Distribution of Radioactive Silver Colloids in Tissues of Rodents Following Injection by Various Routes.* (18017)

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During the last 4 years, colloidal manganese dioxide and colloidal gold containing the radioactive isotopes of the metals have been used in this laboratory in therapy of diseases of the reticulo-endothelial system(1). Colloidal radioactive gold has also been employed in a direct infiltration of tumor masses(2). Since Ag^{111}, a strong β-emitter with half-life of 7.6 days, meets the criteria essential for isotope therapy(3), it was deemed desirable to study the distribution of colloids prepared from this isotope. Voigt(4) used chemical analysis in his studies on colloidal silver localization. Gross microscopic localization was obtained by Shouse and Whipple(5) in their studies on the effects of intravenous injection of colloidal silver on the hematopoietic system in dogs.

Preparation of silver colloids. Due to the non-availability in the literature of methods for preparation of commercial silver colloids, 2 empirically devised procedures were used. Ag^{111} was obtained from neutron-bombarded palladium shipped from the Oak Ridge pile and separated by the method of Rouser and Hahn(6). The acidity of the HNO_3 solution of Ag^{111} was reduced by repeated alternate drying and solution in water and then made up to a convenient volume in a volumetric flask. One ml of a high dilution of the $Ag^{111}NO_3$ in the flask was counted over a period of 2 weeks on the thin mica end-window Geiger-Muller counter and followed the decay curve of Ag^{111} ($T/2 = 7.6$ days). A gelatin-protected silver colloid was prepared by thoroughly mixing 4 ml of the $Ag^{111}NO_3$ with 2 ml of liquid 8% gelatin.† The addition of 4 ml of N/40 NaOH resulted in a brownish appearance due to silver oxide formation. Ascorbic acid solution was added dropwise with thorough mixing to pH 7.4. The added ascorbic acid was considerably more than that calculated to effect reduction of the silver nitrate (containing 1 mg of carrier Ag per ml plus a minute amount of Ag^{111}). This preparation was diluted to 25 ml and allowed to stand overnight and on the next day (date of injection) 1 ml had a count of 310,000 per minute. A dextrin-protected colloid was prepared for the rat series. Separation and preparation of the 25 ml of $AgNO_3$ containing some Ag^{111} was carried out in the same way as with the gelatin-protected colloid. Ten ml of this solution was dried and dissolved in 5 ml of water and mixed thoroughly with 10 ml of a freshly prepared 2% dextrin solution. Next 12.5 ml of N/40 NaOH was added, followed by dropwise addition of ascorbic acid to a pH of 7.85. The dark brownish-red colloid was allowed to stand overnight and was filtered the next morning with loss of some metallic silver. One ml of the filtered colloidal preparation at this time possessed an activity of 1.72×10^6 counts per minute.

Experimental procedure. The gelatin-silver colloid was injected intraperitoneally into 5 white laboratory mice. One ml of the dextrin-silver colloid was injected into the caudal vein of 10 white laboratory rats, intramuscularly

* This work was carried out under contract with the Division of Biology and Medicine, United States Atomic Energy Commission.

1. Hahn, P. F., and Carothers, E. L., *Nucleonics*, 1950, v6, 54.

2. Hahn, P. F., Goodell, J. P. B., Sheppard, C. W., Cannon, R. O., and Francis, H. C., *J. Lab. and Clin. Med.*, 1947, v32, 1442, 1453.

3. Hahn, P. F., *Handbook of Artificial Radioactive Isotope Therapy*, Academic Press, New York, 1950.

4. Voigt, J., *Biochem. Z.*, 1914, v63, 409.

5. Shouse, S. S., and Whipple, G. H., *J. Exp. Med.*, 1931, v53, 413, 420.

6. Rouser, G., and Hahn, P. F., *Ind. and Eng. Chem.*, 1950.

† Knox P-20 gelatin prepared from collagen was furnished through the courtesy of Dr. D. Tourtellotte of the Knox Gelatin Protein Products Co., Camden, N.J.

TABLE I.

Distribution of Ag^{111} Incorporated into a Gelatin-Protected Silver Colloid Injected Intraperitoneally in Mice.

