Metabolism of Cr^{51} by Animals as Influenced by Chemical State.* (20729)

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The knowledge of the factors governing the physiological behavior of a radioisotope in the body may extend the base of its usefulness. Furthermore, such data are needed for the establishment of reasonable maximum permissible levels. Chromium 51 has been used satisfactorily as a tag for plasma proteins or erythrocytes depending upon the valence state (1). As far as is known, only one report on the behavior of this radioisotope in the animal body has appeared in the literature. Kraintz and Talmage(2) have reported the distribution of Cr^{51} in the tissues of 180-200 g rats for intervals up to 24 hours following the intravenous injection of the tracer in isotonic acetate buffer.

Reported in the present paper are studies on the physiological characteristics of this radioisotope over longer time periods as affected by chemical form and route of administration. Data are summarized from more than 200 rats on the uptake, excretion, retention, placental transfer, and tissue distribution of Cr^{51}. The isotope was given in the trivalent state as chloride and chromite and in solutions buffered by acetate and citrate. Hexavalent chromium was given as a solution of sodium chromate. The primary route of administration was intravenous but results are also reported for oral, intraperitoneal and intratracheal administration. In addition, data are presented from two sheep which were intravenously injected with $Cr^{51}Cl_3$. An attempt has been made to correlate the physiological findings with filter paper electrophoretic studies of the dosing solutions.

Methods. Rats of the Rockland and Carworth strains fed commercial rations were used. The age ranged from 7 to 10 months and the minimum weight was 320 g for males and 220 g for females. Solutions for administration to the animals were prepared from a stock of $Cr^{51}Cl_3$ which ranged in specific activity from 200 to 1250 uc per mg. Adjustment of acid concentrations was always made immediately prior to dosing. The chloride, chromite and chromate were given at pH 3 to 4, 11 and 7 respectively and buffered solutions of the isotope were prepared by addition of the $Cr^{51}Cl_3$ to acetate and citrate buffers calculated to be isotonic with physiological saline. For acetate the approximate pH was 5.5 and for citrate 4.0. About 50 microcuries of activity in 0.1 to 0.2 ml of solution was the dose per rat for intravenous, intraperitoneal and intratracheal administration, whereas 5 to 10 times these amounts were used for the oral studies. Intravenous injection was made into the femoral vein, and intratracheal administration was accomplished by exposure of the trachea through a ventral midline incision which allowed insertion of an 18 gauge needle and the deposition of the solution directly into the lungs. Both the latter operations were carried out under surgical anesthesia using nembutal and ether. At the time of administration, diluted reference standards were prepared from the dosing solution. The rats were sacrificed by exsanguination under anesthesia at intervals up to 6 weeks after dosing. Representative tissue samples were placed in tared nickel plated steel planchets and weighed immediately after removal from the carcasses to minimize errors due to dehydration. In all of the intravenously injected animals the site of injection was sampled as a check for quantitative administration. The tissue samples were kept in a frozen state until radioactivity was determined by direct measurement with a scintillation counter. Quantitative fecal and urine collections were made in metabolism cages described by Hansard *et al.*(3). Aliquots of urine were counted directly whereas fecal radioactivity was measured on an aliquot of the ash solution. An additional experiment was carried out with

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TABLE I. Summary of Behavior of Cr^{51} in Rats Following Intravenous Administration in Different Chemical Forms (Tissue Values Represent % of Dose per g Fresh Tissue ± Standard Error; 4 Rats per Value).

