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COPPER METABOLISM IN NORMAL LONG-EVANS RATS

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James K. Ashikawa, Elmer R. Smith, and Harold L. Helwig

September 25, 1956

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ABSTRACT

Copper plays a diverse role in animal metabolism and is highly essential in trace amounts.

As a preliminary to uptake and distribution studies, the copper contents of various normal rat tissues were determined.

The preparation from natural ZnO of carrier-free Cu^{64} used for these studies is described.

The preliminary work on the uptake and distribution of carrier-free Cu^{64} and plasma-bound Cu^{64} in normal adult Long-Evans female rats is also presented.

COPPER METABOLISM IN NORMAL LONG-EVANS RATS

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PART I. THE PREPARATION OF CARRIER-FREE COPPER-64 FOR BIOLOGICAL STUDIES

Copper is highly essential in the diet, but because of its low dietary requirements and wide distribution in nutrients, copper deficiencies in animals are rather uncommon. Copper appears to have erythropoietic properties and plays a role in hemoglobin formation.[1,2,3] It is also involved in the formation of the oxidation enzymes, catalase, cytochrome oxidase, peroxidase, the cytochromes, and many others.[4,5] Thus carrier-free copper is needed in all tracer-study experiments. The existing chemical methods for separating radiocopper from target material are rather hazardous because of the high radioactivity of the cyclotron-bombarded targets. Since micro amounts of material can be efficiently separated from one another by ion-exchange technique, the work described here was undertaken to facilitate the preparation of carrier-free radiocopper for our experiments.

Recent studies by Kraus and Moore[6] on the separation of the chloride complexes of the transition elements Mn to Zn, and our work[7] on the separation of Fe, Co, and Zn from P on the strongly basic (quaternary amine) resin, Dowex-1, indicated the feasibility of this method for separating Cu from Ga and Zn. More recently Herber and Irvine[8] have investigated the anion-exchange behavior of the bromide complexes of Co(II), Cu(II), Zn(II), and Ga(III), and found that a partial separation of Cu(II) and Ga(III) was possible with HBr as the eluant. Herber and Irvine have indicated that HBr is considerably better than HCl as an eluant. However, we have used the much more convenient method of chloride complex formation and column separation, and have consistently obtained Cu^{64} with much less than 1% contamination by longer-lived components (as measured by gamma activity). These contaminations are presumably a mixture of Cu^{67} and Ga^{67}.

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MATERIAL AND METHOD

Preparation of the Ion Exchange Column

The exchange columns were prepared as indicated in our earlier report.[7] The columns used had a cross-sectional area of 1.4 cm^2, and were packed with Dowex-1 to 12 cm length and equilibrated with concentrated HCl.

Preparation of Carrier-Free Copper-64

We have prepared Cu^{64} primarily by bombarding natural zinc oxide in platinum hats, although electroplated zinc was used in later runs. In a typical run, 130 mg of ZnO were placed in a platinum hat and bombarded in an external beam for 4 hr with 19-Mev deuterons at a current of 4μas per hr.

Method of Elution

The ZnO target was dissolved in a small volume of 5M HCl and heated to dryness. The salts were then taken up with 12 M HCl, transferred to the resin bed, and successively eluted from the column with 12 M, 6 M, 0.5 M, and 0.005 M HCl. The acid was fed at a constant flow rate of 10 ml per hr by a mechanically operated syringe pump. The flow rate can be doubled with no apparent effect on the separation.

RESULTS AND DISCUSSION

The theoretical yield of Cu^{64} from natural ZnO thick target is stated to be 300 μc per μa-hr. By bombarding 130 mg of ZnO in an external beam for 4 hr with 19-Mev deuterons at a current of 4 μas per hour, we have obtained about 100 μc of Cu^{64}. A comparable yield of Cu^{64} was obtained by bombarding 90 mg of zinc plated on a platinum hat. In an attempt to obtain higher yields of copper, we have used beam currents above 4 μa per hour. At these higher currents, however, platinum-zinc alloy is produced, and it is very difficult to dissolve.

Since the elution constants E for zinc and copper were known[4] for Dowex-1, it was necessary only to measure the E for Ga(III). This was determined on Dowex-1 in the range of 0 to 12 M HCl. The result is shown in Fig. 1.

The elution histogram for copper, gallium, and zinc is shown in Fig. 2. This was obtained by eluting the dissolved ZnO target with HCl of concentrations shown on the histogram. Each 25-ml fraction of the eluent was collected and counted with a sodium iodide crystal counter. Under these conditions, a small amount of zinc and gallium was removed with 75 ml of 12 M HCl, and of copper with 175 ml of 6 M HCl. Gallium and zinc were readily removed with 100 ml of 0.5 and 0.005 M HCl respectively.

The purity of the eluted fractions was determined by gamma-ray spectroanalysis and by measuring the radioactive decay rates. The radiopurity of the copper fraction was consistently higher than 99% (See Fig. 3).

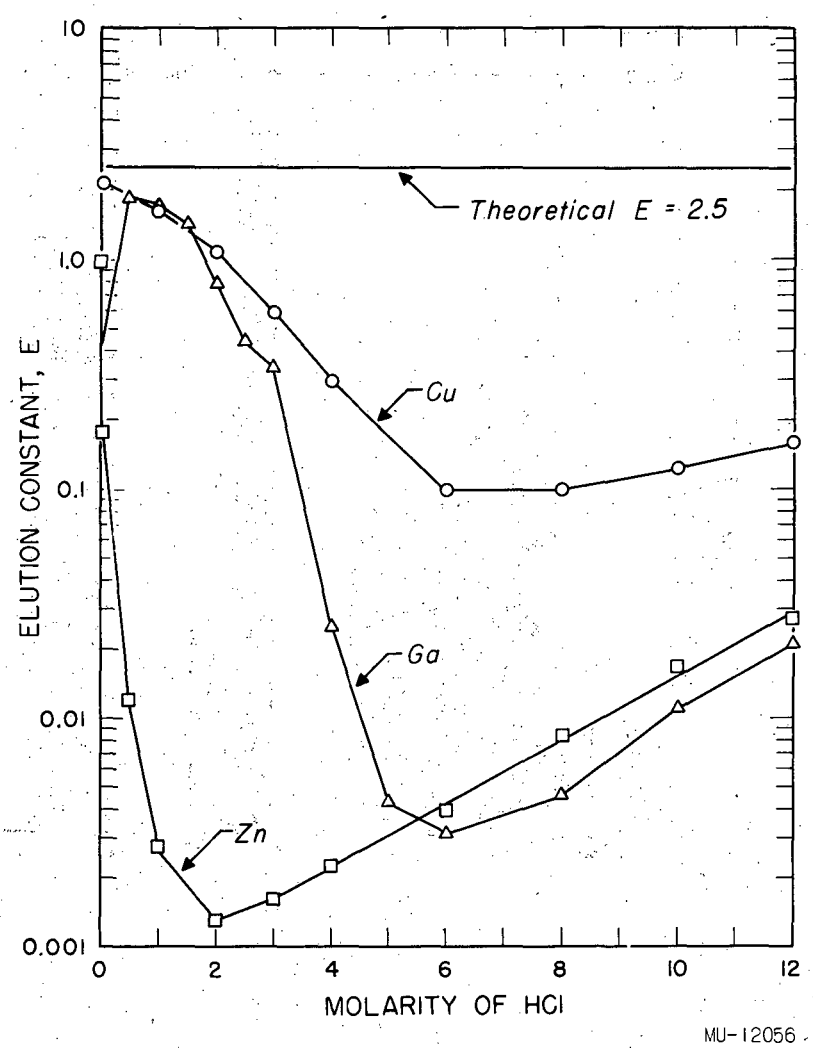

Fig. 1. Elution constants of gallium, copper, and zinc on Dowex-1.