		% total		% total		% total		% total		% total
	CPM	CPM inj.	CPM	CPM inj.	CPM	CPM inj.	CPM	CPM inj.	CPM	CPM inj.
Mouse	A	A	B	B	C	C	D	D	E	E
Inj. sacrifice interval	22 hr		24 hr		30 hr		31 hr		32 hr	
Liver	25540	16.5	7813	6.3	7796	8.4	3865	4.2	11560	7.5
Spleen	1620	1.0	2188	1.8	1038	1.1	5580	6.0	2145	1.4
Heart	43	.0	43	.0					38	.0
	Total	Total	Sternum and 2 femurs		Sternum and 2 femurs		Sternum and 2 femurs		Total	Total
Bone	5350	3.5	255	0.2	108	0.1	197	0.2	4120	2.7
Skin (except of head)	647	0.4							1724	1.1
G. I. (tract and contents)	56500	36.5	13940	10.4	18878	20.1	28100	30.2	28700	18.5
Kidney	263	0.2	288	0.2					325	0.2
Muscle	15820	10.2							15600	10.1
Lungs	618	0.4	420	0.3	681	0.7	141	0.2	1060	0.7
Residual	3970	2.6							9167	5.9
CPM recovered	110471								74401	
% recovery		71.3								47.8

Mice A and E received each 0.5 ml of the gelatin-protected colloid with a count of 155,000; Mouse B received 0.4 ml with a count of 124,000; and Mice C and D received 0.3 ml with a count of 93,000. All counts are corrected to time of injection.

into the right hamstrings of a group of 3, and by cardiac puncture into a group of 2 without any observable toxic manifestations. Chemical analysis indicated that 1 ml of the dextrin-silver colloid contained only about 0.1 mg of silver.

The animals were sacrificed at varied intervals following injection as indicated in Tables I and II. The exsanguinated blood was collected in the beaker reserved for residual carcass and in 3 cases 1 ml was taken for counting. Organs, tissues, and extremities were dissected immediately, weighed and placed in beakers. Dry ashing was carried out in a muffle at from 625-650°C until complete. The ash was then dissolved in a minimum amount of concentrated HNO_3. In the case of organs known from preliminary experiments to concentrate only a small amount of silver and also those with little inorganic salt content in the ash, this HNO_3 solution was directly decanted into a seamless tin counting cup,‡ the bottom of which had been layered with concentrated NH_4OH to prevent corrosion of the cup by HNO_3. Decantations of water and ammonium hydroxide washings of the beakers were made into the counting cup. The NH_4OH was allowed to stand several hours in the beaker to dissolve silver precipitated by tissue chlorides. With organs such as the liver which concentrate large amounts of silver and also with bone, muscle, and residual carcass, in which the inorganic salt content after ashing is high, it was necessary to make decantations into a volumetric flask. The final solution in the flask should be somewhat acid to prevent precipitation of inorganic salts which occurs if the volume of NH_4OH washings renders the solution alkaline. After thorough shaking aliquots of 1 to 3 ml were taken and transferred to counting cups containing a thin layer of NH_4OH. After these samples were dried under an infrared lamp, their activities were determined by placing them under a standard shielded bell-type end-window Geiger-Muller counter.

Experiments. The results obtained with

‡ ¼-oz. size obtained from Buckeye Stamping Co., Columbus, Ohio.

TABLE II.
Distribution of Ag^{111} Incorporated into a Dextrin-Protected Silver Colloid Injected into Rats.

