Lipophilic Colloids of Yttrium-90: Preparation and Tissue Localization in Animals Following I.V. Administration. (26666)

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Essentially all radiocolloids which have been used in palliation and treatment of leukemia and other malignancies have been inorganic and hydrophilic in nature. Such materials (*e.g.* Au-198, chromic phosphate-P-32, yttrium phosphate-Y-90, etc.) are generally accumulated in reticuloendothelial tissues of the body following *i.v.* injection, presumably by phagocytosis. Thus, liver, spleen, bone marrow, and lymph nodes are principal sites of colloid deposition; lung, kidney, and adrenal receive intermediate amounts of activity; while blood, muscle, testis, ovary, brain and other tissues are essentially devoid of radioactivity. In addition to this typical tissue distribution pattern, some radiocolloids may partially dissociate within the body and the resultant "ionic" dissociation products be deposited in bone.

The particle size of the colloid micelle has sometimes been regarded as the property most important in determining the site of tissue localization. Hahn(1) however, recognized the importance of surface coating and contrasted the distributional behavior of gold colloids and silver-coated gold colloids. Lahr (2) also observed this phenomenon and reported different tissue depositions for colloids coated with certain organic materials. In the light of these reports, it was felt that a lipophilic colloid might demonstrate a unique distributional pattern and might, therefore, have unusual therapeutic properties.

Yttrium-90 has been injected *i.v.* with a variety of organic chelating agents(3,4,5). These products were all quite soluble, and their molecular dimensions such that they could hardly be classified as colloidal aggregates. In this investigation, well-dispersed lipophilic colloids have been prepared from yttrium-90 by chelation with (1) oleoylacetone and (2) dodecyliminodiacetic acid. Their tissue localization and excretion have been evaluated in experimental animals.

Materials and methods. Yttrium-Oleoylacetone colloid. Oleoylacetone [CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-CO-CH_2-CO-CH_3, Dow Chemical Co.] chelated yttrium and formed a lipophilic emulsion as follows: One ml of 0.02 M YCl_3 was added to 4.5 ml of an acetone solution containing 0.46 ml of oleoylacetone. This clear solution was then mixed with 10 ml of a solution containing 10% glycerine and 0.3% soluble egg lecithin in distilled water. The turbid emulsion was boiled 5 minutes to expel acetone and allowed to cool. The pH was adjusted to phenolphthalein end-point with 1 N. NaOH. The final product was autoclaved 15 minutes and stored at 5°C.

Yttrium-dodecyliminodiacetic acid colloid. Dodecyliminodiacetic acid (D.I.A.) was synthesized according to Stein(6). Seven ml of 0.02 M. D.I.A. was adjusted to phenolphthalein end-point with NaOH. To this was added dropwise 1.0 ml of 0.02 M. YCl_3. Dilute NaOH was added simultaneously with stirring to maintain alkalinity. To assure isotonicity, 3 ml of 2.3% NaCl was also added. The resultant product was pasteurized at 65°C for one hour and stored at 5°C.

Yttrium-90 colloids. The radioactive yttrium (1 to 25 mc of YCl_3 solution, Oak Ridge Nat. Labs., Union Carbide Nuclear Co.) was equilibrated 15 minutes at room temperature with 1.0 ml of 0.02 M YCl_3 carrier solution and employed for preparation of colloids exactly as described above.

Assay procedures, dialysis technic, animal toxicity and tissue localization methods. As previously employed in these laboratories (7).

Results. Properties. Both products were well-dispersed colloids which showed no flocculation or sedimentation after prolonged

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storage at 5°C. Neither seemed pyrogenic to the animals tested, nor caused hemolysis when mixed with whole citrated human blood.

The yttrium-oleoylacetone reaction product, after dispersion in water, formed a slightly turbid, pale yellow emulsion. Microscopic examination revealed a very few large aggregates (>1 μ), but the majority were less than 0.2 μ in diameter. By contrast, Y-D.I.A. formed an almost completely clear dispersion with minimal Tyndall effect. Individual micelles were not visible by ordinary light microscopy.

To confirm the complete conversion of ionic yttrium to a colloidal product, both chelates were dialyzed against a large excess of saline at pH of 8.0. No $Y(OH)_3$ precipitated during this process. The yttrium-oleoylacetone sol showed 0.05 to 3.0% dialyzable radioactivity, the Y-D.I.A. 3.5 to 5.5%. This tends to substantiate that a complex has been formed by each chelating agent. Such a conclusion also seemed warranted by other observations made during this investigation. Thus, as dilute sodium hydroxide was added to yttrium chloride solution, a heavy, white precipitate of $Y(OH)_3$ formed *in the absence of a suitable complexing agent.* When, however, a potent complexing agent was present, as with oleoylacetone and n-dodecyliminodiacetic acid, no such precipitation occurred. Such behavior strongly suggests that a complex has been formed, and that its dissociation constant is less than the very low solubility product constant of $Y(OH)_3$ at this pH.

