14. Manire, G. P., Sulkin, S. E., Farmer, T. W., *ibid.*, 1950, v73, 341.

15. Kraft, L. M., Melnick, J. L., *J. Exp. Med.*, 1950, v92, 483.

16. Humler, K., *Ann. N. Y. Acad. Sci.*, 1957, v67, 248.

17. Kraft, L. M., Melnick, J. L., *J. Immunol.*, 1952, v68, 297.

18. Melnick, J. L., *Bull. N. Y. Acad. Sci.*, 1950, v26, 342.

Received November 21, 1957. P.S.E.B.M., 1958, v98.

Colloidal Zirconyl Phosphate-P-32. Preparation and Tissue Distribution Following I. V. Administration. (24000)

R. J. SPEER, J. M. HILL, AND J. E. YOUNG*

J. K. and Susie L. Wadley Research Institute and Baylor University Graduate Research Institute, Dallas, Texas

Radiocolloid therapy was first reported by Hahn and co-workers(1). Of the many radiocolloids employed since, radiogold and chromic phosphate-P-32 have been most widely accepted. Chromic phosphate is advantageous because of its pure, high-energy β-emanation as contrasted to the undesirable hard γ-ray from Au-198. Preparation of colloidal chromic phosphate has been achieved but proved time-consuming(2). Colloidal zirconyl phosphate-P-32, first described by Mayer and Morton(3), exhibits low solubility even in concentrated acids and is easy to prepare. However, as reported, the product is unstable and precipitates from neutral media.

This investigation was undertaken 1) to find a means of dispersing and stabilizing zirconyl phosphate colloid, 2) to investigate its fate in man and experimental animals, 3) to establish particle size, and 4) to determine its rate of excretion and radiation toxicity in experimental animals.

Methods. Preparation. By addition of zirconyl ion to aqueous orthophosphate a milk-white colloid is formed. In neutral media this sol is unstable and precipitates unless a suitable dispersant is added. Of the many substances tested for colloid stabilizing action, only 3 have merit: dextran, soluble starch, and glucose. Two of these, glucose and dextran, have been used routinely in the preparation of injectable zirconyl phosphate-P-32 sols.

Dextran-stabilized zirconyl phosphate: 2 ml .1 M K_2HPO_4 was equilibrated with the desired amount of radiophosphorus† (100 mc P-32 in not more than 25 ml) for 10 minutes.

A drop of phenolphthalein indicator and 64 ml 6% dextran‡ were added, and the solution neutralized with 1 N NaOH. After 5 minutes, 7 ml .1 N $ZrOCl_2$ was added dropwise with simultaneous adjustment of pH with NaOH. Volume made to 100 ml with saline. The colloid was then autoclaved for 20 minutes at 15 p.s.i. *Starch-stabilized zirconyl phosphate*: This procedure was similar to that for dextran-stabilized colloid. A 2.5% solution of soluble starch (Merck, according to Lintner) in .9% saline was centrifuged at 5,000 rpm for 20 minutes. Sixty ml of supernatant was employed for 100 ml of colloid. *Glucose-stabilized zirconyl phosphate*: 1) 2 ml .1 M K_2HPO_4 was equilibrated with 100 mc H_3PO_4-P-32 for 10 minutes. 2) 9 ml .1 M $ZrOCl_2$ was mixed with 50 ml 50% dextrose solution. 3) The equilibrium mixture, 1), was added slowly and with constant stirring to 2), followed by 3 rinsings of the container with 50% dextrose. 4) After 10 minutes the solution was titrated with 1 N NaOH to phenolphthalein end point. Volume was adjusted to 100 ml with distilled water, container sealed, and product pasteurized for 1 hour at 65°C. *Fate in Experimental Animals.* Studies were made of tissue localization of zir-

* From thesis in partial fulfilment of requirements for M. S. degree, Baylor Univ. Graduate Research Institute, Dallas, Texas.

† Available as H_3PO_4-P-32, carrier-free, from Oak Ridge National Laboratories, Oak Ridge, Tenn.

