Colloidal Yttrium Hydroxide-Y-90 and Yttrium Phosphate-Y-90. Preparation and Tissue Distribution Following I. V. Administration. (26364)

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Radiocolloid therapy of leukemia and other malignancies, first reported by Hahn (1) and co-workers, has steadily gained acceptance as an advantageous radiation modality. Of the numerous radioisotope preparations proposed for this purpose, only 3 (Gold-198, chromic phosphate-P-32, and zirconyl phosphate-P-32) have been adequately described and tested(2,3,4). These materials share a common attribute. Following *i.v.* administration they concentrate in reticulo-endothelial tissues where they achieve a selective irradiation while sparing other organs of the body (not possible with external irradiation or with commonly employed ionic radioisotopes). Even these, however, have certain disadvantages. Radiogold is undesirable because of its penetrating gamma emission, while chromic and zirconyl phosphates, though pure beta-emitters, have for certain situations an undesirably long half-life (14.3 days). Thus, the latter produce their biological effects rather slowly and leave appreciable residual activity in the patient for a considerable period. For the treatment of acute and chronic leukemia and in particular for selective sublethal irradiation of leukemic patients prior to attempted bone marrow transplantation, a more rapidly acting radiocolloid which would decay promptly was especially desired. Radioactive yttrium (Y-90) with its short half-life (2.54 days) and its energetic beta emission (2.18 MEV) seems uniquely suited for this purpose.

Simple methods for preparation of stable colloids of yttrium hydroxide and yttrium phosphate have been developed. Their properties, toxicities and tissue deposition following *i.v.* administration have been studied in mice, and terminal leukemic patients. Their therapeutic application is described elsewhere (5).

Methods. Screening tests. Many surfactants and suspending agents were evaluated for their effectiveness in preparation of colloidal yttrium hydroxide and yttrium phosphate *in situ*. Those which permitted flocculation or sedimentation of the colloid within 48 hours were rejected. Of the remainder, carboxymethylcellulose and Igepon T-77† served to disperse both phosphate and hydroxide, while albumin, dextran,‡ maltose, and dextrin were effective only with the latter. Igepon T-77 was eliminated for lack of information as to its toxicity. Albumin was subsequently eliminated when it was found to interfere with complete conversion of yttrium to the insoluble hydroxide form.

Tests of completeness of conversion. Completeness of chemical conversion of radioyttrium to the desired colloidal form was evaluated in low specific activity pilot experiments. By methods such as described below, yttrium hydroxide was prepared in presence of each dispersent listed above, as was yttrium phosphate. After pasteurization at 65°C for one hour, aliquots of each colloid were dialyzed against 250 volumes of physiologic saline at a pH of 8.1. After 24 hours the dialysate and original colloid were assayed for radioactivity, and the percent dialyzable radioyttrium calculated. Results are presented in Table I. From these studies albumin was rejected, and the preparations of yttrium hydroxide in maltose and of phosphate in carboxymethylcellulose were selected for additional study.

Preparation of therapeutic colloidal yttrium hydroxide, $Y(OH)_3$-Y-90. To radio-

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† Antara Chemicals Div., General Dyestuff Corp., New York.

‡ Plavoflex, 6% Aqueous, Wyeth Labs.

active yttrium chloride§ (75-275 millicuries) in its original shipping container, 1.0 ml yttrium chloride carrier solution (0.01 M YCl_3 in 0.1 N • HCl) was added. These were mixed, allowed to equilibrate at room temperature for 10 minutes, and transferred to a suitable 20 ml vial, using sterile hypodermic needle and syringe. The original container was then washed with 5.0 ml of 50% maltose solution, the wash being added to the radioactive material and mixed. A drop of phenolphthalein solution was added, and the mixture titrated to its endpoint with 1.0 N • NaOH. Total volume was adjusted to 10.0 ml with sterile 0.9% NaCl. Sterilization was achieved by pasteurization at 65°C for one hour and the product stored at 5°C.

TABLE I. Dialyzability of Various Radioyttrium Colloids.