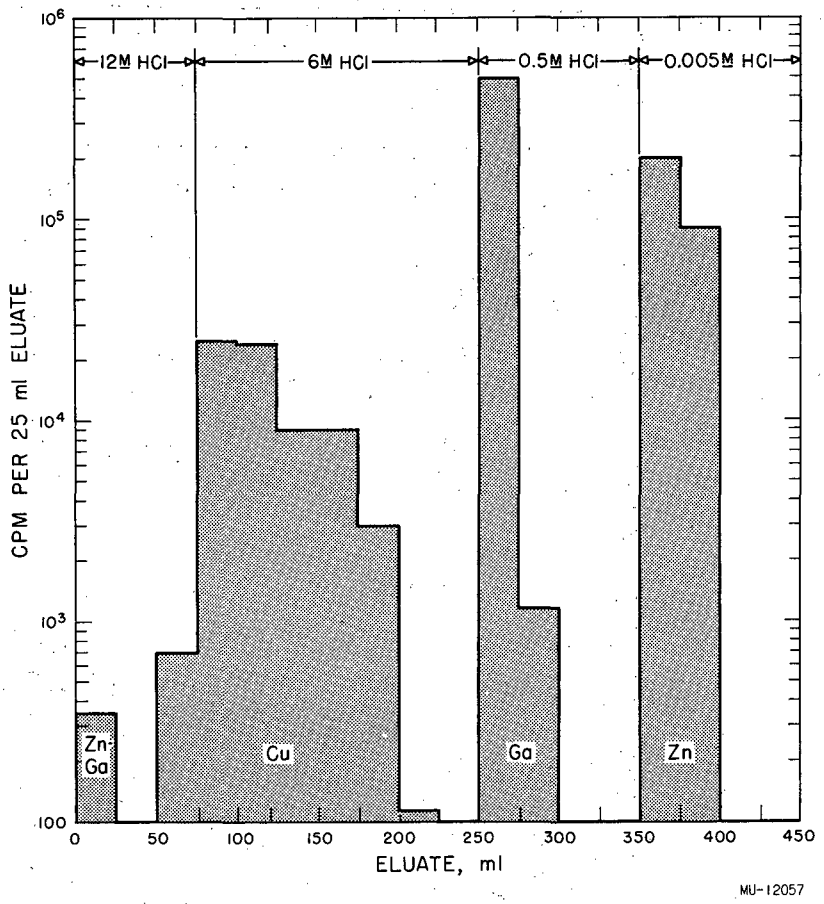

Fig. 2. Elution histogram of gallium, copper, and zinc on Dowex-1 ion-exchange column.

If further purification is desired, the contamination can be reduced to less than 0.1% by dithizone extraction of the Zn and Ga.[9] The gallium and zinc fractions (Figs. 4 and 5) appeared to be completely separated from each other.

Since Cu^{67} with a half life of 61 hours is preferable to Cu^{64} for biological studies, attempts were made to produce this longer-lived copper by bombarding enriched Zn^{67} with deuterons according to the reaction $Zn^{67}(d, 2p)\ Cu^{67}$. However, Cu^{64} predominated, as in the natural ZnO target.

PART II. THE DETERMINATION OF COPPER IN THE TISSUES OF NORMAL LONG-EVANS ADULT FEMALE RATS

In 1929, Lindow et al.[10] reported the distribution of copper in the tissues of adult rats raised on stock diets and on diets containing varying amounts of copper. Owing to the availability of a more sensitive and modern method of analyzing copper, we have determined the normal copper content in several organs of the rat preliminary to tracer studies utilizing carrier-free Cu^{64}.

MATERIAL AND PROCEDURE

Preparation and Digestion of Samples

Adult female Long-Evans rats weighing from 115 to 145 g (average weight about 130 g) were used. The animals were anesthetized with ether, and blood was withdrawn by heart puncture with a hypodermic needle and syringe rinsed with heparin. The blood was centrifuged for 10 min at 3000 rpm and the plasma removed. The cells were washed twice with double their volume of isotonic saline. The organs were removed, weighed, and transferred to flasks for digestion.

Tissue samples of about 2 g (the entire organ if necessary), were transferred to 250-cc Erlenmeyer flasks with small funnels placed in the mouth to act as reflux condensers. Five ml of concentrated nitric acid and 2 ml of cencentrated sulfuric acid were added to each sample and each flask was heated on a large hot plate, gently at first to prevent too vigorous a reaction. As the digestion progressed, the temperature was slowly raised to maintain a moderate rate of reaction. Digestion was continued until a colorless to light-straw-colored solution was attained. In case of charring, additional nitric acid was added and the ashing continued to completion. After digestion, the flask was cooled, 2 to 3 ml of doubly distilled water added, and the flask reheated to destroy any remaining nitrosyl-sulfuric acid. This step was repeated, with heating, until dense white fumes of SO_2 were evolved. Finally, 10 ml of doubly distilled water was added to the cooled flask and the contents were brought to boiling to facilitate solution of the salts.