‡ *Plavoflex*, 6% aqueous, Wyeth Labs.

conyl phosphate-P-32 following intravenous administration in Swiss albino mice, Wistar albino rats, New Zealand white rabbits, and mongrel dogs. Tracer doses (20 to 50 μc/kg) suspended in dextran were administered to mice, rats, and dogs. Two rabbits were given lethal doses (1 mc/kg) of starch-stabilized colloid. Changes in the distribution pattern with time were studied in mice and rats by sacrificing groups of animals (2 or 3 animals/group) at varying intervals following injection. Tissues for radioassay were handled as follows: Animals were sacrificed with ether or sodium nembutal and the major organs removed and weighed. Duplicate samples (.05 to .20 g) of these tissues were introduced into weighed planchets, the weights determined, and incinerated at 500°C for several hours. This process facilitated radioassay by eliminating self-absorption correction. After ashing, radiophosphate content of each sample was determined with an end-window GM detector attached to model 172 Nuclear-Chicago scaling unit. Detector was calibrated with N.B.S. primary standards of P-32. *Excretion.* Total excretion per day of P-32 by the rat was investigated. Two animals were given 1 mc/kg of radiocolloid by vein, and thereafter daily collections of total excreta were obtained for 9 consecutive days. Each collection was homogenized, diluted to known volume, and assayed. *Radiation toxicity.* Toxicity of P-32 was studied in 8 male rats. Intravenous doses ranged from .1 to 2 mc/kg. Hematologic studies were made on 4 of these animals for 42 days. *Localization in Man.* Six patients with terminal acute leukemia and 1 patient with Hodgkin's disease were injected with small amounts of radiocolloid (.1 to 2 mc). In addition, 2 chronic leukemia patients, who had received the isotope therapeutically (4 to 7 mc), were included in this study. Seven of the 9 patients were given dextran-stabilized zirconyl phosphate and 2 were injected with glucose-stabilized preparations. In order to determine total radiocolloid content in the tissues studied, it was necessary that their total weights be known. Determination of gross weights of bone, bone marrow, lymphatics, muscle, gastro-intestinal tract, and blood was impracticable; so estimates were made on the assumption that a given tissue weight is proportional to total body weight(4). The tissues studied included most major organs and glands of the body. Those tissues of primary interest were liver, bone marrow, lymph nodes, spleen, lungs, and blood which are concerned with hematopoiesis, phagocytosis, and storage of the cellular entities of the blood.

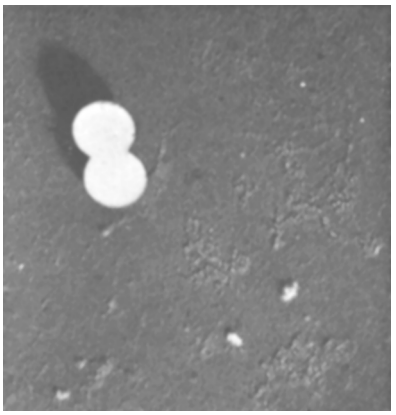

FIG. 1. Electron photomicrograph of preparation of zirconyl phosphate. Latex reference spheres .26 μ diameter.

Results. Zirconyl phosphate sols stabilized with dextran, starch, and dextrose are quite stable even after prolonged storage at 5°C. Dextran and starch-stabilized colloids can be autoclaved, but those stabilized with glucose must be pasteurized to avoid caramelization. When dialyzed against excess .9% saline, the preparations routinely contained less than 5% dialyzable P-32. Sterility of these preparations has been repeatedly demonstrated by thioglycollate culture methods. In no case was a pyrogenic response elicited when these products were administered intravenously to man and experimental animals.

An electron photomicrograph of a typical colloidal preparation is shown in Fig. 1. The average particle size has been estimated at .013 μ diameter. Uniformity and size of the colloidal particles were independent of suspending agent.

Tissue distribution data of P-32 following i.v. zirconyl phosphate-P-32 are presented in Table I. P-32 distribution appeared to reach equilibrium within a 5 hour period in mice

TABLE I. Tissue Distribution of P-32 in Experimental Animals following I.V. Zirconyl Phosphate-P-32.

