Distribution of Radioactivity Following Intravenous Administration of Trivalent Chromium 51 in the Rat and Rabbit.* (19919)

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The use of chromium^{51} as a biological tracer has recently been reported by Gray and Sterling(1). They suggested that the hexavalent form was taken up by the red cells and the trivalent form combined with plasma proteins *in vivo* and *in vitro*. In view of the possible application of chromium^{51} labelled proteins to biological and medical studies the uptake and distribution of trivalent chromium^{51} alone must be established in order to interpret these studies. Since trivalent chromium is relatively insoluble at the pH of blood, and readily forms colloid complexes, it seemed of interest to determine whether the distribution of radioactivity after administration of trivalent chromium^{51} would be similar to that of other colloids.

Materials and methods. The Cr^{51} was obtained from Oak Ridge and the Abbott Laboratories as the chloride with a specific activity of approx. 100 μc per mg. For injection, an aliquot of this solution was added to isotonic acetate buffer at pH 5.5. Twenty-four male rats, weighing between 180-200 g were used for the first set of experiments. Each animal received 0.2 mg of chromium containing approx. 20 μc of Cr^{51} via caudal vein injection. The animals were sacrificed by exsanguination under anaesthesia at time intervals up to 24 hours. An *additional experiment* was carried out utilizing 2 male rabbits weighing approx. 2 kilos each. The rabbits received, intravenously, 2.0 mg of chromium containing approx. 200 μc of Cr^{51}. One rabbit was sacrificed 12 hours after injection, the other at 24 hours. Of the *tissues* studied, weighed representative portions were placed in vials and digested with a constant volume of 10% NaOH, while bone samples were digested in concentrated HNO_3. Diluted standards of the injected material were prepared in similar vials. Radioactive determinations were obtained by the use of a cup-type torroidal gamma counter.‡ In order to determine *sites of localization* in the bone, the tibia of each rabbit was prepared for radioautography. The bone was frozen in liquid air, sawed longitudinally with a carborundum wafer, and placed on no-screen X-Ray film for 7 days. In order to determine whether the radioactivity was bound to protein in the plasma and various tissues, the proteins were precipitated from the plasma and saline extracts of the liver and spleen homogenates with 10% TCA. Marrow was removed from a portion of the femur, suspended in 10 volumes of isotonic saline, and centrifuged to remove the cellular components. 10% TCA was then used to precipitate the protein dissolved in the saline. The fatty material obtained by centrifuging the marrow suspension was extracted with a 3:1 alcohol-ether mixture.

Results. The results of the rat experiment are summarized in Table I. At the end of the first hour the radioactivity (expressed as % of injected dose per g of tissue) of the bone and liver approximated that of the blood. The highest concentration of Cr^{51} was found in the kidney while the spleen and muscle showed negligible uptake. During the ensuing 24 hours, the Cr^{51} was lost rather rapidly from the blood and liver, and decreased somewhat in the kidney. The bone, however, showed considerable retention, and at the end of 24 hours contained the major portion of the remaining radioactivity. The concentration in the kidney is a reflection of the rapid excretion of part of the injected chromium for 40% of the total activity was found in the urine at the end of 24 hours. No activity was noted in the feces. Only after 24 hours did the concentration of Cr^{51} in the spleen of the rat approach that of blood.

Extension of these studies to determine the sites of localization of radioactivity in the bone showed most of the Cr^{51} to be concen-

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TABLE I. Radioactivity of Tissues of Rats after Injection of $Cr^{51}Cl_3$.

Time after inj., hr	% of inj. dose/g of tissue. Each value represents avg of 4 animals ± stand. error					
	Blood	Bone	Spleen	Liver	Kidney	Muscle
1	1 ± .08	1 ± .11	.28 ± .01	1 ± .10	1.60 ± .10	.10 ± .0
2	1.27 ± .11	1.57 ± .18	.30 ± .14	1.10 ± .10	1.70 ± .10	.09 ± .01
4	1.23 ± .10	1.65 ± .07	.40 ± .10	.80 ± .14	1.65 ± .18	.09 ± .01
6	.57 ± .05	1.39 ± .04	.18 ± .01	.68 ± .13	1.03 ± .19	.06 ± .01
12	.54 ± .03	1.30 ± .01	.26 ± .03	.50 ± .04	1 ± .17	.07 ± .01
24	.34 ± .02	1.70 ± .08	.40 ± .10	.48 ± .04	1.18 ± .16	.08 ± .01

TABLE II. Radioactivity of Tissues of Rabbit after Injection of $Cr^{51}Cl_3$.

Time after inj., hr	% of inj. dose/g of tissue. Each value represents one animal							
	Blood	Bone	Bone without marrow	Marrow	Spleen	Liver	Kidney	Muscle
12	.15	.13	.08	.25	.35	.14	.14	.01
24	.07	.22	.16	.41	.25	.17	.08	.003

trated in the marrow. Since complete removal of the marrow in the rat femur and tibia was difficult, a similar experiment was performed using the rabbit. In the larger animal removal of the marrow was more easily accomplished and large distinct radioautographs of split long bones could be obtained.