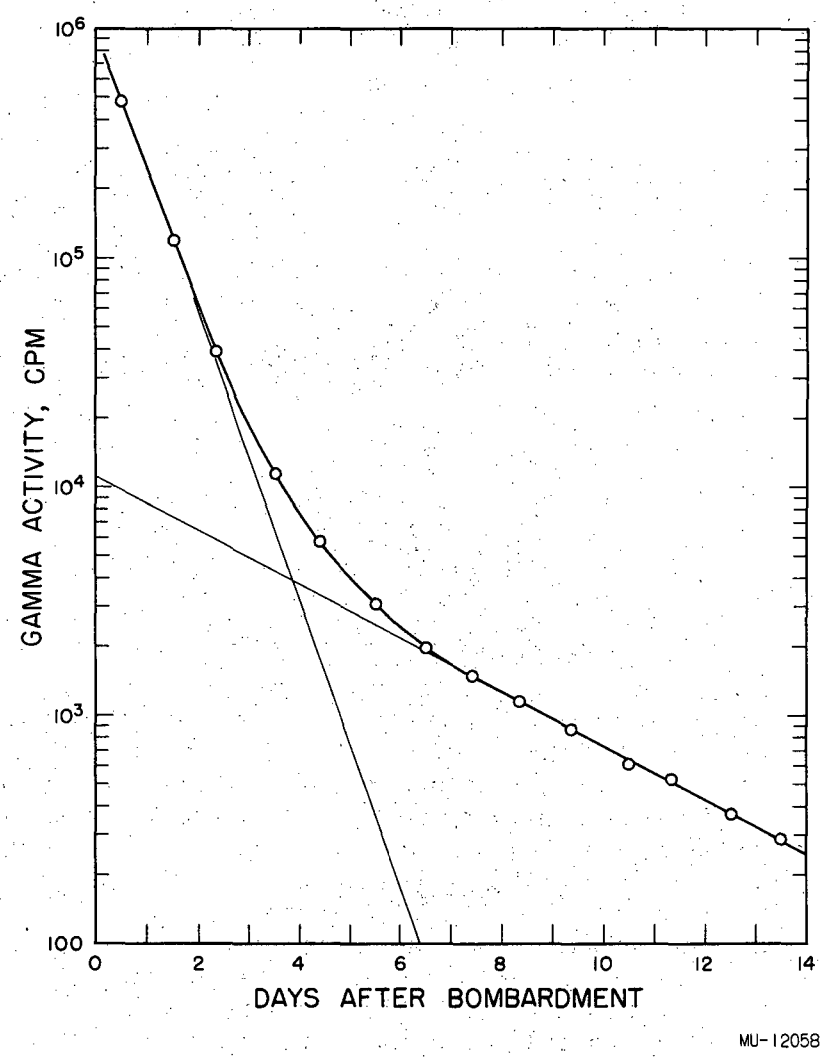

Fig. 3. Copper fraction, showing a mixture of Cu^{64} and Cu^{67} with half lives of 12.8 hr and 61 hr respectively.

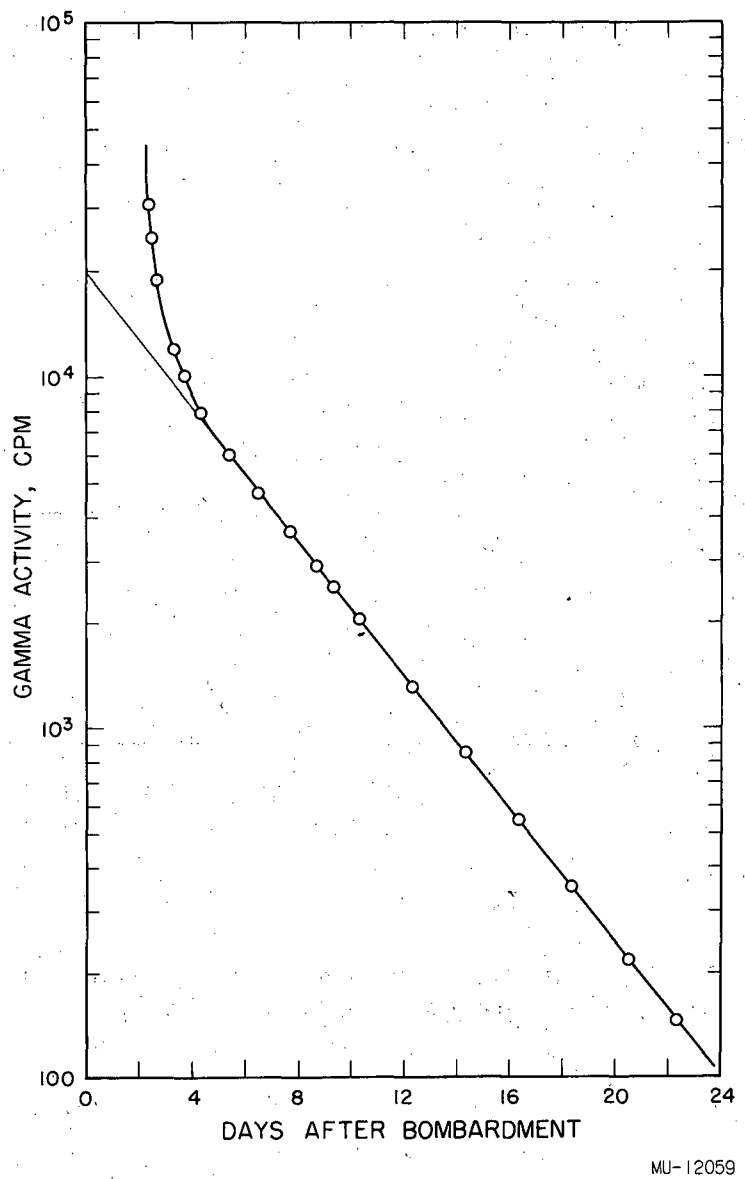

Fig. 4. Gallium fraction, showing a mixture of Ga^{66} and Ga^{67} with half lives of 9.4 hr and 78 hr respectively.

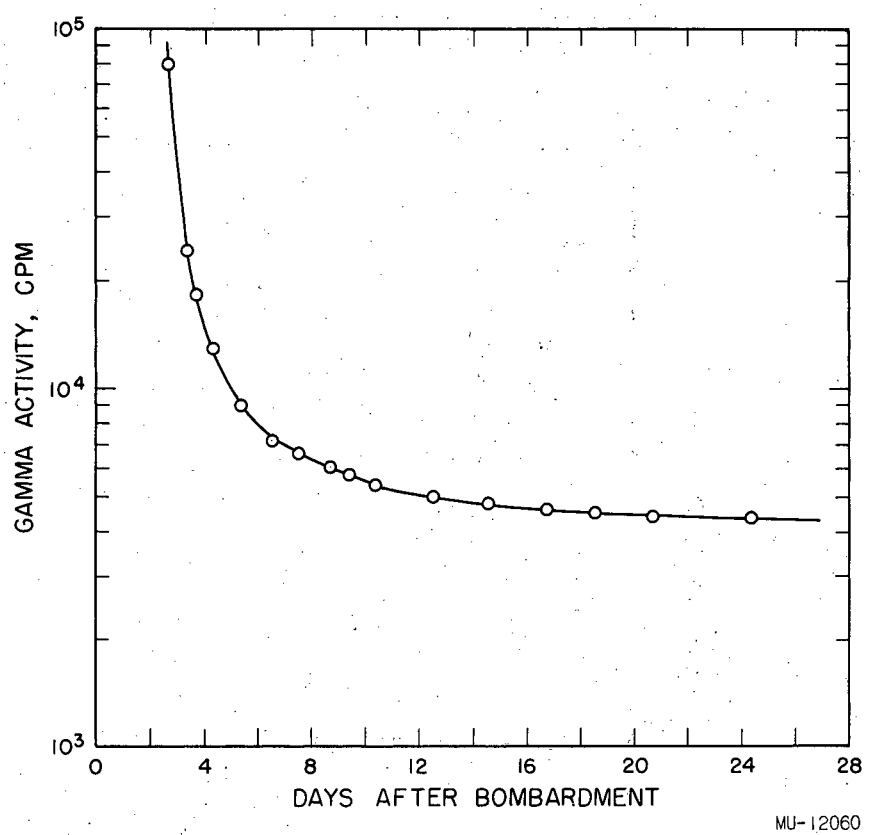

Fig. 5. Zinc fraction, showing a mixture of Zn^{62} and Zn^{65} with half lives of 9.3 hr and 250 days respectively.