Chemical form	Dispersant	% dialyzable Y-90
YPO_4	Carboxymethylcellulose	.02%
$Y(OH)_3$	"	.44
"	Albumin	75.9
"	Dextran	1.8
"	Dextrin	1.2
"	Maltose	1.3

Preparation of therapeutic colloidal Yttrium phosphate, (YPO_4-Y-90). One ml yttrium chloride carrier solution was added to the radioactive yttrium chloride in its original shipping container, mixed, and allowed to equlibrate for 10 minutes at room temperature. The mixture was transferred to a suitable 20 ml vial and the container washed with 5.0 ml 4.0% carboxymethylcellulose solution. The washings were added to the radioactive mix, 0.5 ml of 0.1 M Na_3PO_4 was added, mixed, and the solution titrated to the phenolphthalein endpoint with 1.0 N • NaOH. The volume was adjusted to 10 ml with sterile 0.9% saline, and the product pasteurized at 65°C for one hour and stored at 5°C.

Fate in experimental animals. Tissue distribution of these radiocolloids in mice 1, 3 and 7 days following their *i.v.* administration was evaluated by methods described previously(4).

§ Obtained as carrier-free YCl_3-Y-90 in HCl from Oak Ridge Nat. Labs., Union Carbide Nuclear Co., Oak Ridge, Tenn.

TABLE II. Toxicity of $Y(OH)_3$-Y-90 for Swiss Albino Mice.

Dose (mc/kg)	28-day survival	Dose (mc/kg)	28-day survival
200	.0%	18	.0%
150	.0	16	.0
100	.0	14	.0
50	33.3	12	100.0
40	.0	10	100.0
30	33.3		

Radiation toxicity. Toxicity of yttrium hydroxide-Y-90 was evaluated in white mice by *i.v.* administration of varying doses (10 to 200 mc/kg). These animals were observed during a 28-day period and percent survival in each group calculated, Table II.

Excretion. Total excretion per day of Y-90 by mice was investigated during the study described above. Methods were those described previously(4) (Table III).

Localization in man. Six patients received each radiocolloid. Of these, 11 were terminal acute leukemia, while one had inoperable C.A. of the rectum. Tissue assay methods were those previously described(4). Diagnoses, doses, and survival times of all patients are indicated in Tables IV and V.

Excretion by man. From 9 of the above patients, complete 24 hour urine specimens were collected for assay of excreted Y-90.

Results. These radiocolloids had remarkable stability; after prolonged storage at 5°C, no visible sediment was observed. Electron microscopy revealed well-dispersed, uniform micelles ranging from 0.04 to 0.08 μ in diameter. The products were routinely demonstrated to be sterile by thioglycollate culture methods. In no instances were pyrogenic reactions elicited upon their *i.v.* administration to experimental animals or man. The tissue distribution of maltose-suspended

TABLE III. $Y(OH)_3$ Excretion by Mice.

Day	% excreted
1	.49%
2	.13
3	.05
4	.03
5	.03

TABLE IV. Tissue Deposition of Y-90 in Leukemic Patients after I.V. $Y(OH)_3$.*

Patient	SMB†	MLD†	SR†	VP†	MM	JM
Days survival	17	20	20	23	26	57
Dose, mc	53.3	54.6	75.0	56.9	6.6	20.0
Body wt, kg	25.9	18.1	19.1	15.9	61.0	nm
Diagnosis	L	L	L	L	M	L
Tissue	% of recovered Y-90/g of wet tissue					
Marrow	.0346	.062	.075	.067	.012	.026
Bone	.020	.012	.015	.023	.006	nm
Spleen	.014	.030	.028	.055	.013	.009
Liver	.006	.015	.007	.004	.012	.007
Lung	.0007	.002	nm	.002	.002	.009
Kidney	.004	.020	"	.005	.001	.007
Adrenal	.0001	.002	"	.002	.0006	nm
Heart	.0003	.0005	"	.0002	.0002	.0063
Node	.0005	.004	"	.001	.0008	nm
Muscle	.0001	.0006	"	.0002	.0003	"

* Postmortem results.
† Massive irradiation prior to attempted marrow transplantation.
nm = Not measured. L = Lymphatic. M = Myelogenous.