It was found that the Y-D.I.A. was precipitated by addition of an equal volume of absolute methanol. The solubility characteristics of this material in water, methanol, and 50% methanol showed clearly that it was neither $Y(OH)_3$ nor n-dodecyliminodiacetic acid. Further, when dried under nitrogen, weighed, and ignited to constant weight, its ash content (presumably Y_2O_3) corresponded closely to a 1:1 molar ratio of reactants. Preliminary evidence suggests that yttrium and oleoylacetone have combined in a 1:3 molar ratio in this product.

Tissue deposition of yttrium-oleoylacetone. Tissue localization of yttrium-oleoylacetone following *i.v.* administration to albino rats (8 animals) is presented in Table I. Results are expressed in Relative Specific Activity (R.S.A.), *i.e.*, counts per minute per gram of tissue under consideration divided by total counts per minute per gram of animal as a whole. Suitable corrections have, of course, been introduced to account for radioisotope decay. Results so reported seem to facilitate comparisons between animals and between species, independent of dose injected and organ size. With this colloid, despite its lipophilic character, tissue distribution involved primarily the R.E. system in a manner quite analogous to the more common, hydrophilic radiocolloids. Some enhanced lung uptake was apparent; perhaps a few larger than average aggregates have been trapped in the capillary bed as suggested by the work of Gabrieli(8).

TABLE I. Tissue Deposition of Y-90 in Rats after I.V. Oleoylacetone-Y-90 Chelate.

Tissue	Relative specific activity* Day 1	Days 2–8
Liver	12.3	11.9
Spleen	9.1	13.8
Marrow	5.3	4.4
Bone	4.9	4.9
Lung	2.7	.8
Kidney	3.9	2.5
Blood	.30	.12
Muscle	.06	.02
Skin	.07	.05
Brain	.02	.01

* Calculated on basis of recovered activity.

Following *i.v.* administration, carrier-free yttrium salts are deposited primarily in bone and show an accelerated urinary excretion (9,12). With the quantities employed in these experiments (15 μg/rat), one might anticipate such results if the chelate were rapidly dissociated *in vivo.* Indeed, some enhanced bone and kidney deposition of yttrium suggests partial metabolic dissociation of this chelate. Presumably, the resultant "ionic" dissociation products would demonstrate a tissue deposition pattern similar to that of carrier-free yttrium salts.

Tissue localization of yttrium-D.I.A. colloid. Results, expressed as R.S.A., are presented in Table II for 8 FBW mice and in

TABLE II. Tissue Deposition of Y-90 in Mice after I.V. Injection of Y-D.I.A.

Tissue	Relative specific activity* Day 1	Day 3	Day 7
Liver	9.8	14.0	14.2
Spleen	9.7	18.9	37.0
Bone	.73	1.0	1.1
Lung	.87	1.0	1.3
Kidney	.91	.71	.69
Blood	.06	.01	.003
Muscle	.06	.03	.03
Skin	.15	.09	.10

* Calculated on basis of injected activity.

Table III for 2 mongrel dogs following *i.v.* injection of Y-D.I.A. These R.S.A. values are based on total injected activity. As before, this lipophilic colloid gave essentially a typical R.E. system distribution, but with less uptake by lung, bone, and kidney than noted above. The slow blood clearance of colloid in dogs was unexpected and somewhat at variance with previous experience in this institution.

In mice the increase in R.S.A. of spleen after the first day does not represent an actual increase in total amount of activity in this organ. This change appears to arise from a decrease in weight of the spleen. Thus, average spleen weight decreased as a function of time after colloid injection (Table IV), and one must conclude that the "tracer" doses employed in this experiment (10 μc/20 g mouse) caused some radiation injury. In light of our own toxicity experiments and the experience of others, it seems most unlikely that chemical or pharmacologic effects of non-radioactive yttrium could produce such a result.

Combined excreta from 3 mice were assayed to evaluate excretion rates. Excretion of Y-90 decreased from 5.6%/day to 3.1%/day during a 7-day interval following injection.

TABLE III. Tissue Localization of Y-90 in Dogs after I.V. Injection of Y-D.I.A.