Spectrophotometric Determination of Copper[9, 11, 12]

To the sample or aliquot having a volume of 10 ml and containing 1 to 4 μg of copper, 3 drops of cresol red indicator were added and the solution adjusted to a yellow color (pH ~ 2) with concentrated ammonium hydroxide. To this, 1 ml of phosphate-citrate buffer and 10 ml of 0.001% dithizone (weight/volume) in CCl_4 was added. The sample was shaken for 15 min on a mechanical shaker. The CCl_4 layer was transferred to a centrifuge cone and then to a Beckman cuvette for reading. Double transfer was used to prevent the transfer of water with the CCl_4. The optical density was obtained at 520 mμ with 1-cm cells in a Beckman DU spectrophotometer. Readings were compared with a standard copper curve prepared each day. The curves were reproducible over a period of several weeks if the same stock dithizone was used.

Blanks consisting of buffer, indicator, and water were used to prepare the standard curves. In addition, reagent blanks (with water replacing the tissue sample) were ashed.

Reagents used:

Stock copper standard: 100 μg of copper per ml in double-distilled water (0.0982 g $CuSO_4 \cdot 5H_2O$ per 250 ml).

Working copper standard: A dilution of stock standard to give from 1 to 4 μg per ml in double-distilled water.

Cresol red indicator: 0.02 g per 100 ml double-distilled water.

Concentrated nitric acid, sulfuric acid, and ammonium hydroxide.

Buffer solution: 8.3 g of anhydrous disodiumphosphate and 38 g of citric acid in 250 ml of double-distilled water. Traces of copper were removed by washing with stock dithizone solution.

Stock dithizone solution: 8 mg of dithizone (diphenylthiocarbazone) dissolved in 100 ml CCl_4 to give 0.01% (weight/volume) solution. Dithizone is usually only about 90% pure. Refrigerated in the dark.

Working dithizone solution: A 1:10 dilution of the stock dithizone with CCl_4. Refrigerated and kept in the dark.

RESULTS

The results of the experiment are summarized in Table I. In a number of instances the samples were lost or contaminated during the digestion. Consequently there is a variation in the number of samples of different tissues analyzed. Attempts were made to determine the copper content of adrenals, pituitaries, ovaries, and bone marrow. However, the size of the sample was far too small and the copper concentration much too low for accurate determination. Results for these tissues are not included in the table.

Table I

Copper content of various normal rat tissues

Tissue	Number of samples	Copper content ± standard deviation
Red blood cells	28	1.62 ± 0.44 μg/ml
Plasma	29	2.20 ± 0.57 μg/ml
Brain	40	4.53 ± 1.56 μg/gm wet tissue
Kidney	37	10.19 ± 2.21 μg/gm wet tissue
Muscle	30	2.37 ± 1.14 μg/gm wet tissue
Spleen	28	4.80 ± 1.88 μg/gm wet tissue
Liver	44	6.26 ± 1.45 μg/gm wet tissue
Reagent blank	7	0.15 μg/10 ml of dithizone

Our data were obtained on a relatively large number of animals. Earlier work was limited to ten or less. A comparison of the values reported by various authors for normal rat liver is given below. Lorenzen and Smith[11] have reported the values listed in Table II.

Table II

Copper content of rat liver as reported by various authors

Workers	Liver copper μg/g wet tissue
Cunningham (1931)	3.01
McHargue (1925)	5.15
Lindow, et al. (1929)	3.12
Lorenzen and Smith (1947)	9.37
This paper	6.26

In Table III are given the data from Lindow et al. for a variety of normal rat tissues.

Table III

Copper content of various rat tissues as reported by Lindow et al.

Tissue	% Water	Copper μg/g dry	Copper μg/g wet
Brain	76.2	9.14	2.27
Kidney	74.4	12.41	3.18
Muscle	70.3	2.00	0.59
Spleen	75.6	3.41	0.83
Whole Blood			0.54 μg/ml

As can be seen, our results are consistently higher than those reported by Lindow et al. This may be due to a dietary factor or to a difference in the method of analysis. The results presented here are used as a basis for carrier-free Cu^{64} tracer experiments.

PART III. THE UPTAKE AND DISTRIBUTION OF CARRIER-FREE COPPER-64 IN NORMAL ADULT LONG-EVANS FEMALE RATS

Although some information is available on the distribution of copper in the organs of the adult rat,[10] there has been little work done on the state of copper in these organs. Keilen and Mann isolated the copper proteins, hepatocuprein and hemocuprein, from the liver and blood respectively.[13] Holmberg and Laurell have isolated the copper-containing alpha$_2$ globulin protein from the blood serum and named it coeruloplasmin.[14] Normally about 90% of the copper in the serum is bound in coeruloplasmin. More recently, Earl et al. have used Cu^{64} to study copper metabolsim in hepatolenticular degeneration in humans.[15]

The availability of carrier-free Cu^{64} made it seem advisable to make a further study on copper metabolism in rats by the tracer technique.

PROCEDURE

Preparation of Tissues

Adult female rats weighing between 115 and 145 g (average weight about 130 g) were injected via the tail vein with $Cu^{64}Cl_2$. A dose of 650,000 cpm as measured in a well-type sodium iodide crystal counter was used.

At various times, animals were ether-anesthetized and blood samples were obtained by heart puncture with a heparin-rinsed hypodermic needle and syringe. The blood was centrifuged for 10 min at 3000 rpm and the plasma removed. The cells were washed twice with normal saline. Tissue samples were obtained, weighed, and placed in 1.5-dram vials for counting. All counts were obtained with a well-type sodium iodide crystal counter and were corrected back to the same initial time.

Blood samples were also obtained by periodically bleeding the animals via the tail vein, and 50-gamma samples of whole blood were counted.

RESULTS AND DISCUSSION

The results of the experiment are summarized in Tables IV and V. The values for Table V were obtained by dividing the values in Table IV with the average copper content for that organ as reported in Part II.

The whole-blood copper level plotted in Fig. 6 as a function of time levels off in about 1 hr. Although there is some indication that the copper level slowly rises again, additional data are necessary to ascertain this point. In Figs. 7 and 8 are given the time-concentration curves for several tissues. The plasma Cu^{64} level follows the same pattern as in the whole blood. This is in agreement with the work of Earl et al. on their control individuals.[15] There is little or no exchange of Cu^{64} with the red cell

