ant development in *Codium Cranwelliae* may add information to be considered in any attempt to solve the problem.

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*STUDIES IN MINERAL METABOLISM WITH THE AID OF INDUCED RADIOACTIVE ISOTOPES. IV—MANGANESE**

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This communication is a report of a test study of the suitability of radioactive manganese for biological tracer investigations. The requirement for manganese of the animal organism is very small. Consequently, a high specific radioactivity is required in the samples in order to keep the test dosages within physiological limits.

Various investigators have demonstrated that manganese is essential for the health and well-being of the animal organism.[1, 2, 3, 4] However, little is known about its specific biological functions. Orent and McCollum[5] found that manganese aids lactation and prevents degeneration and atrophy of the testes in the rat. In the chick, it is markedly effective in preventing the development of the bone condition known as perosis.[6] Manganese has been found to serve as an activator of certain enzymes, notably arginase,[7, 8] phosphoglucomutase,[9] and certain peptidases.[10]

The results reported here demonstrate that radioactive manganese may be usefully employed in the elucidation of many problems connected with its metabolism.

Experimental Methods.—The isotope Mn^{54}, with a half life of 310 days,[11] was employed in this investigation. It was prepared in the Radiation Laboratory of the University of California by bombardment of iron with deuterons. The reaction involved is:

$$_{26}Fe^{56} + {}_{1}H^{2} \longrightarrow {}_{25}Mn^{54} + {}_{2}H^{4}$$

Radioactive manganese was isolated from the residue after extraction of the radioactive iron. Traces of radioactive phosphorus formed from iron phosphide were removed by dissolving the iron-free residues in $0.5M$ HNO_3, adding small quantities of NaH_2PO_4 to serve as a carrier, and precipitating twice with a solution of $Bi(NO_3)_3$ in $0.5M$ HNO_3. The filtrate was evaporated to dryness, dissolved in $16M$ HNO_3, and the manganese precipitated as MnO_2 with a few crystals of $KClO_3$. The MnO_2 was filtered off on a Jena sintered glass filter, then dissolved with H_2O_2, and reprecipitated twice by

the above procedure to free it from traces of radioactive cobalt that were also present in the original residues.

Finally the MnO_2 was dissolved in dilute HCl, evaporated to dryness to remove excess acid and then dissolved in enough water to give a concentration of about 1 mg. of manganese per ml. of solution. The radioactivity of the resulting preparation was of the order of 0.1 μc. per mg. of Mn. All radioactivity measurements were made with a thin copper wall Geiger-Müller counter and a scale of eight circuit.

TABLE 1

EXCRETION OF LABELED MANGANESE

TIME, HOURS	ORAL ADMINISTRATION, PER CENT OF TOTAL DOSE	INTRAPERITONEAL INJECTION, PER CENT OF TOTAL DOSE
	Feces	Feces
0–23	4.2 ± 0.26	*
23–48	50.2 ± 0.45	53.9 ± 0.60
48–75.5	39.9 ± 0.40	36.8 ± 0.35
Total excreta	97.2† ± 0.68	90.7 ± 0.70

* Amount found was not statistically significant.

† Total excreta includes 2.9 per cent labeled manganese found in the urine in the time interval between 11 and 23 hours. This was the only statistically significant amount found in any of the urine samples.

For the metabolic tests, 1-ml. doses of the labeled manganese chloride (1 mg. Mn) were administered to each of two rats weighing about 160 gm., one by stomach tube, the other by intraperitoneal injection. During the experiment, the animals were maintained on a synthetic control diet.

The feces and urine were obtained separately at the desired intervals through use of glass separators.[12] At the end of 75.5 hours, the animals were anesthetized with ether, blood was drawn by cardiac puncture and the animals were sacrificed. The various organs were dissected out, the skin was removed and the muscles and bone were separated by boiling the residual carcass in 1:5 NH_4OH, and then allowing the solution to stand for several days.

All tissues were dried and dry ashed at 500°C. One mg. of inactive manganese as the sulfate was added to each ashed sample as a carrier for the radioactive Mn. The ash was dissolved in a minimum of dilute HCl and filtered. The total filtrate was evaporated to dryness with concentrated HNO_3 three times to remove the chloride. The residue was then dissolved in hot concentrated HNO_3, and a few crystals of $KClO_3$ were added to precipitate the MnO_2. The precipitate was filtered off, another mg. of inactive Mn was added to the filtrate and MnO_2 was again precipitated. This second precipitate was collected on the same filter paper so that all of the radioactivity in a single tissue was on the one filter paper.

In the case of the bones, it was necessary first to separate the manganese from the large amounts of calcium present. The bone ash was dissolved in dilute HCl and evaporated to dryness. The chlorides were dissolved in water in an Erlenmeyer flask and NH_4Cl was added to the neutral solution. A freshly prepared $(NH_4)_2S$ solution was added to precipitate the manganese sulfide. The flask was filled with boiled water and stoppered. After standing for 12 hours, the precipitate was filtered off. The MnS was dissolved in a small amount of dilute HCl, and then the MnO_2 was separated by the same procedure as in the case of the other tissues. An aliquot of the original radioactive $MnCl_2$ solution was treated in the same manner as the test samples to serve as a standard for comparison of radioactivity measurements.

