $\pm$ 1.1	29.9 $\pm$ 2.2	37.9 $\pm$ 3.0	38.9 $\pm$ 4.2	35.3 $\pm$ 4.1
Bones	22.1 $\pm$ 3.5	10.5 $\pm$ 2.5	14.4 $\pm$ 1.7	12.0 $\pm$ 2.7	10.3 $\pm$ 2.5
Spleen	1.3	1.1	1.4	1.9	2.1
Skin	3.6	3.8	5.5	6.5	5.5
Gastrointestinal tract	2.4	2.2	2.7	2.3	3.8
Excretion	0.4	0.3	0.2	0.5	0.4

effect on the distribution of ^{59}Fe , but to a lesser degree. On the other hand, with EDTA, an entirely different pattern of distribution is encountered. EDTA causes an increased deposition of radioactivity in the skin, a negligibly low concentration in the liver, a low concentration in the blood and a large increase of activity in the excretion. The distribution of iron is thus susceptible to wide alterations by different chelating agents.

The movement of ^{59}Fe in the body, after administration as the diethyldithiocarbamate chelate, can be inferred from the results presented in Table 5. Initially there is a large deposition of radioactivity in the liver, over 75 % in 5 min. The activity then falls gradually, but even at the end of 6 days an appreciable amount is retained in the liver. The activity in the blood fluctuates considerably during the first few hours, but increases steadily later on, about half of the injected activity being present in the blood at the end of 6 days. It is thus obvious that considerable amounts of ^{59}Fe originally deposited in the liver have moved into the blood

during this period. The activity in the bones is low in the beginning, increases gradually to attain a maximum at about the fifth hour and then gradually falls off. The changes observed are qualitatively similar to those with ^{59}Fe alone, but at considerably lower values.

From Table 6 it will be observed that ^{59}Fe administered along with EDTA is rapidly removed from the blood stream and is deposited mainly in the bones, the muscles and the skin, but not in the liver. The activity in the liver is negligible, being less than 1 % at all times after 15 min. The activity in the blood continues to fall till it reaches a minimum at about 5 hr. after administration. During this period, the skin, the bones and the muscles account for most of the radioactivity in the body. A rapid excretion of the isotope is observed initially, more than half of the injected activity being excreted during the first hour. But subsequently the rate of excretion slows down so much that only 10 % more of the activity is excreted during the next 71 hr.

Table 3. *Distribution of ^{59}Fe at various times after its administration with 500 μg . of inert carrier iron*

Each value, expressed as a percentage of injected activity, is the mean for five rats. Standard deviations for the values of blood, liver and bones are also given.

	5 min.	10 min.	15 min.	30 min.	1 hr.	3 hr.	5 hr.	16 hr.	3 days	6 days	8 days	10 days
Blood	58.7 ± 4.9	57.7 ± 5.1	43.5 ± 4.1	45.6 ± 4.3	32.0 ± 2.5	14.8 ± 1.5	7.5 ± 1.2	5.1 ± 1.0	16.0 ± 1.8	19.6 ± 1.3	25.4 ± 2.4	24.7 ± 2.6
Liver	17.4 ± 1.0	18.1 ± 0.9	25.7 ± 2.2	29.0 ± 3.2	38.2 ± 4.1	54.4 ± 3.2	57.2 ± 1.2	55.8 ± 5.6	56.4 ± 3.4	48.1 ± 2.8	38.9 ± 2.3	41.5 ± 4.7
Bones	8.6 ± 0.8	14.3 ± 1.0	11.3 ± 1.9	13.0 ± 1.5	13.2 ± 1.3	13.7 ± 2.6	16.3 ± 1.4	14.5 ± 2.0	12.2 ± 2.5	11.7 ± 1.3	2.6 ± 0.9	7.2 ± 2.3
Spleen	0.6	0.8	1.5	1.8	2.8	2.7	3.0	2.2	3.0	2.6	4.1	2.7
Muscles	0.0	1.8	5.1	1.8	4.1	5.1	6.0	5.1	2.3	3.7	3.9	5.3
Skin	4.2	3.1	5.8	7.2	5.6	4.7	5.4	4.8	3.5	5.6	3.5	5.3
Gastrointestinal tract	1.8	1.8	2.7	3.6	3.6	4.2	3.9	6.1	7.2	2.7	2.6	2.7
Kidneys	1.3	1.6	1.5	1.8	1.2	1.5	2.1	1.8	1.5	1.2	1.4	1.6
Lungs	1.2	1.9	1.2	1.8	0.9	0.9	0.6	2.1	0.7	0.5	0.8	0.8
Excretion	0.0	0.0	0.1	0.2	0.0	0.0	0.6	3.2	7.8	13.1	16.0	19.7