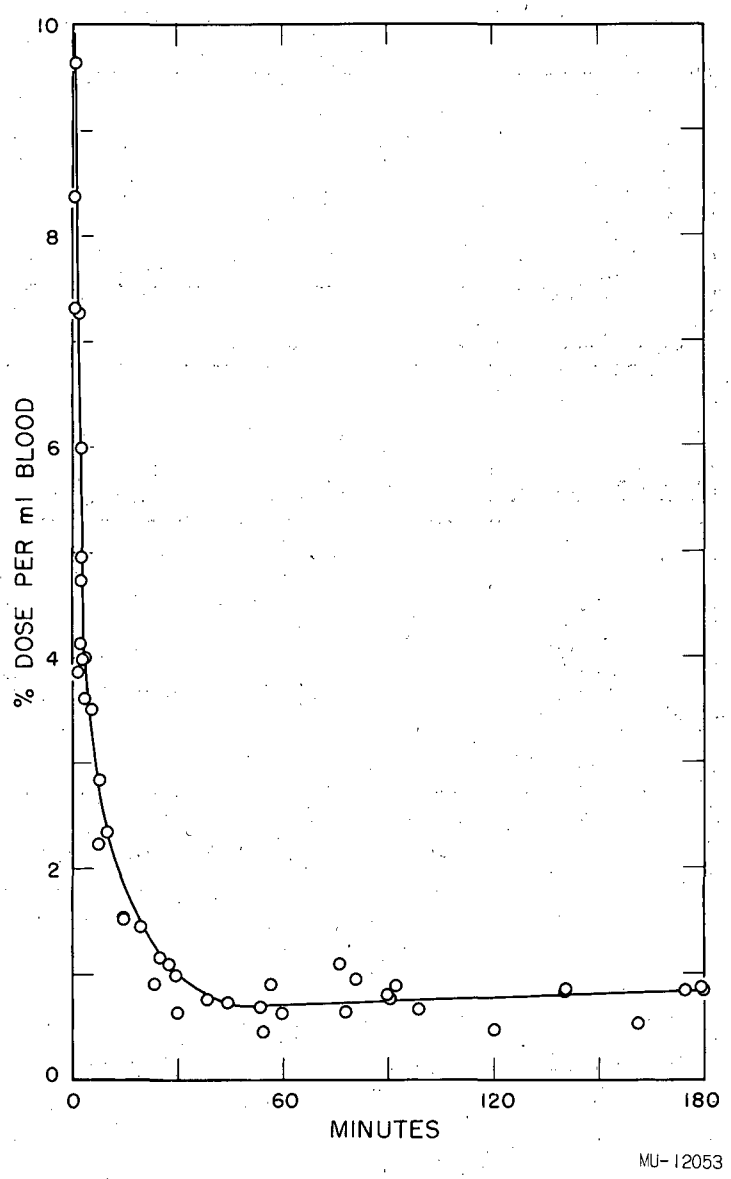

Fig. 6. Uptake of Cu^{64} in the whole blood of normal adult Long-Evans female rats following intravenous injection of isotonic copper chloride solution.

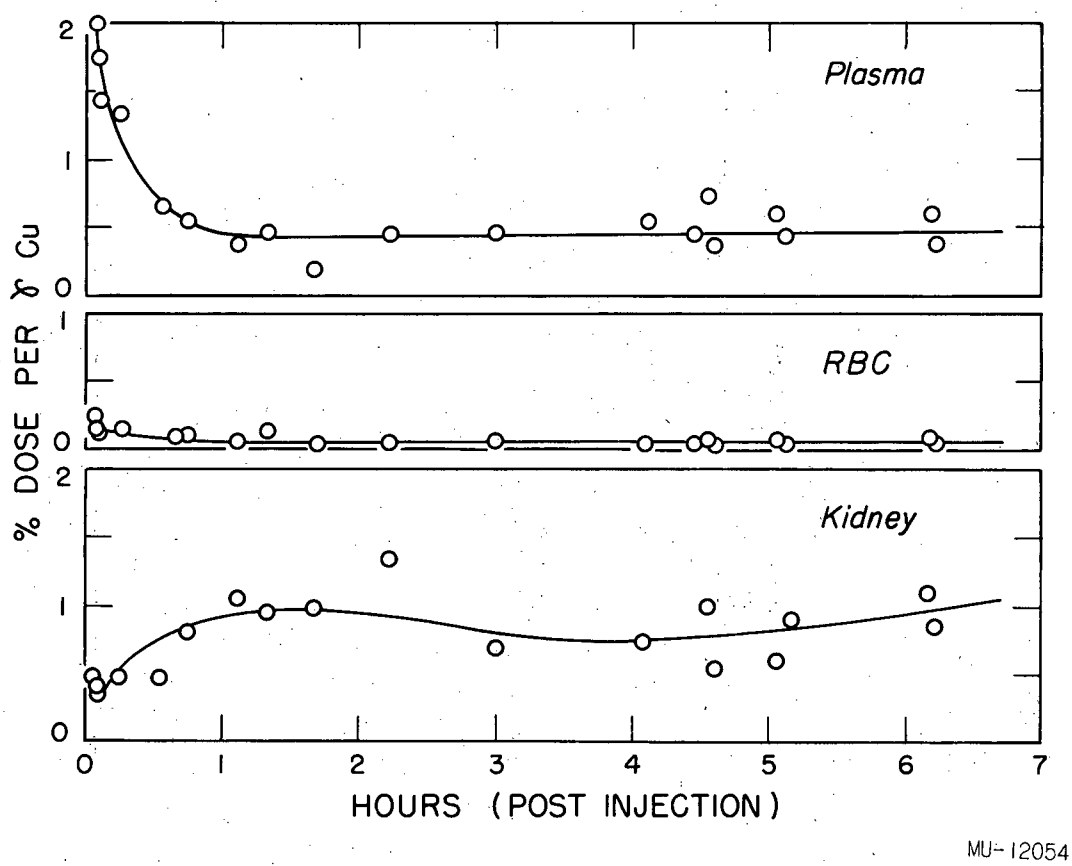

Fig. 7. Uptake of Cu^{64} in plasma, RBC, and kidney. (Experimental conditions same as in Fig. 6.)

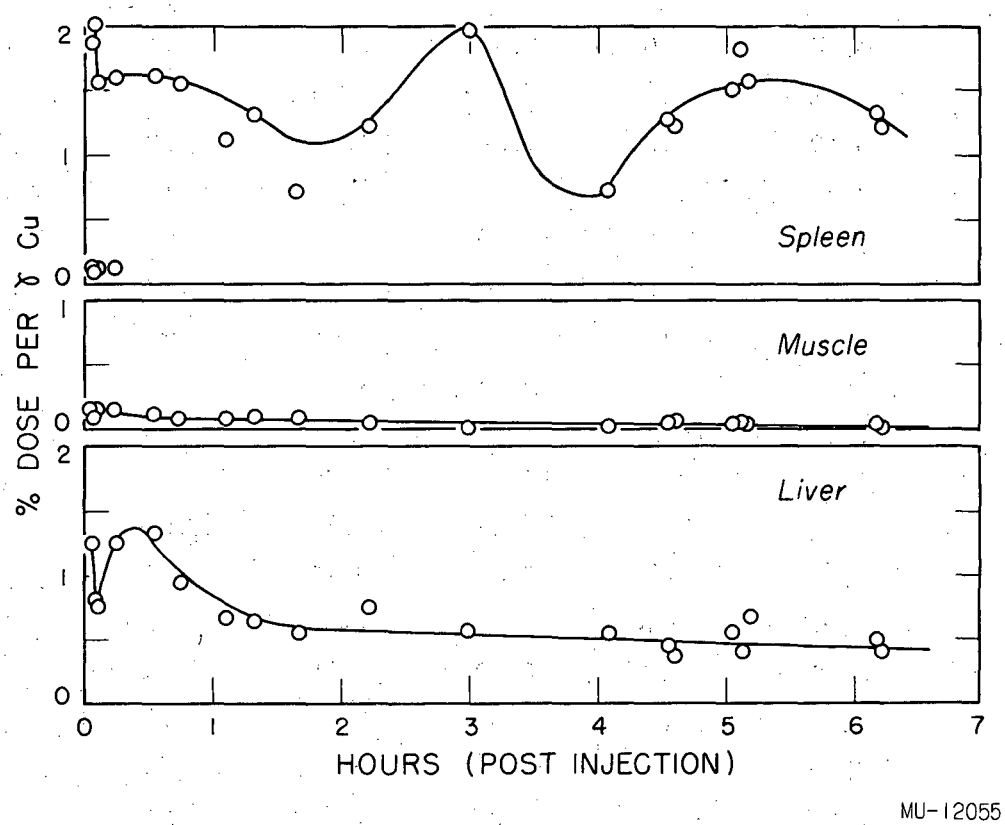

Fig. 8. Uptake of Cu^{64} in spleen, muscle, and liver. (Experimental conditions same as in Fig. 6.)