	$Na_3Cr^{51}O_2$	$Cr^{51}Cl_3$†	$Na_2Cr^{51}O_4$	$Cr^{51}Cl_3$ buffered by acetate	$Cr^{51}Cl_3$ buffered by citrate
% excreted in urine 4d*	.6 ± .12	15 ±1.3	35 ±1.9	56 ±5.1	75.1 ±9.3
% excreted in feces 4d*	1.6 ± .22	20 ±1.5	17 ±1.6	8 ±3.7	17.4 ±4.8
Liver 4d	9.5 ± .12	5.5 ± .69	.52± .06	.16 ± .01	.31 ± .12
" 42d	6.4 ± .39	2.6 ± .22	.07± .00	.19 ± .01	.17 ± .01
Spleen 4d	17 ±3.9	3.2 ± .35	.91± .16	.30 ± .03	.76 ± .28
" 42d	8.3 ± .61	4.3 ± .45	4.8 ±1.6	.41 ± .05	.23 ± .04
Bone marrow 4d	6.4 ± .84	1.5 ± .23	.56± .14	.17 ± .13	.13 ± .08
" " 42d	.94± .36	1.5 ± .28	.16± .16	<.02	<.02
Tibia epiphysis 4d	1.5 ± .33	1.09± .19	.38± .03	1.0 ± .17	.20 ± .06
" " 42d	.22± .08	.63± .05	<.02	.97 ± .12	.35 ± .06
Lungs 4d	1.6 ± .24	.24± .02	.39± .26	.11 ± .01	.21 ± .14
" 42d	.15± .01	.11± .03	.05± .01	.08 ± .01	<.02
Kidneys 4d	.06± .01	.44± .02	2.1 ±1.1	.62 ± .06	.13 ± .07
" 42d	.04± .00	.26± .03	.24± .05	.25 ± .03	.07 ± .00
Blood 4d	<.02	.05±0	.52± .07	.03 ± .01	<.02
" 42d	<.02	<.02	.04± .01	<.02	<.02
Behavior	Colloidal	Colloidal under these conditions	Probably ionic under these conditions	Complex	Complex
Electrophoretic pattern	Colloidal in serum	Protein bound	Ionic in serum bound by hemoglobin in cells	"	

* Avg of 8 animals ± stand. error.
† Animals receiving $Cr^{51}Cl_3$ were sacrificed at 45 days instead of 42.

two sheep on a normal ration of hay and grain for purposes of comparison with laboratory animals. The activity in the form of $Cr^{51}Cl_3$ was administered by jugular catheter (1 mc per 100 lbs of body weight) and the metabolism procedures were those described by Hansard *et al.*(4).

Results. Table I summarizes the distribution of radioactivity in the tissues and excreta of rats following intravenous injection of Cr^{51} in various chemical forms. The data presented show the Cr^{51} excretion and tissue concentration at 4 days and the tissue concentration of 42 days after dosing. Animals were sacrificed at six hours after dosing and at various intervals thereafter for 42 days. Only the 4- and 42-day values are reported for the liver, spleen, bone marrow, lung, kidney, tibia epiphysis and blood. With the exception of the value for the pancreas following chromate injection, all preparations of radiochromium resulted in concentration values for the pancreas, tibia shaft, incisors and mandible plus teeth which were about 50 percent of the tibial epiphysis value. For chromate the concentration in the pancreas was equal to that in the epiphysis of the tibia.

In preliminary experiments involving intravenous injections of $Cr^{51}Cl_3$ the following were found to contain insignificant concentrations of radioactivity: small intestine, large intestine, hide and hair, brain, eyes, skeletal muscle, ovaries, prostate gland, uterus and testes.

Sodium chromite. It is clearly evident that the Cr^{51} from sodium chromite was picked up in extremely large quantities by the organs of the reticulo-endothelial system and the liver accumulated approximately 90% of the dose. The excretion values were correspondingly low. A summation of the excretion values and the organ values gave recovery of the total dose within ±10%. Considering the extremely small amount excreted, and high liver and spleen values at 21 and 42 days after injection, it may be considered that 100% of the dose was held in the body during the early times after injection. The tibial epiphysis showed a higher concentration of Cr^{51} than did the other skeletal tissues, and the activity was slow in leaving all of the organs except the

blood and lungs. The liver and spleen contained 33 and 50% of their 4-day activity at the end of 42 days, respectively, whereas the lung showed only 10% of its 4-day activity. No detectable radioactivity was found in the blood after 21 days.