Table 4. *Influence of some chelating agents on the distribution of ^{59}Fe after 15 min.*

Each value, expressed as a percentage of injected activity, is the mean for five rats. Standard deviations for the values of blood, liver and bones are also given.

	Control (tracer only)	Sodium diethyldithio- carbamate		1-Nitroso-2-naphthol		EDTA	
		0.3 ml.	1.0 ml.	0.3 ml.	0.8 ml.	0.3 ml.	0.8 ml.
Blood	58.1 $\pm$ 4.3	26.9 $\pm$ 3.1	7.1 $\pm$ 2.4	61.3 $\pm$ 4.9	31.1 $\pm$ 4.2	17.1 $\pm$ 2.5	11.1 $\pm$ 3.0
Liver	5.6 $\pm$ 1.4	57.7 $\pm$ 6.6	72.6 $\pm$ 7.1	23.3 $\pm$ 3.4	43.0 $\pm$ 3.8	1.0 $\pm$ 0.3	0.8 $\pm$ 0.4
Bones	21.1 $\pm$ 1.3	6.0 $\pm$ 1.0	4.5 $\pm$ 2.0	12.0 $\pm$ 2.5	10.4 $\pm$ 2.6	9.2 $\pm$ 2.3	13.4 $\pm$ 1.5
Spleen	0.7	1.6	1.8	0.5	0.5	0.1	0.1
Muscles	0.3	2.0	1.3	0.0	8.9	13.1	14.7
Skin	3.9	2.0	16.7	3.4	2.1	21.3	18.2
Gastrointestinal tract	2.0	1.2	0.4	3.3	3.0	2.2	3.2
Kidneys	1.5	0.3	0.4	1.2	1.7	2.6	5.9
Lungs	2.1	1.6	3.9	0.9	0.6	0.8	0.8
Excretion	0.3	0.2	0.1	0.6	0.6	34.5	28.7

Table 5. *Distribution of ^{59}Fe at various times after administration as the sodium diethyldithiocarbamate chelate*

Each value, expressed as a percentage of injected activity, is the mean for five rats. Standard deviations for the values of blood, liver and bones are also given.

	5 min.	10 min.	15 min.	30 min.	1 hr.	3 hr.	5 hr.	16 hr.	1 day	3 days	6 days
Blood	8.1 ± 3.7	32.6 ± 4.5	15.0 ± 2.5	33.3 ± 5.3	25.0 ± 4.7	12.0 ± 2.2	9.4 ± 3.1	18.1 ± 2.6	25.7 ± 1.8	32.3 ± 1.8	49.4 ± 3.3
Liver	77.5 ± 2.7	44.8 ± 6.2	56.2 ± 5.1	31.4 ± 4.0	48.6 ± 2.2	51.7 ± 2.3	31.5 ± 1.5	23.6 ± 1.9	28.6 ± 2.1	22.9 ± 3.1	15.7 ± 2.9
Bones	2.4 ± 0.8	9.0 ± 2.2	7.9 ± 3.8	17.6 ± 1.4	15.0 ± 3.2	28.7 ± 2.3	23.8 ± 3.6	29.0 ± 3.5	18.6 ± 2.0	15.1 ± 2.9	13.0 ± 1.1
Spleen	4.0	2.6	2.6	1.8	3.2	2.8	4.9	3.6	3.3	3.6	3.0
Muscles	0.0	1.7	6.2	0.0	3.8	1.8	4.3	3.3	1.4	1.2	1.6
Skin	0.6	2.6	1.7	1.6	2.3	1.8	2.3	6.0	4.2	3.1	5.0
Gastrointestinal tract	0.0	1.8	4.8	1.6	1.8	2.8	7.2	6.0	5.0	3.0	1.6
Kidneys	0.0	0.9	0.6	1.0	1.6	1.2	0.8	1.2	1.0	0.6	1.2
Lungs	1.0	2.6	1.5	3.3	1.8	3.6	2.6	1.2	1.9	2.3	1.4
Excretion	0.0	0.4	0.8	0.6	0.5	0.6	1.6	1.6	3.8	16.7	17.6

Table 6. *Distribution of ^{59}Fe at various times after intravenous administration as the EDTA chelate*

Values are expressed as the percentages of injected activity. Each value is the mean for five rats.