Table IV

Uptake of intravenous Copper-64 in normal rat tissue: percent dose per wet tissue

Time after injection (min)	Plasma	RBC	Kidney	Liver	Spleen	Muscle	Brain
4	4.38	0.40	5.04	7.93	9.08	0.41	0.07
5	3.85	0.25	4.17	5.08	9.82	0.31	0.07
6	3.17	0.22	3.34	4.93	7.59	0.36	0.08
14	2.99	0.24	5.03	7.77	7.72	0.28	0.09
32	1.44	0.15	5.73	8.28	7.91	0.29	0.04
44	1.16	0.16	8.36	5.92	7.58	0.23	0.05
66	0.86	0.10	10.82	4.25	5.44	0.20	0.03
79	1.01	0.21	9.78	4.13	6.43	0.27	0.12
100	0.45	0.08	10.24	3.50	3.53	0.15	0.05
133	1.01	0.08	13.74	4.76	5.99	0.09	0.04
180	1.05	0.12	7.03	3.61	9.58	0.02	0.06
245	1.23	0.08	7.72	3.47	3.49	0.08	0.07
267	0.96	0.08	---	---	---	---	---
273	1.62	0.15	10.32	2.76	6.31	0.15	0.05
276	0.86	0.07	4.51	2.37	6.13	0.16	0.06
303	1.41	0.14	6.18	3.52	7.52	0.17	0.06
3.07	0.98	0.11	1.07	2.58	8.84	0.16	0.05
311	---	---9.32		4.32	7.71	0.15	0.05
371	1.38	0.17	11.43	3.20	6.42	0.14	0.05
373	0.83	0.10	8.73	2.60	6.02	0.11	0.04

Table V

Uptake of intravenous Copper-64 in normal rat tissue: percent dose per μg copper in tissue

Time after injection (min)	Plasma	RBC	Kidney	Liver	Spleen	Muscle	Brain
4	1.99	0.25	0.49	1.26	1.89	0.17	0.016
5	1.75	0.15	0.41	0.81	2.04	0.13	0.016
6	1.44	0.14	0.33	0.78	1.58	0.15	0.018
14	1.34	0.15	0.49	1.24	1.61	0.12	0.020
32	0.65	0.09	0.47	1.32	1.65	0.12	0.010
44	0.53	0.10	0.82	0.94	1.58	0.10	0.010
66	0.39	0.06	1.06	0.68	1.13	0.08	0.006
79	0.46	0.13	0.96	0.66	1.34	0.11	0.025
100	0.20	0.05	1.00	0.56	0.73	0.06	0.011
133	0.46	0.05	1.35	0.76	1.25	0.04	0.009
180	0.48	0.07	0.69	0.57	1.99	0.007	0.013
245	0.56	0.05	0.75	0.55	0.73	0.032	0.015
267	0.44	0.05	---	---	---	----	----
273	0.74	0.09	1.00	0.44	1.31	0.062	0.010
276	0.39	0.04	0.44	0.38	1.28	0.069	0.013
303	0.64	0.09	0.61	0.56	1.56	0.070	0.014
307	0.44	0.07	0.10?	0.41	1.84	0.066	0.011
311	---	---	0.91	0.69	1.60	0.061	0.010
371	0.63	0.10	1.12	0.51	1.34	0.058	0.010
373	0.38	0.06	0.86	0.41	1.25	0.045	0.009

copper during the first 6 hr. The late rise in the plasma Cu^{64} level may be due to the release of labeled coeruloplasmin from the liver or some other tissue. The short half life of Cu^{64} forbade any attempt to ascertain the state of the copper in the plasma at some latter times.

The kidney level rises slowly and then tends to oscillate. This is undoubtedly due to variations in the animal's excretion. The liver level rises sharply and falls rather slowly after 2 hr. This may indicate a binding of the Cu^{64} in the liver cells. Splenic Cu^{64} levels varied considerably during the entire experiment, probably owing to varying degrees of blood pooling in this organ in the different animals. Muscle and brain have little affinity for tracer carrier-free copper; the uptake is slight and the rate of turnover very slow.

Since each point on the curves represents no more than three animals at the most, and since the animals were not litter mates, one could expect considerable variations in the results obtained.

PART IV. THE DISTRIBUTION OF COPPER-64 PLASMA IN NORMAL ADULT LONG-EVANS FEMALE RATS

Results on the distribution of $Cu^{64}Cl_2$ following intravenous administration are given in Part IV. However, less than 10% of the plasma copper is normally present in labile form. The rest is bound to an $alpha_2$ globulin called coeruloplasmin.[14] Hence the question immediately arises whether or not the protein-bound copper would be distributed differently from $CuCl_2$. Labeled with coeruloplasmin Cu^{64} is very difficult to make because coeruloplasmin normally present in the blood is completely saturated with copper. Furthermore, the half life of Cu^{64} is far too short to allow time for the formation of the labeled coeruloplasmin and its separation from the blood. However, if the $Cu^{64}Cl_2$ is allowed to equilibrate with plasma for a time, the copper is bound to the blood proteins. This plasma can then be used as the protein-bound copper system.

PROCEDURE

Preparation of Copper-64 Plasma

Five ml of plasma was obtained by bleeding a normal rat via heart puncture with a heparin-rinsed hypodermic needle and syringe. The blood was centrifuged and the plasma removed. The radiocopper was then added to the plasma and the mixture allowed to stand for 2 hr. After dialysis the activity of the plasma was determined by means of a sodium iodide crystal counter.

Preparation of Tissues

Tissue samples and blood samples were prepared as described in Part III.

RESULTS AND DISCUSSION

The results of the experiment are summarized in Tables VI and VII. The values for Table VII were obtained by converting the values in Table VI, using the average copper content for each organ as determined earlier.

The whole-blood Cu^{64} level is plotted in Fig. 9. The Cu^{64}-plasma curve is nearly the same as that of $CuCl_2$. When $Cu^{64}Cl_2$ is used the blood copper level appears to rise again after 1 hr, whereas when Cu^{64} plasma is used the blood copper level seems to fall at a very slow rate.