Chromic chloride. The radioisotope was found to be in highest concentration in the liver, spleen, and bone marrow, undoubtedly a reflection of reticulo-endothelial pickup but not as great as with sodium chromite. Although there was considerable variation between animals, it is obvious that the activity once deposited was removed very slowly. The value for the liver at 45 days was 35% of its maximum value which was observed at 24 hours after dosing. In contrast, the concentration in all of the other tissues remained practically constant throughout the 45-day period with the exception of the blood and lungs. The blood contained practically no activity after the seventh day and the lungs at 45 days contained about 15% of their maximum value which was observed at 6 hours following injection. The skeletal tissues showed practically equal amounts of activity in the tibial shaft, mandible plus teeth and incisors with approximately twice as much in the epiphysis of the tibia. Interestingly, the spleen appeared to gain activity over the 42-day period. With $Cr^{51}Cl_3$ other routes of administration were studied, but only the overall results are presented. Seven days after intratracheal administration it was found that 55% of Cr^{51} was excreted in the feces and 7% in the urine which indicated that the material probably had been transported from the region of deposition in the lungs to the oral cavity where swallowing took place and subsequently the isotope was handled as after ingestion. Tissue concentrations indicated that probably less than 5 percent was absorbed from the lungs. The pattern of distribution in the tissues was more like that of the buffered Cr^{51} solutions. Orally administered $Cr^{51}Cl_3$ was almost totally excreted in the feces at the end of 4 days. Less than 0.5% of the dose was absorbed from the gastrointestinal tract as indicated by tissue distribution studies. Although the urinary excretion indicated a higher level of absorption, the urine radioactivity was probably due to fecal contamination. After intraperitoneal injections of $Cr^{51}Cl_3$, extensive necrosis developed at the site of injection due to the high acidity of the solution and the tissue uptake was variable and extremely small.

Sodium chromate. The major uptake was by the liver but in much lower amounts than for the chromic chloride or sodium chromite and the excretions were correspondingly higher. Twenty-five percent of the injected activity became deposited in the liver in 30 minutes after administration and at the end of 42 days less than one percent was present, which again represented a marked difference in behavior from the chromite and chloride. All of the other organs and tissues lost activity with time except the spleen which seemed to gain. The skeletal tissues showed relatively small concentrations of Cr^{51} and the tibia epiphysis contained twice the concentration of the other skeletal tissues sampled during the earlier time periods. Interestingly, the concentration in blood at the end of 21 days was about one-fifth of the 30-minute value but declined to practically zero at 42 days. This greater retention by blood of hexavalent Cr^{51} versus trivalent Cr^{51} was probably because the former is bound by erythrocytes which turnover relatively slowly and the latter by plasma proteins. The chromate solution, however, is the only one which gave evidence of blood radioactivity at the end of 42 days.

Chromic chloride in acetate and citrate buffers. The results following intravenous injection of $Cr^{51}Cl_3$ when isotonically buffered by acetate (pH 5.5) and citrate (pH 4) followed the same general pattern. The organ of greatest accumulation was the liver for both solutions. On a concentration basis, however, there seemed to be a difference between the 2 solutions, the epiphysis of the tibia showing the highest accumulation for acetate with the spleen showing the highest for citrate. The concentrations in all of the tissues and organs except for the lungs and blood tended to remain constant throughout 21 days and appeared to have declined in some of the other tissues by the end of 42 days. The blood contained no detectable activity at the end of 21 days. In the case of citrate, all of the bones

TABLE II. Distribution of Cr^{51} in Sheep Following Intravenous Injection of $Cr^{51}Cl_3$.

Tissue	% of dose per g fresh tissue $\times 10^4$ Sheep 107	Sheep 68
Blood	16.6	7.5
Lung	114.	727.
Liver	78.8	28.4
Spleen	73.9	33.2
Bone marrow	42.0	25.6
Kidney	55.7	43.4
Lymph node	40.8	20.9
Pancreas	6.7	3.3
Loin muscle	2.6	.61
Gastrocnemius muscle	1.1	.4
Vertebra	27.6	29.4
Metatarsus shaft	4.3	5.2
Metatarsus epiphysis	9.2	9.5
Incisors	8.1	9.4
Angle mandible	33.3	32.5
White bone marrow	3.8	1.7
Excretions (total % of dose in 7 days)		
Urine	32.7	60.3
Feces	3.3	2.2

were about equal in Cr^{51} concentration whereas with acetate, there was a higher deposition in the tibial epiphysis than in any other tissue. The lower uptake by the organs of the reticulo-endothelial system in the case of these 2 solutions is probably the result of relative absence of large particles. Of special interest was the large urinary excretion of the activity when administered intravenously in the buffered solutions. Fifty-six percent of the acetate buffered Cr^{51} was excreted in the urine in 4 days and 8% in the feces. Respective values for the citrate buffered solution were 80% and 13%.