Tissue	% recovered radioactivity: Rats (Mean % ± S.D. of mean)*	Mice	Rabbits (Mean %)†	Dogs
Liver	63.68 ±2.55	87.14 ±3.00	78.12	78.93
Spleen	30.56 ±2.47	4.39 ± .60	1.92	6.22
Blood	3.49 ±1.04	2.63 ±1.43	13.42	4.96
Kidneys	1.00 ± .18	1.27 ± .49	3.52	.70
Lungs	.62 ± .17	2.97 ± .96	2.13	.80
Heart	.31 ± .04	.52 ± .33	.87	.42
Testes	.33 ± .07	.10 ± .04		.06
Adrenals	.03 ± .003	.06 ± .01		.01

* 8 rats and 9 mice sacrificed between 5 hr and 14 days from inj. time (2 to 3 animals each at 5, 24, and 48 hr and 14 days).

† 2 animals each group. Dogs sacrificed at 3 days after inj.; rabbits at 10 days.

and rats and did not change appreciably thereafter. Concomitantly, blood levels of P-32 declined rapidly during this period, especially during the first hour. In the mouse, circulating P-32 was less than 5% of the total within 30 minutes after administration. The liver had the greatest capacity for radiocolloid accumulation in all species, exceeding 78% in mice, rabbits, and dogs. Total P-32 uptake by rat spleen was high compared to that of other species, though specific activity (μc P-32/g) of this tissue was high in all experimental animals. P-32 concentration in the bone marrow appeared to be high in the rabbit, minimal in the mouse and dog. All other tissues studied in the 4 species accounted for small increments of total recovered dose of radiocolloid. Accumulation in brain and testes was especially minute. In the mouse, specific activity of P-32 in bone was very low and did not increase with time. Total excretion of P-32 by the albino rat amounted to 1.11 ± .16%/day (Mean % ± S.D. of mean) of the total injected. These observations attest to the insolubility and chemical stability of colloidal zirconyl phosphate *in vivo*.

Radiation toxicity of zirconyl phosphate-P-32 in the rat was minimal. At the end of 8 months following injection, all animals were in excellent health and showed no evidence of permanent ill effects. The LD_{50} of zirconyl phosphate-P-32 is apparently greater than 2 mc/kg in the male albino rat (though 2 rabbits were killed by 1 mc/kg). Hematologic changes were characterized by a precipitous drop in leukocyte (primarily lymphocyte) concentration and a slight decrease in erythrocyte count. Hemoglobin concentration dropped in those animals receiving 2 mc/kg but did not change appreciably in the .1 mc/kg group. Variability in platelet counting was such as to obscure any significant change in this cellular component; however, no apparent thrombocytopenia developed.

In man, the primary sites of colloid accumulation were liver, bone marrow, spleen, muscle, lymph nodes, and lungs in that general order (Table II). These tissues contained between 85 and 96% of *recovered* radioactivity. Percentage recovery of *administered doses* of P-32 are listed at the bottom of Table II. Figures on each individual patient are given to show the pronounced variability of tissue uptake and to demonstrate the effect of time.

In terms of concentration of P-32 per gram of tissue, liver and spleen were comparable in most instances. Concentration in lymph nodes, bone marrow, adrenals, kidneys, and lungs decreased in that order. One patient (V.H.K.), who received glucose-suspended zirconyl phosphate, demonstrated an increased concentration of P-32 in the kidneys and diminished accumulation in bone marrow and lymphatics. The other patient (J.G.B.), who received a glucose-suspended preparation, demonstrated an uptake pattern not unlike those shown with dextran-suspended colloid.

Dextran-suspended zirconyl phosphate-P-32 has been employed successfully in the management of more than 100 cases of chronic leukemia and related blood dyscrasias at the Wadley Research Institute. Although this product was in general satisfactory, a few patients showed hypersensitivity to dextran; because of this, its use has been discontinued. Glucose-suspended zirconyl phosphate has proved to be completely satisfactory and is now used exclusively. The starch-suspended sols were not administered to human patients. Details of clinical evaluation of the products described above will be published elsewhere.