	Intravenous group										Intramuscular group			Cardiac puncture group	
Rat No.	IV-1	IV-2*	IV-3	IV-4	IV-5	IV-6	IV-7	IV-8	IV-9	IV-10	IM-1	IM-2	IM-3	CP-1	CP-2
Inj.-sacrifice interval, days	1 da.	5 da.	6 da.	8 da.	9 da.	12 da.	12 da.	12 da.	14 da.	14 da.	2 da.	6 da.	13 da.	5 da.	9 da.
Liver	1219.00	124.60	502.40	170.30	118.20	335.80	152.40	106.00	106.20	163.10	25.70	16.50	Lost	141.00	77.80
Spleen	46.61	10.26	9.95	6.94	10.40	Lost	7.26	12.32	3.81	5.12	4.50	2.35	5.76	10.72	2.02
Heart	.56	.24	.09	.15	.29	.44	.13	.28	.28	.04	.23	.15	.13	Lost	.27
Bone		RF .38	17.10T			39S	6.40T			.65S	.20S	.22S		31.40T	26.20T
Inguinal Lymph nodes	.14	.49†	.08	.08							.21				
Skin‡	52.20	8.59	52.60	44.80	42.40	31.80	25.20	14.25		19.20	36.30	23.25	13.00	59.40	41.60
G.I. tract and contents	192.70	19.53	33.70	26.70	45.90	63.70		25.10	23.90					64.92	45.35
Mesentery	5.42				1.00	3.85			2.05						
Kidney		4.45	1.79				1.18	.71		.54				3.09	4.85
Lungs						4.25			3.23		.96	.49		8.02	2.05
Right leg (site of i.m. inj.)											1529.00	1248.00	1089.00		
Left leg											4.20	4.70	.73		
Residual carcass	70.65	233.70	51.58	44.18	72.90			48.31			62.50	54.20	35.80		118.60
CPM recovered	1587.28	1339.80*	669.29	294.18	291.09			218.88§			1663.80	1349.86			360.08
% recovery	92.3	77.9	38.9	17.1	16.9			12.7			96.7	78.4			20.9

Rats received 1 ml of a dextrin-protected silver colloid calculated to contain 1.72×10^6 CPM on date of injection by the route indicated. Thousands of CPM for all tissues are corrected for decay to the date of injection (8/4/49) for comparison.

T—Total; S—Sternum; RF—Right Femur.

1 ml of blood in IV-2 gave 1360 CPM; in IV-6, 684 CPM; in IV-10, 160 CPM. Total recoverable muscle in IM-1 gave 50.60 thousand CPM; in IM-2, 41.34 thousand CPM. 90 mg of fecal pellets gave 1020 CPM in IV-4; 819 mg, 1100 CPM in IV-7; 300 mg, 850 CPM in IV-10.

* Rat IV-2—From results, it is probable that the injection for the most part was subcutaneous in the tail. (936.20 thousand CPM in tail.)

† Includes axillary nodes.

‡ Exclusive of head skin.

§ Includes 11.91 thousand CPM in the tail.

the intraperitoneal injections into mice are summarized in Table I. Excluding the intestinal tract through which elimination is taking place, the liver leads in content of silver, followed by muscle, bone, spleen, skin, and lungs. However, when activity is referred to counts per minute per unit of wet weight, the order of decreasing activity is found to be spleen, liver, bone marrow, lungs, muscle, and skin. The highest silver concentration is thus found in organs of the reticulo-endothelial system and resembles the distribution of colloidal iron following intravenous administration(7). In determining the bone marrow activity, it was assumed that the bone content of silver is primarily in the marrow(5) and that the bone marrow weight is 2-3% of the body weight(8). The feces were not collected, but complete recoveries of the silver were attempted from the carcasses of mice A and E. The lower percentage (47.8%) of silver activity recovered from Mouse E at 32 hours after injection as compared with recovery of 71.3% from Mouse A 22 hours after injection, together with the high content in the gastro-intestinal contents suggests elimination of ionic silver by that route. This elimination via the gastro-intestinal tract may be due: 1. to a large ionic silver content in the colloidal preparation§, 2. to possible *in vivo* conversion of colloidal silver into the ionic form, or 3. to elimination of colloidal silver. There is no known evidence for the elimination of more than trace amounts of silver in the urine.

The data obtained with the injection of the dextrin-silver colloid into rats is presented in Tables II and III. The most striking results were obtained with the 3 rats receiving the colloid intramuscularly in the right hamstrings. Almost 90% of the injected activity was recovered in the vicinity of the site of injection in one rat 6 days following injection and 63.3% of the injected activity was re-

TABLE III.