Placental transfer. Insignificant amounts of Cr^{51} were found to cross the placenta of rats in the 24 hours following intravenous injection regardless of the chemical form of the isotope, its valence state, the gestational stage of (15 to 20 days) the animal or the number of fetuses per litter. In no case did the recovery of Cr^{51} per total litter exceed 0.13% of the injected dose. In all of the determinations, radioactivity was measured only on the fetuses exclusive of the placental membranes or fluids.

Chromic chloride in sheep. Sheep 107, weighing 92 lb and Sheep 68, weighing 120 lb, both 5-year-old ewes, were dosed intravenously by jugular catheter with $Cr^{51}Cl_3$ and sacrificed 7 days later for tissue distribution studies. During the 7-day interim all excretions were collected for radioassay. The results are presented in Table II. There was considerable variation in the results between the 2 animals which may have been caused by a difference in the mass of chromium contained in the 2 doses since Sheep 107 received 4 mg of chromium, whereas Sheep 68 received 8 mg. Though, in preliminary experiments, the mass of chromium injected was found to affect the uptake of Cr^{51} by the lungs of rats, it is questionable whether the differences in the dosage masses of the sheep were sufficient to cause the differences in lung uptake observed. Both sheep showed maximum retention in the lungs with the liver being next highest. The pattern of tissue distribution was quite similar for both animals although some of the actual values were not in close agreement. Sheep 107 deposited 7% of the activity in her lungs and 5% in the liver, whereas the respective values for Sheep 68 were 33% and 2%. The spleen and bone marrow showed appreciable concentrations of Cr^{51} and the lymph nodes fell in a range comparable to the bone marrow, vertebrae and angle of the mandible. The white bone marrow was extremely low in activity in contrast to the red bone marrow. Of the skeletal tissues studied, the vertebrae and angle of mandible were highest and the tibia shaft and epiphysis and incisors were very low. All of the muscle tissues sampled were extremely low in activity. The excretions also showed considerable variation between animals with Sheep 107 excreting 33% in the urine and 3% in the feces in 7 days. Respective values for Sheep 68 were 60.0 and 2.2. Over 80% of the 7-day urinary excretion took place on the first day after injection.

Paper electrophoretic studies on various Cr^{51} solutions. Paper electrophoresis was performed with the apparatus designed by Gordon *et al.*(5) using 0.05 Na barbital or homologous serum as a buffer. The procedure followed and a more complete report of the findings are included in another publication (6). Sodium chromite incubated with rat

serum and applied to paper strips buffered by the homologous serum was found to lack mobility which supported the view that the chromite was colloidal in this medium. This evidence led to some limited studies in which sodium chromite was incubated with rat serum and intravenously injected. A high uptake by the reticulo-endothelial system resulted which strengthened the view that chromite behaved as a colloid *in vivo*. Chromic chloride incubated in rat serum and applied either to a barbiturate or serum buffered strip moved predominantly with the serum protein indicating protein binding. These results were surprising and led us to intravenous injection of the incubated mixture which resulted in less than 10% of the activity being taken up by the liver in contradistinction to approximately 50% when the solution was injected directly into the blood stream. Apparently direct injection of the solution caused the formation of particles which were phagocytized before protein binding could take place. When chromic chloride was complexed with acetate before incubation with rat serum the resulting electrophoretic patterns did not show appreciable protein binding. Sodium chromate incubated for 16 hours with whole rat blood showed the activity in the red cells to be bound to the hemoglobin fraction. Chromate was found to move as a free ion after incubation in serum.

Discussion. These experiments emphasize the variations in physiological behavior of chromium which may be brought about by its physico-chemical state. The behavior of radiochromium varied as a function of chemical form prior to injection, the solvent used and the route of administration. These factors assume great importance with elements that are prone to form colloids as pointed out by Schubert(7). Differences in the physiological behavior of the chromium in the animal resulting from the various solutions were in fair correlation with the predictions one might make from the paper electrophoretic patterns observed in serum and lysed erythrocytes *in vitro*(7-11).