The total radioactive manganese recovered from each animal was computed from the measured activities of the excreta and of all the tissues. The recovery was 96 per cent in the case of oral administration, and 78 per cent in the case of intraperitoneal injection, so that the data have been corrected by the factor 100/96 and 100/78, respectively, to make them comparable.

Results.—The course of excretion of the labeled manganese over the 75.5 hours is shown in table 1. Most of the manganese is excreted in the feces whether it is administered orally or by injection. This agrees with Skinner, Peterson and Steenbock,[13] who have reported that from 80 to 99 per cent of orally fed manganese was excreted in the feces, depending upon the amount ingested. Except for statistically insignificant traces, labeled manganese was found in the urine only in the second collection period of the animal given the dose orally.

From the data it is not possible to decide to what extent manganese is absorbed from the gastro-intestinal tract when it is administered orally, although it is probable that it is small. When administered intraperitoneally, 90.7 per cent of the labeled manganese appeared in the feces, showing that there is a preferential excretion into the alimentary tract.

Table 2 shows the distribution of the labeled manganese that was retained by the animal. When administration was oral, the 2.8 per cent retained manganese was found in the liver, bone, muscle and blood, the liver showing the largest uptake.

When administration was by injection, the retained manganese was found in the skin, bone, liver, muscle, small intestine and stomach, the skin and bone showing rather large amounts. Other tissues showed no significant amount. In general, these observations also agree with the findings of Skinner, Peterson and Steenbock.[13]

The manganese found in the stomach and small intestine possibly represents manganese in the process of being excreted. Muscle and skin, apparently, are important sites for the storage of manganese that is absorbed,

especially as these tissues represent a large portion of the mass of the animal. Bone and liver also seem to be important in the storage of manganese. The manganese found in the liver may be indicative of its excretion into the bile, or it may be connected in some manner with the activation of certain enzymes found in the liver.

Summary.—1. Radioactive manganese, Mn^{54}, is suitable for "tracer" studies on the metabolism of manganese.

2. On a normal control diet the rat excreted over 90 per cent of the manganese within 75 hours, when administered either by stomach tube or by intraperitoneal injection.

3. Very little, if any, of the absorbed manganese is excreted in the urine.

4. Liver, bone and muscle take up appreciable quantities of the absorbed manganese. Other tissues may take up varying amounts of the manganese, due to storage or to processes of excretion.

TABLE 2

DISTRIBUTION OF LABELED MANGANESE
(In Per Cent of Total Dose, 75.5 Hours after Administration)

	ORAL ADMINISTRATION			INTRAPERITONEAL INJECTION		
TISSUES	WEIGHT, GM.	CONTENTS IN WHOLE TISSUE	CONTENTS PER GM., FRESH WEIGHT	WEIGHT, GM.	CONTENTS IN WHOLE TISSUE	CONTENTS PER GM., FRESH WEIGHT
Muscle	95	0.7 ± 0.12	0.007 ± 0.0012	97	0.8 ± 0.16	0.008 ± 0.0016
Bone	8.6	0.7 ± 0.11	0.081 ± 0.013	9.8	2.0 ± 0.17	0.20 ± 0.017
Skin	30.66	*	*	28.94	3.7 ± 0.22	0.13 ± 0.008
Whole blood	5.14	0.5 ± 0.14	0.097 ± 0.027	5.07	*	*
Heart	0.50	*	*	0.56	*	*
Liver	6.49	0.9 ± 0.15	0.14 ± 0.023	8.54	1.2 ± 0.20	0.14 ± 0.023
Small intestine	5.37	*	*	6.36	0.8 ± 0.27	0.13 ± 0.042
Large intestine	1.13	*	*	1.23	*	*
Stomach	4.75	*	*	3.67	0.8 ± 0.27	0.22 ± 0.074
Spleen	0.35	*	*	0.89	*	*
Kidney	1.38	*	*	1.36	*	*
Lung	0.88	*	*	1.16	*	*

* Radioactivity measurements made, but amounts found were not statistically significant.

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A REVERSION TO WILD-TYPE ASSOCIATED WITH CROSSING-OVER IN DROSOPHILA MELANOGASTER

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In a study of two alleles of lozenge eye in *Drosophila melanogaster*, a low frequency of reversion to the wild-type has been observed. The reversion involves the color and structure of the eye, and also the genital tracts which are abnormal in the mutant females.[1] In each case in which the reversion has occurred, crossing-over between the *X*-chromosomes has also occurred.

Glossy and spectacle are sex-linked, recessive mutants, alleles of lozenge, which were induced by irradiation. Glossy (lz^g) individuals have eyes which are blood-red in color, with fused facets making a glossy surface. Spectacle (lz^s), as reported by Dr. J. T. Patterson,[2] is characterized by a light brown color of the eye, and the facets are run together to cause a smooth surface of the eye. In the compound, heterozygous glossy-spectacle females, glossy is more dominant in its expression; and the character of the eye is nearly that of homozygous glossy. Spectacle is associated with the *dl*-49 inversion, and the mutant gene is located within that inversion. Glossy is also located within an inversion which is very similar to, probably the same as, the *dl*-49 inversion.

From the mating of lz^g Bx/lz^s f females to lz^g Bx males, a total of 5584 offspring have been inspected. Most of the offspring were of the expected types. Males were glossy or spectacle. Females were phenotypically