Counts Per Minute Per Milligram of Wet Weight Tissue in Rats Injected with Silver-Dextrin Colloid.

Group	Intravenous										Intramuscular			Cardiac puncture	
Rat	1	2	3	4	5	6	7	8	9	10	1	2	3	1	2
Liver	292.9	16.1	69.4	22.3	15.5	44.8	15.8	11.7	12.6	26.9	5.7	2.4	—	16.9	13.1
Spleen	350.5	31.1	35.3	16.3	28.1	—	11.7	28.9	10.1	22.8	19.9	12.7	11.6	27.7	6.2
Heart	1.7	0.4	0.1	0.2	0.3	0.7	0.1	0.4	0.4	—	0.4	0.3	0.2	—	0.5
Lungs						3.8			3.3		1.2			7.3	2.4
Bone Marrow			4.1											7.0	7.0
Mesentery	7.5				1.7	3.9			1.9						

7. Hahn, P. F., and Whipple, G. H., *Am. J. Med. Sci.*, 1936, v191, 24.

8. Maximow and Bloom, *Textbook of Histology*, 5th Edition, p. 85.

§ Conductivity measurements for ionic silver content were not carried out.

covered in another rat 13 days following injection. This high degree of localization suggests that radio-silver might be of considerable value in a direct infiltration of a tumor mass. Also, in the intramuscular group in the two animals for which data are available for both liver and spleen, the spleen possessed considerably greater activity per milligram of wet weight than the liver. Slower release to the circulation is possibly involved.

With the intravenous and cardiac puncture injections of the dextrin-silver colloid, again there is seen a diminishing percentage of injected activity recoverable with increasing injection-sacrifice intervals. Also, activity levels in the various organs show a general downward trend. Excretion of silver in the feces is again indicated by the high activity of the gastro-intestinal tract and also by the activity found in fecal pellets passed during the ether anesthesia preceding death. The activity of the stomach and its contents was measured in three animals and found to be insignificant. Assuming silver excretion in the bile, it seems probable that some is reabsorbed since the mesenteric content of silver per unit of wet weight is several times that of the heart or residual carcass. The silver content of the inguinal lymph nodes is slight. The blood level of silver is seen to decrease with time. The continued presence of silver in the blood is suggestive of translocation of silver deposited in the various tissues.

Reference to Table III indicates high but variable concentrations of silver in the liver and spleen. The silver concentrations of the liver and spleen for all the rats in the intravenous group except 1 and 6 and for the 2 in the cardiac puncture group were averaged. No. 1 was omitted due to the brevity of the injection-sacrifice interval and No. 6 since the spleen sample was lost. The average for liver was 22.0 cpm/mg of wet weight and for spleen was 21.8. This seems to indicate that, in spite of wide individual variations, the phagocytic propensities of the 2 organs per unit weight are approximately the same for colloidal silver if a large group is considered. Bone marrow, lungs, and mesentery followed in order of decreasing activity. It is of interest, however, that with the mice the activity of bone marrow followed quite closely that of spleen and liver; whereas, with the rats it was considerably lower. However, the colloidal preparation, species, and mode of injection were different.

Summary. 1. Intramuscular injection of a dextrin protected radio-silver colloid results in a high concentration of the injected material at the site of injection. 2. Intravenous injection of a dextrin-silver colloid into rats and intraperitoneal injection of a gelatin-silver colloid into mice result in deposition of the silver in highest concentration in the reticuloendothelial system. 3. Considerable silver was eliminated through the intestines and feces. The possible causes of such elimination under the conditions of this experiment are discussed. 4. A method of preparing tissues of small bulk for determining radio-silver activity with the Geiger-Muller counter is described.

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Experimental Pulmonary Edema. IV. Pulmonary Edema Accompanying Trauma to the Brain.* (18018)

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Boyd in his textbook notes that "Pulmonary edema is a very common condition" and ". . . occurs in a bewildering variety of conditions . . ."(1). One of these is skull fracture

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1. Boyd, W., *The Pathology of Internal Diseases*, 4th Ed., Lea & Febiger, Phila., 1944, 213.