	5 min.	10 min.	15 min.	30 min.	1 hr.	3 hr.	5 hr.	17 hr.	1 day	3 days
Blood	17.7	10.8	11.5	9.1	5.4	3.3	2.0	3.8	4.4	8.0
Liver	1.6	1.2	1.2	0.8	0.4	0.8	0.5	0.9	0.9	0.9
Bones	23.3	21.5	20.5	15.6	7.4	12.7	12.6	6.0	7.4	6.2
Spleen	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.4	0.4	0.2
Skin	15.7	13.7	14.7	13.5	6.8	7.1	6.8	8.3	5.4	4.1
Muscles	27.3	23.7	11.2	13.3	5.3	6.8	10.7	4.2	4.2	2.7
Gastrointestinal tract	5.5	3.0	3.1	3.3	2.6	12.1	2.1	12.5	1.9	1.9
Kidneys	3.4	4.8	5.8	2.6	1.8	1.2	0.9	0.9	0.9	0.9
Lungs	0.7	0.7	0.8	0.4	0.1	0.1	0.1	0.0	0.2	0.2
Excretion	0.5	16.8	30.3	42.3	60.6	59.0	62.9	65.2	67.4	70.0

Table 7. *Relative distribution of ^{59}Fe in the plasma and the cells at different times after intravenous administration, either in the ionic form, with or without 500 μg . of carrier, or as diethyldithiocarbamate chelate*

Values are expressed as percentages of total activity in blood.

Time	Control (tracer alone)		Carrier added		Diethyldithiocarbamate chelate	
	Plasma	Cells	Plasma	Cells	Plasma	Cells
5 min.	85.1	14.9	89.2	10.8	71.1	28.9
3 hr.	50.0	50.0	37.0	63.0	44.5	55.5
24 hr.	1.0	99.0	4.0	96.0	3.3	96.7
10 days	0.1	99.9	0.2	99.8	0.1	99.9

The distribution of radioactivity in the blood between the cells and the plasma at various times after administration of ^{59}Fe , alone or with carrier iron or with diethyldithiocarbamate, is shown in Table 7. It will be observed that in all these cases, whereas most of the activity is present in the plasma initially, as is to be expected, the proportion in the cells rapidly increases in the first few hours and at the end of 24 hr. over 95 % of the activity is present in the cells. After a few days the cells alone are radioactive. It is obvious from these results that ^{59}Fe , initially transported mainly by the plasma, is rapidly incorporated into the cells. The binding in

the cells seems to be firm, for even after prolonged dialysis for 72 hr. less than 1 % of the radioactivity has been removed (185 out of 19 500 counts/sec. in one experiment).

DISCUSSION

In these studies, the actual amount of iron in the ^{59}Fe administered to any rat is less than 1 μg . and is thus truly at the tracer level. As such, the changes observed in the distribution of iron at various times with the help of this isotope could reasonably be assumed to depict normal physiological processes

occurring in this organism. This would imply that the ingested iron absorbed in the blood (from the gut) in the rat passes into the bones and returns to the blood to the extent and at the rates observed with this isotope. This also suggests that normally the bones are an important tissue in iron metabolism, second only to the blood quantitatively. We have not determined the activity in the bone marrow and the bone tissue separately in these experiments, but it is of interest to examine the available evidence concerning the relative roles of these bone components in iron metabolism. The importance of the bone marrow in iron metabolism was early recognized by Copp & Greenberg (1946*b*), who observed the highest specific activity to be in the bone marrow. An initial accumulation of nearly 60 % of intravenously administered ^{59}Fe in the bone marrow in adult rats has been noted by Huff *et al.* (1950). Past (1963) has demonstrated through radioautography the uptake of ^{59}Fe by osseous tissue in rat fetuses and in suckling rats at a stage of development at which there was new bone but little or no marrow. He has also observed that, when once the bone marrow is formed: 'the strongest reaction of all occurred in the well-developed bone marrow'.