TABLE II. Tissue Distribution of P-32 in Leukemia and Hodgkin's Disease following I.V. Zirconyl Phosphate-P-32.

	% recovered radioactivity								
Tissue	J.Z.C.	J.G.B.*[h]	S.D.B.	V.H.K.*	O.C.B.	J.R.E.	W.J.D.	E.L.D.	E.W.
Liver	31.58	29.25	34.62	49.08	64.17	31.28	39.73	56.72	44.19
Bone marrow	35.08	19.72	10.32	1.64	7.18	18.64	13.16	18.22	7.48
Spleen	11.06	9.23	12.04	10.33	12.99	13.16	25.62	‡	15.27
Muscle	5.92	7.34	4.24	12.04	9.11	nm	10.19	12.96	20.41
Lymph nodes	5.02	2.53	3.99	.51	1.15	24.35	4.12	5.96	6.83
Lungs	6.10	3.75	19.83	4.18	1.22	6.85	2.11	2.34	1.52
Kidneys	3.39	2.54	5.54	11.84	.92	1.13	1.24	nm	1.33
Blood	nm	22.79	5.51	5.54	1.51	2.72	.77	.71	.69
G.I. tract	1.08	1.89	1.90	2.82	.95	nm	2.30	——nm——	
Bone	——nm——		1.10	———	———nm———			2.01	1.06
Heart	.42	.32	.35	.75	.25	.57	.36	.34	.45
Brain	.07	.19	.09	.27	.15	.71	.18	.12	.43
Pancreas	.13	.23	.16	.75	.23	.23	.17	.43	.21
Adrenals	.16	.17	.24	.23	.16	.11	.05	.13	.05
Thyroid	.02	nm	.02	.05	.01	.01	nm	.04	.07
Ovaries		.04			nm			nm	.01
Testes	nm		.02	nm		.07	nm		
Time†	2 hr	4 hr	12 hr	30 hr	3 days	6 days	8 days	10 days	22 days
Recovered dose (%)	47.3	61.3	61.2	51.7	97.5	80.8	117.5	114.8	84.1

* Given glucose-stabilized zirconyl phosphate. † Time elapsed from inj. to death of patient. ‡ Previous splenectomy.

[h] Hodgkin's disease. nm = not measured.

Summary. 1. Several technics of preparing stabilized sols of zirconyl phosphate-P-32 have been described using dextran, soluble starch, and glucose as suspending agents. All preparations exhibited good stability, small and uniform particle size, low solubility, and metabolic "inertness." High concentrations of glucose have proved superior to all other suspending agents for the preparation of injectable zirconyl phosphate sols. 2. In experimental animals, zirconyl phosphate-P-32 was concentrated rapidly in the liver, spleen, and bone marrow, when given intravenously. In the rat, a marked depression of the leukocyte count was effected. The LD_{50} for the rat was greater than 2 mc/kg but less than 1 mc kg in the rabbit. Combined urinary and fecal excretion of P-32 by the rat was 1.11 ± .16%/day. 3. In human leukemia patients the radiocolloid was localized principally in liver, spleen, and bone marrow. Other tissues as muscle, brain, skeleton, and reproductive organs exhibited little affinity for the colloid. 4. This radiocolloid may be used to advantage in the rather selective irradiation of the reticulo-endothelial system in the management of chronic leukemia and related blood diseases.

Authors wish to acknowledge invaluable assistance and advice of Dr. G. J. Marshall, Miss June Dawson, and Dr. J. L. Rabinowitz.

1. Hahn, P. F., Carothers, E. L., Hilliard, G. W., Bernard, L., Jackson, M., *Therapeutic Use of Radioisotopes,* John Wiley and Sons, Inc., 1956.

2. Hill, J. M., Speer, R. J., Marshall, G. J., Lajous, J. R., *Le Sang,* 1955, v26, 195.

3. Mayer, S. W., Morton, M. E., *Nucleonics,* 1952, v13, 98.

4. I. C. R. P. Supplement on Maximum Permissible Amounts of Radioisotopes, *Nucleonics,* 1951, v8, 74.

Received January 17, 1958. P.S.E.B.M., 1958, v98.