Tissue	Relative specific activity*	Tissue	Relative specific activity*
Liver	8.3	Lung	0.7
Spleen	12.2	Kidney	2.1
Marrow	5.0	Thyroid	0.4
Blood	0.6	Ovary	1.2
Bone	0.9	Muscle	0.05

* Mean for 2 animals sacrificed at 2 days. Calculated on basis of injected activity.

Six mice received 0.1 ml of Y-D.I.A. (10 μc) *i.p.* Upon sacrifice at 3 and 7 days, these animals showed good localization of Y-90 within the peritoneal cavity. Of the little which left the cavity, most was accumulated within bone. Mean R.S.A. = 0.37.

Discussion. In Table V is summarized the tissue distribution of lipophilic colloids of this investigation together with the hydrophilic colloids previously studied in this Institute (7,10,11). All previous data have been recalculated and results expressed in R.S.A. to facilitate comparisons. Since blood R.S.A. decreased with time in all cases, values of less than 24 hours post-injection are not included. Averages for other organs include data from one hour to several weeks post-injection, for there appears little redistribution of activity with time. R.S.A. values for spleen in the 7-day mice were corrected for organ weight loss in the case of Y-D.I.A. All averages were taken from R.S.A. values based on total recovered activity. Many tissues having very low R.S.A. have been omitted for sake of clarity.

TABLE IV. Splenic Atrophy Following 0.5 μc/g Y-D.I.A. in Mice.

Days after inj.	Spleen wt, g*
1	.0793
3	.0535
7	.0181

* Mean for 3 mice/group.

Though the number of individuals included in each mean value is small and the variance between individuals considerable, some observations appear warranted: (1) All radiocolloids were accumulated readily by liver and spleen in all species considered, though this effect is less marked with YPO_4 and $Y(OH)_3$. (2) Lung and kidney show low R.S.A. except for glucose-suspended zirconyl phosphate in man. The enhanced kidney concentration in this case may reflect kidney damage and leukemic infiltration in these terminal patients, though an accelerated excretion of radioactivity cannot be excluded. (3) R.S.A. of bone marrow is probably lower than one would desire for radiation therapy

TABLE V. Comparison of Tissue Distributional Behavior of Certain Radiocolloids after *I.V.* Administration.

Radiocolloid and recipient	Relative specific activity*							
	Liver	Spleen	Lung	Kidney	Marrow	Blood	Bone	Ref.
Chromic phosphate (in Kelcosol)								
Rat	22.2	18.2	1.3	.57	2.6	.08	.69	(11)
Man	13.0	13.2	3.3	.82	2.8	—	—	(11)
Zirconyl phosphate (in dextran)								
Rat	19.0	146.0	1.5	1.4	—	.32	—	(7)
Dog	27.3	11.6	.92	1.1	2.4	.75	—	(7)
Man	9.7	14.4	2.2	2.9	3.7	.28	.14	(7)
Zirconyl phosphate (in glucose)								
Man	9.1	8.0	2.0	8.6	2.6	.8	—	(7)
Yttrium hydroxide (in maltose)								
Mouse	6.8	3.6	.53	1.6	—	.13	6.6	(10)
Man	2.7	6.2	.52	1.5	10.5	—	3.5	(10)
Yttrium phosphate (in carboxy-methyl-cellulose)								
Mouse	4.1	1.4	.26	.81	—	.16	10.0	(10)
Man	5.9	7.2	3.6	1.1	4.4	—	3.0	(10)
Yttrium oleoylacetone (Aq. glycerol and lecithin)								
Rat	12.1	11.4	1.9	3.2	4.8	.29	4.9	
Yttrium dodecyliminodiacetic acid								
Mouse	18.2	16.2	1.4	1.0	—	.03	1.3	

* Calculated on basis of recovered activity.

of chronic leukemia and related blood dyscrasias, except for yttrium hydroxide, (4) bone accumulation of radioactivity very probably reflects partial metabolic dissociation of the colloids followed by redistribution of "ionic" dissociation products. This is certainly suggested by the data for yttrium hydroxide, phosphate and oleoylacetone chelate. (5) The lipophilic colloids of yttrium show essentially the same tissue distribution pattern as the inorganic, hydrophilic colloids. Lipophilic character would not, therefore, seem to be a major determinant of tissue deposition of radiocolloids.

Summary. Two new lipophilic colloids of radioyttrium, Yttrium-Oleoylacetone Chelate-Y-90 and Yttrium-Dodecyliminodiacetic Acid Chelate-Y-90, have been prepared. Their tissue deposition in experimental animals following *i.v.* administration has been shown to compare closely with that of the more common inorganic, hydrophilic radiocolloids. Lipophilic character appeared to confer on these products no unique tissue distributional behavior.

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