These observations seem to confirm the vital role of bone marrow in iron metabolism, at the same time indicating appreciable uptake by the bone tissue also, at least in young animals. The values obtained by us for the excretion of the isotope, whether administered alone or with the carrier, are considerably higher than those of Copp & Greenberg (1946*a*), who noted an excretion of only 1.5 % in 96 hr. The reason for this discrepancy is not known; it may be due to the presence of citrate in the radioactive preparations used by Copp & Greenberg. Our observations suggest that the excretion of iron over a period of time is quite appreciable.

Copp & Greenberg (1946*b*) have noted a maximum deposition of over 40 % of the injected activity in the liver, whereas in our experiments with the tracer alone the activity in the liver does not exceed 9 % over the period of observation. The higher value obtained by Copp & Greenberg (1946*b*) is because the tracer dose administered by them contained over 50 μg . of iron. As is evident from our experiments with carrier iron, the distribution of iron is strictly governed by the amount of iron administered, any excess of iron tending to be deposited in the liver and mobilized at a later stage. Granick & Hahn (1944) have shown that the liver could act as a storehouse for iron. The low but steady values obtained for the activity in the bones, with the carrier iron, may imply that the bones have a limited capacity for the amount of iron that they can handle for blood-cell formation in any

given time. The steady values obtained in these experiments probably represent such a maximal amount, about 75 μg . The percentage excretion with the carrier is of the same order as with the tracer alone. This suggests that the amount of iron excreted during any particular period is a fixed proportion of the total iron being metabolized in the body.

With ^{65}Zn and ^{60}Co we have observed that administration of the inert carrier causes a greater retention of radioactivity in the blood, the deposition in the various organs being considerably lowered. Iron differs sharply from cobalt and zinc in this regard. But most of the chelating agents tested have quite similar effects on the distribution of these metals. Thus diethyldithiocarbamate produces a large deposition of all these isotopes in the liver immediately after administration; the pattern of distribution of the EDTA chelates of these metals with time are nearly identical. With EDTA and any one of these isotopes, there is always an initial accumulation of radioactivity in the skin, the bones and the muscles, a negligibly low activity in the liver and in the blood, and a large excretion. It is thus obvious that, when once a firm chelate is formed, the chelating agent rather than the metal decides the organs of preferential localization.

The metabolism of ^{14}C -labelled EDTA in the rat has been studied by Foreman, Vier & Magee (1953), who observed an appreciable retention of the activity in the skin only, among the various tissues, and also the rapid excretion of the radioactivity. Unfortunately, these workers do not present any data for the amount of activity in the skeleton and the muscle at 1½ hr., nor any data for distribution at earlier periods. It is just possible that the initial distribution of EDTA itself is quite similar to that of its chelates.

It is interesting that ^{59}Fe , whether administered alone or with carrier iron or with diethyldithiocarbamate, is rapidly removed from the plasma and incorporated into the cells. The nature of this incorporation has not yet been studied in detail. With the tracer alone, and possibly with carrier iron also, it may be due to the utilization of the injected radioactive iron for the formation of erythrocytes by the bone marrow, the ^{59}Fe having been transported into the bones by the plasma earlier and subsequently leaving as cells. The mutually correlated variations in the activities of the blood and the bones observed at different times after administration of the tracer iron seem to support this idea. Whether there is any incorporation of the injected radioactivity into the cells in the blood itself, different from that of regular cell formation, is not definitely known. Even when administered as the diethyldithiocarbamate chelate, nearly half of the injected ^{59}Fe is present in the cells after 6 days.

Whether the chelate as a whole is incorporated into the cells or whether there is a partial release of the metal in the system, which then undergoes 'normal' metabolism, is not known. The fact that the changes observed in the activities of the bones with the diethyldithiocarbamate chelate are qualitatively quite similar to those with the tracer alone rather suggests the second possibility.