TABLE V. Tissue Deposition of Y-90 in Patients after I.V. YPO_4.*

Patients	MB	AMH	CDS	LFD	EM†	PM
Days survival	1	3	5	6	21	72
Dose, mc	3.2	2.0	19.2	19.2	125	15
Body wt, kg	61.7	48.5	66.2	63.5	64.9	47.6
Diagnosis	CML	CLL	AML	ALL	CR	AGL
Tissue	% of recovered Y-90/g of wet tissue					
Liver	.021	.012	.010	.006	.006	.005
Marrow	.0052	.0011	.015	.017	.0012	.002
Lung	.005	.014	.0012	.005	.0002	.016
Bone	.001	.001	.0003	.0035	.011	.015
Spleen	nm	.031	.007	.004	.005	.022
Kidney	.0037	.0044	.001	.001	.0007	.0005
Node	.0006	.0005	.0011	.0008	.0002	.0004
Muscle	.0005	.0003	.0007	.0000	nm	nm
Adrenal	.002	.002	.002	.0006	"	"
Heart	.001	.001	.0005	.0002	.0001	"
Brain	.0000	.0000	.0000	nm	.0000	"

* Postmortem results.
† Massive irradiation prior to attempted marrow transplantation.
nm = Not measured. A = Acute. C = Chronic. LL = Lymphatic leukemia.
G = Granulocytic. ML = Myelogenous leukemia. CR = Carcinoma of rectum.

radioyttrium hydroxide in Swiss albino mice is presented in Table VI. The corresponding data for carboxy-methylcellulose-suspended yttrium phosphate in AKR mice are presented in Table VII. Results are reported as percent of recovered activity per gram of wet tissue. Each figure represents the mean of 3 animals for each sacrifice date. Considerable variance among animals of any one group was observed so that small differences between groups probably have little significance. Despite these limitations some conclusions appear warranted. Following *i.v.* administration, both radiocolloids disappeared rapidly from the circulating blood. With both colloids, much of the Y-90 was deposited in liver and in bone (including marrow). Of the remaining tissues, spleen, kidney, adrenal, and lung revealed significant accumulation of radioactivity. Other tissues contained only very minute quantities. These data suggest that both colloids are deposited primarily in the reticulo-endothelial system (presumably by phagocytosis). However the high specific activity of bone is suggestive of a competing mechanism. Efforts at separation of bone and bone marrow for independent assay were unsuccessful, and one is un-

TABLE VI. Tissue Deposition of $Y(OH)_3$ in Mice after I.V. Administration.

Tissue	% of recovered Y-90/g wet tissue Day 1	Day 3	Day 7
Liver	22.7%	36.7%	36.4%
Bone	24.0	42.1	25.1
Muscle	1.4	.4	.2
Spleen	6.0	10.3	33.1
Blood	.5	.6	.1
Kidney	6.0	10.6	1.5
Lung	2.0	2.2	2.4
Adrenal	5.9	5.8	1.2
Heart	3.3	1.6	.3
Brain	.1	.2	.5

able to estimate the relative contribution of activity in the individual tissues. Such high specific activities associated with bone, however, are suggestive that these colloids may be undergoing some dissociation within the animal body with subsequent deposition of "ionic" yttrium in bone substance in a manner such as that previously described(6,7).

Toxicity of radioyttrium hydroxide. Though a few animals survived a much larger dose, the mouse LD_{50} for this colloid appears to be 12-14 mc/kg of body weight. The small number of test animals does not permit a more precise evaluation of toxicity (Table II).

Excretion of radioyttrium hydroxide. Radioactivity is excreted very slowly, that is 0.5% in the first 24 hours, decreasing to 0.03% at 5 days (Table III). Thus, less than 1% of the administered dose is excreted during an interval of 5 days.

Distribution of radioyttrium colloids in man. Consideration of Table IV relative to tissue deposition of maltose-suspended yttrium hydroxide in man, and Table V concerning the deposition of carboxymethylcellulose-suspended yttrium phosphate in man, warrants certain observations. As with previously described radiocolloids, these products deposit primarily in the reticulo-endothelial tissues of the human body. Thus, marrow, spleen and liver accumulate appreciable quantities. To a lesser extent bone, kidney, and lungs also demonstrate radiocolloid uptake. Other tissues, including adrenal, heart, lymph nodes, and muscle, show little uptake. In these experiments, where bone and bone marrow could be successfully separated and assayed independently, it was noted that bone deposition was not a prominent feature in distribution of yttrium phosphate. It did, however, account for a greater portion of the distribution of yttrium hydroxide by comparison. Such results would tend to confirm the metabolic stability of yttrium phosphate and to a less extent the yttrium hydroxide, a conclusion which could not be reached on the basis of mouse data alone. With this rather limited group of subjects, no correlations were evident between size of dose administered, diagnosis, or time of survival after administration of radiocolloid and the particular tissue deposition observed in that individual patient.