Trivalent Cr^{51} might be expected to differ in its physiological behavior from the hexavalent form since the former is bound by plasma proteins and the latter by erythrocytes(1). However, the variations in the behavior of trivalent chromium require further explanation. The trivalent chromium, namely chromite, chromic chloride, and buffered chromic chloride showed respectively an increasing excretion and a decreasing pickup by the reticulo-endothelial system. This behavior may be explained on the basis of the colloidal nature of the chromite, the protein binding of the chromic chloride, and the complexing action of the buffers. Since acetate and citrate apparently form complex ions with chromium there is a possibility that acetate and citrate compounds might be used as therapeutic agents to increase the urinary elimination of Cr^{51} from the body as has been done with plutonium(7). The preparation showing greatest deposition in the reticulo-endothelial system was the chromite which apparently formed large particles as shown by paper electrophoresis, and for this reason would be expected to be so distributed *in vivo*(7-11). The liver did not always show the highest concentration of Cr^{51}. In the case of the acetate buffered Cr^{51} the epiphysis of the tibia showed the highest concentration. Thus, an indication is given as to the manner in which the localization of Cr^{51} may be governed by the chemical form used. For example, if a high liver concentration were desired, sodium chromite would be the logical compound to use. On the other hand, a buffered chromic chloride solution, or chromic chloride after incubation with serum would yield a more generalized distribution. Of particular importance from a health physics viewpoint is the observation that once the radiochromium was deposited in an organ it was slow in leaving. The fact that $Cr^{51}Cl_3$ incubated with serum showed a much lower reticulo-endothelial uptake after injection as compared to that of the solution itself points out the effects which form of injection may have on the experimental results.

Kraintz and Talmage(2) observed an apparent species difference between rats and rabbits in the behavior of Cr^{51} buffered with acetate. In their studies the rat showed the highest concentration of Cr^{51} in the bone at 24 hours, whereas in rabbits the spleen was

highest. In our studies with rats, the organ of maximum uptake following intravenous injection of acetate buffered $Cr^{51}Cl_3$ was also the bone. With $Cr^{51}Cl_3$ in rats and sheep the organs of maximum concentration were the liver and lung, respectively. The high uptake by the lung of the sheep may have resulted from increased phagocytosis of particulate material by the lungs of these animals due to a lung infection which had been prevalent in the flock.

Summary. The physiological behavior of trivalent and hexavalent Cr^{51} has been observed over a 45-day period in rats as affected by chemical form and route of administration. When intravenously administered as sodium chromite, practically 100% of the dose was picked up by the organs of the reticuloendothelial system. Chromic chloride resulted in a pickup by the liver of about 55% of the dose. When $Cr^{51}Cl_3$ was added to isotonic acetate and citrate buffers, less than 5% of the dose reached the liver and most of the activity was excreted in the urine. The use of sodium chromate solutions resulted in about 25% of the dose reaching the liver. However, the activity left this organ rapidly in contradistinction to the other forms studied. Oral, intraperitoneal and intratracheal administration of $Cr^{51}Cl_3$ were also studied. None of the forms of Cr^{51} used were found to cross the placenta of rats ranging from 15 to 20 days in gestation. In addition, data are presented from 2 sheep intravenously dosed with $Cr^{51}Cl_3$. The above physiological observations are correlated with the colloidal behavior, complex formation, protein binding, and red cell binding, and are in general agreement with interpretations of filter paper electrophoretic studies of the dosing solutions.

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Decreased Resistance of Vitamin B_{12}-Deficient Rats to Cold Stress.* (20730)

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Considerable data are available indicating that requirements for a number of nutrients are increased and resistance to cold stress is decreased in the nutritionally-deficient animal. In the present communication data are presented on 1) the effects of prolonged exposure to cold on the vit. B_{12} requirement of the rat and 2) the effects of vit. B_{12} deficiency on resistance to cold stress in the rat.

Procedure. The basal ration employed in the present experiment consisted of expeller-processed soybean flour,† 66%; sucrose,

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† Soy Flour Lo-Fat, A. E. Staley Mfg. Co., Decatur, Ill.