SUMMARY

1. When administered alone to albino rats there is a rapid passage of the ^{59}Fe from the blood into the bones and a subsequent slow retransfer. This process is presumably a normal occurrence in iron metabolism. At all times, the blood and the bones account for most of the activity. The activity in the liver remains fairly constant at a low level. The excretion, initially negligible, becomes appreciable subsequently.

2. Simultaneous administration of stable iron changes the pattern of distribution completely. A gradual accumulation of radioactivity in the liver, instead of in the bones, occurs followed by a slow mobilization later on. The low but constant amount in the bones seems to indicate a limited capacity of the bone components for iron.

3. Both 1-nitroso-2-naphthol and sodium diethyldithiocarbamate cause an immediate deposition of radioactivity in the liver, the deposition being more pronounced with the latter. Subsequently, a large fraction of the isotope appears in the blood.

4. Whether administered as the sodium diethyldithiocarbamate chelate or in the ionic form with or without the carrier, the tracer in the blood, though initially present mostly in the plasma, occurs almost completely in the cells after 24 hr. in the bound form.

5. EDTA causes a rapid removal of the isotope from the blood, a negligibly low deposition in the liver but considerable accumulation in the skin, the bones and the muscles, followed by a large excretion. This distribution pattern is presumed to be characteristic of all EDTA chelates.

It is a pleasure to thank Dr S. Krishnamurthi, Director of this Institute, for his keen interest and constant encouragement. We are very grateful to the Department of Atomic Energy, Government of India, for financing this research scheme.

REFERENCES

- Beutler, E. (1961). *Annu. Rev. Med.* **12**, 195.
Copp, D. H. & Greenberg, D. M. (1946*a*). *J. biol. Chem.* **164**, 377.
Copp, D. H. & Greenberg, D. M. (1946*b*). *J. biol. Chem.* **164**, 389.
Foreman, H., Vier, M. & Magee, M. (1953). *J. biol. Chem.* **203**, 1045.
Granick, S. & Hahn, P. F. (1944). *J. biol. Chem.* **155**, 661.
Hahn, P. F. (1948). *Advanc. biol. med. Phys.* **1**, 288.
Huff, R. L., Bethard, W. F., Garcia, J. F., Roberts, B. M., Jacobson, L. O. & Lawrence, J. H. (1950). *J. Lab. clin. Med.* **36**, 40.
Huff, R. L. & Judd, O. J. (1956). *Advanc. biol. med. Phys.* **4**, 223.
Lamerton, L. F., Belcher, E. H. & Harris, E. B. (1959). In *The Kinetics of Cellular Proliferation*, p. 301. Ed. by Stohman, F., jun. New York: Grune and Stratton Inc.
Past, W. L. (1963). *Amer. J. Path.* **42**, 285.
Rubin, M. & Princiotto, J. V. (1960). *Ann. N.Y. Acad. Sci.* **88**, 450.
Sivaramakrishnan, V. M., Sekhara Varma, T. N., Brahmanandam, S. & Nagarajan, B. (1962*b*). *Proc. Soc. exp. Biol., N.Y.*, **110**, 817.
Sivaramakrishnan, V. M., Sekhara Varma, T. N. & Lakshmanan, M. R. (1961). In *Symposium on Proteins*, p. 239. Ed. by Rao, M. V. L. Mysore: C.F.T.R.I.
Sivaramakrishnan, V. M., Sreenivasan, N. G., Sekhara Varma, T. N. & Brahmanandam, S. (1962*a*). *Nature, Lond.*, **193**, 1195.

Biochem. J. (1964), **92**, 537

The Enzymic Formation of Aminoacetone from Threonine and its Further Metabolism

BY MARGARET L. GREEN* AND W. H. ELLIOTT

Department of Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., Australia

(Received 6 December 1963)

Aminoacetone was first isolated from a biological system by Elliott (1959, 1960*c*), who obtained it in crystalline form from the medium in which

* Present address: The Rockefeller Institute, New York 21, N.Y., U.S.A.

Staphylococcus aureus cells had been incubated aerobically with threonine. Evidence for its existence in trace amounts in human urine had been obtained previously by Mauzerall & Granick (1956). The compound is also produced by *Rhodospseudo-*