The plasma and erythrocyte Cu^{64} levels are shown in Fig. 10. Several differences from the results observed with $Cu^{64}Cl_2$ are apparent. One must note that this experiment was carried out to 30 hr, whereas in that with the $Cu^{64}Cl_2$ the sampling was stopped at 6 hr. However, it is quite apparent from this work that the erythrocyte Cu^{64} level tends to rise slowly as exchange with the red cell copper occurs or as new erythrocytes are formed containing Cu^{64}. Undoubtedly both occur, with the first probably predominating. In contrast to the study with $Cu^{64}Cl_2$, the plasma curve with Cu^{64} plasma has an apparent peak at about 3 hr. After 4 hr there is a rather slow but steady decrease in the plasma Cu^{64} level. The reason for this difference is not known at this time.

The time distribution curves for Cu^{64} plasma in the kidney, spleen, and liver are shown in Fig. 11. The kidney data are quite scattered, but the concentration appears to reach a maximum at about 3 hr. It is also somewhat higher than that observed for $CuCl_2$. The splenic Cu^{64} level is fairly constant and at a relatively low level. This is in contrast to the work using cupric chloride, the dose level in this experiment is about 1/10 that observed in the cupric chloride work and is much steadier, following a smoother curve. No obvious interpretation can be made at this time, but this variation in the spleen warrants further investigation. The level of Cu^{64} in the liver is nearly the same as that observed using $CuCl_2$.

As in the earlier experiment, the Cu^{64} uptake in the muscle and brain are again very low. In this instance the specific activity in these organs is about the same as was observed with $CuCl_2$.

On a basis of percent dose per g of tissue, at 3 hr the bone marrow contained 0.180%, pancreas 0.53%, the pituitary 2.0 to 5.3%, and the adrenal 0.52%. Data for the adrenal, pituitary, and bone marrow were, in general, uneven and unreliable owing to the low activity observed and the errors involved in sampling, weighing, and counting such small organs. The pituitary dose level was surprisingly high compared with that observed in the other endocrine organs.

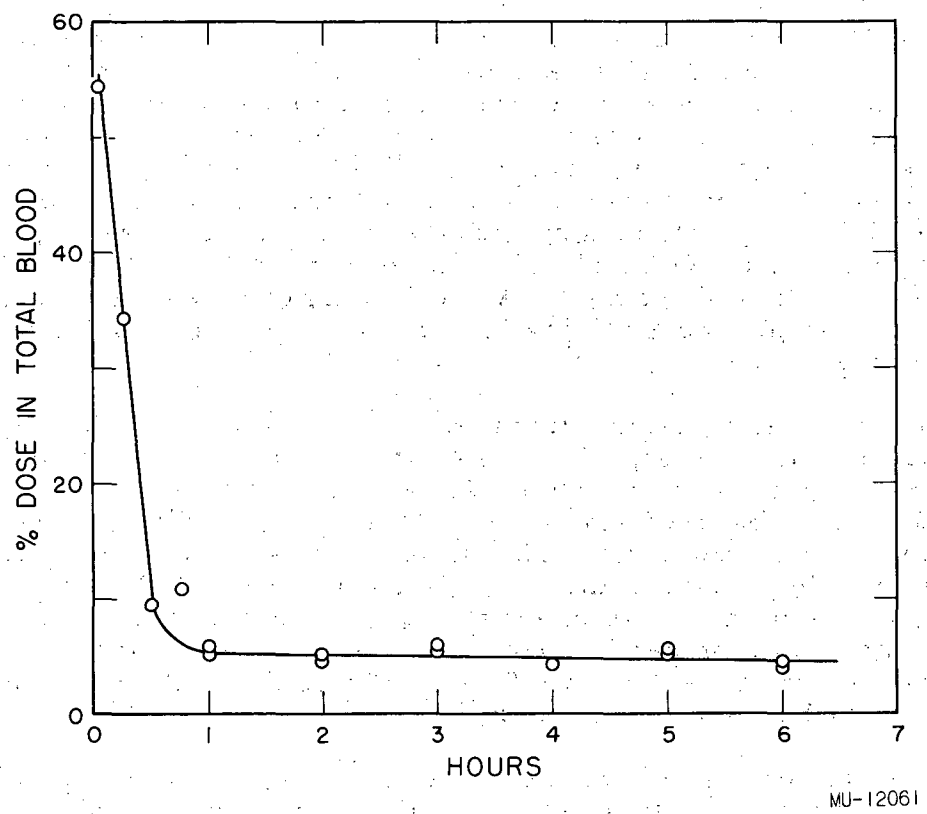

Fig. 9. Uptake of Cu^{64} plasma in the whole blood of normal adult Long-Evans female rats following intravenous injection of plasma proteins tagged with Cu^{64}.

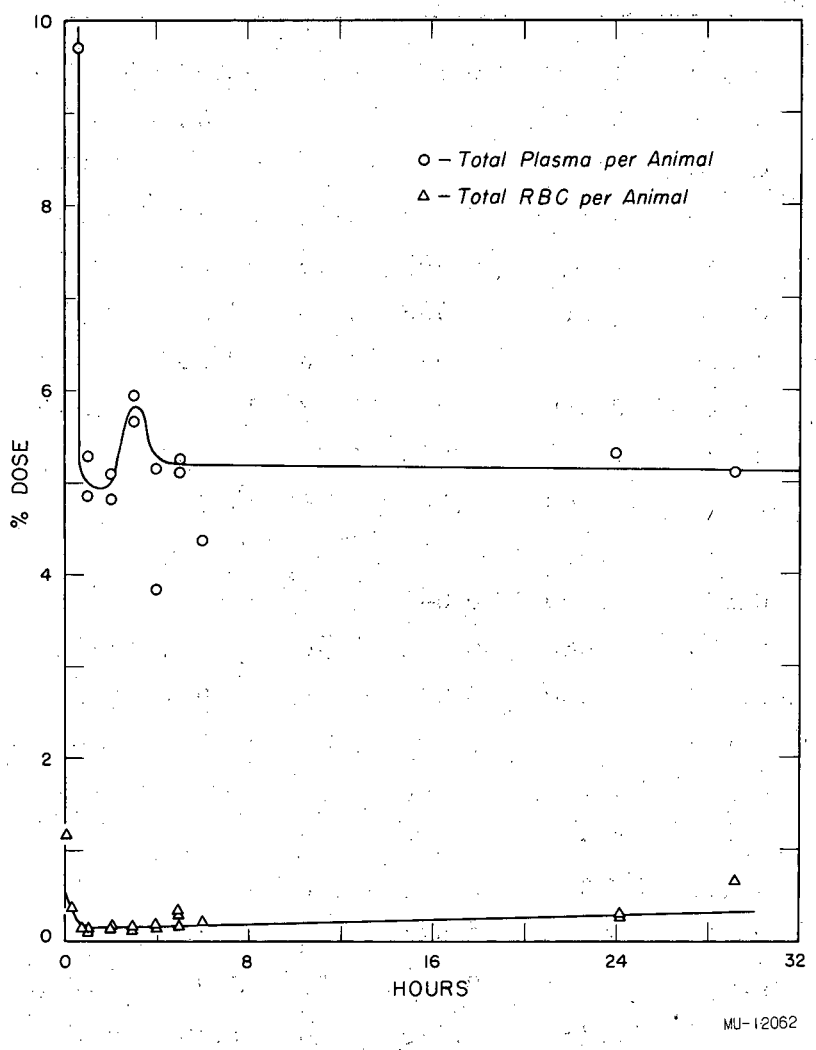

Fig. 10. Distribution of Cu^{64} plasma in total body fluids. (Experimental conditions same as in Fig. 9.)