The uptake by the various tissues of the rabbit is summarized in Table II. The highest concentration of Cr^{51} of the rabbit tissues studied was found in the spleen, which is in marked contrast to the findings of the rat. The apparent differences between the other values for the rat and the rabbit are due primarily to a difference in total blood volume in relation to organ size and body mass. Most of the Cr^{51} taken up by the intact bone of the rabbit was concentrated in the marrow. This was borne out by a radioautograph of a split tibia (Fig. 1) which showed also an additional concentration of activity in the region of the epiphysial plate.

Studies were undertaken to determine whether or not the Cr^{51} was bound to protein. For this purpose tests were made on the blood serum, liver, spleen, and bone marrow of a rabbit killed 24 hours after injection with radio-chromium. In the plasma, 80-90% of the radioactivity was found to be associated with the TCA precipitate. Paper strip electrophoresis of this plasma at a pH of 8.6 showed Cr^{51} activity associated with all the serum proteins, but in addition to this, a region of non-protein-associated activity was found migrating toward the cathode. Relative to protein bound Cr^{51} in liver and spleen, it was found that the saline extracts of homogenates of these organs contained only 40% of their total radioactivity, and 80-90% of this was precipitable with TCA.

Centrifugation of a suspension of the bone marrow in saline and subsequent determination of radioactivity of the sediment, supernatant, and the top fatty layer revealed that the supernatant and the insoluble fatty layer contained almost all of the activity with only a negligible amount associated with the cellular elements. TCA precipitation of the saline supernatant demonstrated that the Cr^{51} remained with the protein precipitate. After treating the insoluble fatty layer with Bloors solution (3:1 alcohol-ether mixture) to remove the lipids, the radioactivity was retained in the residue. Microscopic examination of marrow smears did not reveal the presence of particulate matter in the macrophages.

Discussion. Chromium, when administered intravenously as $CrCl_3$, in addition to its retention in the plasma, is taken up by the reticulo-endothelial system, primarily the bone marrow. This is shown by the fact that in both the rat and rabbit, the marrow, 24 hours after injection, contains more of the injected Cr^{51} than does any other organ or tissue, not only in total activity but also in activity per g of wet tissue.

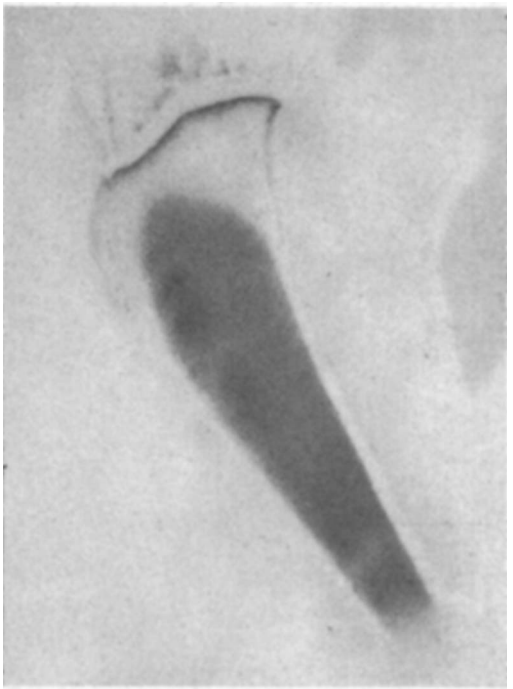

FIG. 1. Radioautograph of split tibia of rabbit 24 hr after administration of Cr^{51}.

The distribution of Cr^{51} throughout the various tissues of these animals resembles the distribution of various radioactive colloids, particularly Yttrium and Zirconium colloids (2,3). Selective localization of colloids in the reticulo-endothelial system is greatly dependent on the particulate size of the colloid. Small size particles localize to a great extent in the bone marrow and spleen. In view of the physical characteristics of the trivalent chromium, these experiments suggest that the chromium might form a small size colloid as indicated by the localization sites. However, we have been unable to demonstrate the actual presence of such colloidal aggregates or particles in the tissues or the serum by ordinary methods.

Since these experiments indicated that much of the chromium was bound to protein in the spleen, liver and bone marrow, it is suggested that the chromium is removed by these tissues and bound to protein located in these sites. It has been shown that trivalent chromium will bind to a variety of serum proteins *in vitro*(1). Some pituitary protein preparations have also been combined with chromium *in vitro*(4), therefore it would not seem unlikely that there would be *in vivo* tagging of tissue proteins as well as those in serum.

In addition, these experiments indicate that the Cr^{51} in the bone marrow is not in the red cell precursors and that the membranes of these cells are impermeable to trivalent chromium as are those of mature red cells.

Summary. Using Cr^{51} as the tracer, chromic chloride was administered intravenously to rats and rabbits for distribution studies. At the end of 24 hours up to 40% was excreted by the kidneys, while the greatest concentration of Cr^{51} occurred in the bone marrow. It is suggested that in addition to binding with plasma proteins, the trivalent chromium deposits in the reticulo-endothelial system in a pattern similar to that of small size colloids of a slow clearing nature. The Cr^{51} in the sites of localization appeared to be bound to protein.

1. Gray, S. J., and Sterling, K., *J. Clin. Invest.*, 1950, v29, 1604.

2. Gofman, J. W., *J. Lab. and Clin. Med.*, 1949, v34, 297.

3. Dobson, E. L., Gofman, J. W., Jones, H. B., Kelly, L. S., and Walker, L. A., *J. Lab. and Clin. Med.*, 1949, v34, 305.

4. Kraintz, L., and Talmage, R. V., unpublished.

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