TABLE VII. Tissue Deposition of YPO_4 – Y-90 in Mice after I.V. Administration.

Tissue	% of recovered Y-90/g wet tissue Day 1	Day 3	Day 7
Bone	57.9%	58.2%	50.6%
Liver	18.4	22.9	31.2
Lung	1.8	1.6	.8
Muscle	2.1	.7	.1
Blood	1.9	.1	.1
Kidney	9.1	3.4	2.0
Heart	1.1	.6	.3
Brain	.3	.0	.1
Spleen	2.2	8.9	14.5
Adrenal	—	2.2	2.4

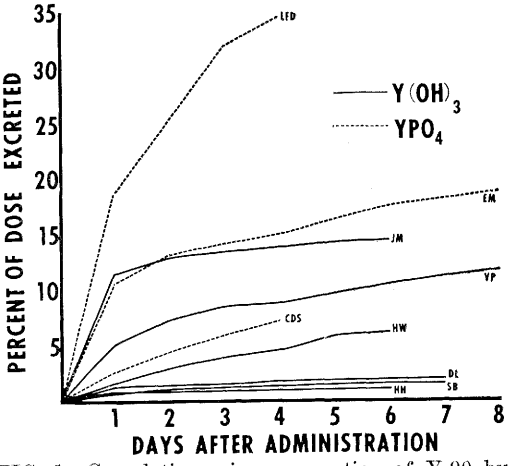

FIG. 1. Cumulative urinary excretion of Y-90 by man after i.v. colloidal $Y(OH)_3$ and YPO_4.

Urinary excretion of radioactivity by man. It is evident from Table III and Fig. 1 that urinary excretion of radioactivity by man exceeds that observed in mice quite ap-

preciably. Thus, of some 9 patients studied, only 3 showed total excretion of 2% or less during the first 5 days, while the remaining 6 patients demonstrated a cumulative excretion of 5 to 35% of administered dose during this same time interval. Quite probably this latter figure (35% in 4 days) was associated with gross hematuria in this particular patient. There seems no obvious correlation between tissue deposition in the individual patients and the excretory patterns shown by them.

Summary. 1. Simple technics have been described for preparation of 2 new colloids of radioyttrium $Y(OH)_3$ and YPO_4. Maltose has been employed as suspending agent for the former, carboxymethylcellulose for the latter. Both products have been demonstrated to have a small, uniform particle size, good emulsion stability, and low percentage dialyzable radioactivity. 2. The LD_{50} of $Y(OH)_3$ – Y-90 in mice was approximately 12 to 14 mc kg. 3. Tissue deposition in mice and terminal human patients has been predominantly reticulo-endothelial in both. Evidence is suggestive that some metabolic dissociation occurs with subsequent deposition of yttrium in bone. 4. Urinary excretion of activity has been evaluated for mice and man.

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Normal Growth of Rat Mammary Glands During Pregnancy and Early Lactation.* (26365)

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Kirkham and Turner(1) first suggested desoxyribonucleic acid (DNA) may be employed as a quantitative index of mammary gland development under normal and experimental conditions. By this method, mammary gland proliferation has been observed to occur through pregnancy in the mouse(2, 3), and during lactation in the rat and mouse (3,4). This paper presents a more detailed study of mammary gland growth during pregnancy and early lactation in the rat.

Methods. Data were obtained from primiparous Sprague-Dawley-Rolfsmeyer rats on days 5, 10, 15, 18-20 of pregnancy and on days 1, 2, 3, and 4 of lactation. Days of pregnancy were determined by presence of sperm within the vagina. Six abdominal-inguinal mammary glands were removed from each rat and placed in a deep freeze for 4 days, extracted in 95% ethanol for 3-5 hours and in ether for an additional 3-5 hours. Dry, fat-free tissue (DFFT) was weighed and ground to a fine powder in a Wiley mill. DNA of a 25 mg aliquot of tissue was determined by method of Webb and Levy(5). Product of quantity DNA/mg DFFT and DFFT/100 g body weight (B.W.) was estimated as "total DNA".

Results. Mammary gland DNA/100 g B.W. of normal non-pregnant rats has been

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