Table VI

The distribution of Cu^{64} in various tissues. Percent dose per g wet tissue

Time after injection	Muscle	Pancreas	Brain	Liver	Kidney	Total plasma	Total RBC	Spleen
4 min	0.214	0.360	0.184	2.67	2.32	51.22	1.174	0.95
16 "	0.228	0.470	0.110	4.87	4.00	29.86	0.192	0.83
17 "	0.498	0.720	0.147	---	7.65	31.90	0.355	1.17
31 "	0.183	----	0.044	3.14	4.57	9.23	0.073	0.40
31 "	0.144	----	0.053	3.93	7.00	7.16	0.162	0.60
45 "	0.207	0.610	0.068	4.33	11.26	9.71	0.130	0.64
47 "	0.226	----	0.075	4.70	10.64	---	---	0.68
1 hr	0.122	0.680	0.037	2.53	12.88	4.86	0.082	0.39
1 "	0.179	----	0.060	5.76	10.48	5.29	0.104	0.66
2 "	0.183	0.480	0.063	3.66	11.79	5.04	0.124	0.47
2 "	0.179	0.590	0.009	2.83	15.61	4.81	0.140	0.46
3 "	0.124	0.490	0.046	3.21	17.85	5.67	0.124	0.34
3 "	0.130	0.560	0.058	3.40	13.19	5.98	0.142	0.65
4 "	0.149	0.350	0.047	5.44	9.08	5.14	0.153	0.70
4 "	0.110	0.---	0.035	2.84	12.70	3.85	0.115	0.39
5 "	0.122	----	0.056	3.15	17.86	5.25	0.300	0.40
5 "	0.158	0.580	0.054	3.99	7.19	5.11	0.160	0.64
6 "	0.126	0.31	0.049	3.16	16.88	4.37	0.200	0.45
6 "	0.119	0.83	0.044	2.46	5.91	---	----	0.35
5 " 10'	0.264	1.04	0.092	4.21	11.94	---	0.330	0.97
24.5 hr	0.165	0.35	0.075	2.38	6.69	5.32	0.270	0.64
24.5 "	0.204	0.460	0.032	2.80	2.78	7.94	0.310	0.92
29 "	0.130	0.390	0.071	2.56	8.23	5.15	0.670	0.66

Table VII

The distribution of Cu^{64} in various tissues. Percent dose per μg copper

Time after injection	Muscle	Brain	Liver	Spleen	Kidneys
4 min	0.09	0.04	0.42	0.20	0.23
16 "	0.10	0.02	0.77	0.17	0.39
17 "	0.21	0.03	---	0.24	0.75
31 "	0.08	0.10	0.50	0.08	0.45
31 "	0.06	0.012	0.62	0.12	0.69
45 "	0.09	0.015	0.69	0.13	1.10
47 "	0.10	0.017	0.75	0.14	1.04
1 hr	0.05	0.008	0.40	0.08	1.26
1 "	0.08	0.013	0.92	0.14	1.03
2 "	0.08	0.014	0.58	0.10	1.16
2 "	0.08	0.002	0.45	0.10	1.53
3 "	0.05	0.010	0.51	0.07	1.75
3 "	0.05	0.013	0.54	0.14	1.29
4 "	0.06	0.10	0.86	0.15	0.86
4 "	0.05	0.008	0.45	0.08	1.24
5 "	0.05	0.012	0.50	0.08	1.75
5 "	0.07	0.012	0.63	0.13	0.70
6 "	0.05	0.011	0.50	0.09	1.65
6 "	0.05	0.010	0.39	0.07	0.60

CONCLUSION

The foregoing data on the distribution of Cu^{64} in various tissues of the rat after intravenous adminstration of Cu^{64} plasma have been reported with some misgivings. Some differences were observed when these results were compared with those found after the administration of $Cu^{64}Cl_2$. These differences are difficult to explain merely on the basis of the form of the injected copper. It would appear that injected $CuCl_2$ would very quickly combine with the plasma proteins *in vivo* and that the same situation would exist as observed in this experiment. Some slight differences could be expected in the first hour or so, but most of the observed differences occurred at a later time in the experiment. The splenic values are quite different in the two experiments and certainly warrant further investigation.

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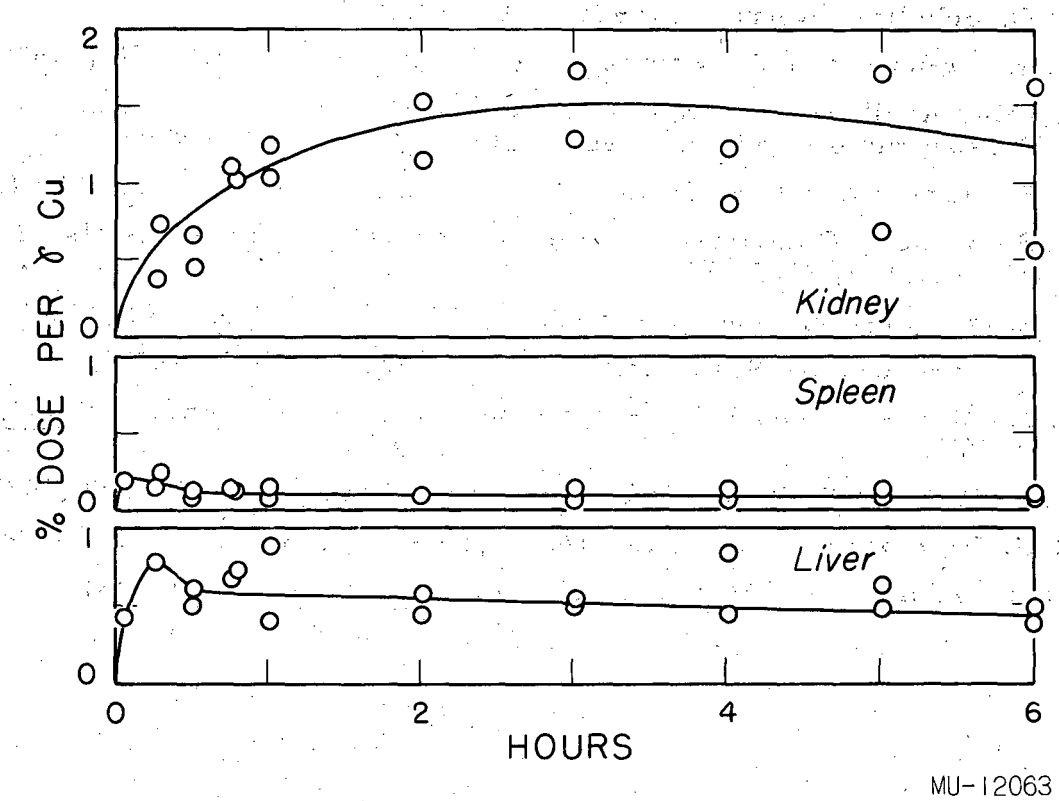

Fig. 11. Uptake of Cu^{64} plasma in kidney, spleen, and liver. (Experimental conditions same as in Fig. 9.)

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