Distribution in Blood and Excretion of Zn^{65} in Man.* (25847)

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In studying radiozinc localization in the prostate gland, it was desired to learn more about distribution and excretion of this radioisotope in man, since relevant literature(1-10) leaves many questions unanswered.

Materials and methods. 17 patients were given Zn^{65} chloride (specific activity greater than 75 mc/g) in intravenous doses, ranging from 50 μc to 100 μc. All patients but one suffered from various neoplastic diseases; none had serious impairment of liver or kidney function. Two of the group were receiving steroid therapy. Activities at various intervals up to 60 days of whole blood, plasma and red cells were determined. No studies of Zn^{65} concentration of leucocytes were attempted, since this has been extensively studied by others(9). Erythrocytes were hemolyzed with distilled water, and the supernatant fluid as well as the sediment, consisting of red cell ghosts (and leucocytes), were counted separately. Red cells were washed 3 times before assay; their sediment was washed until colorless. Plasma was dialyzed against normal saline. Urine and stool were collected to 2 weeks, as completely as possible. Daily urine samples less than 700 ml and 24 hr stool specimens weighing less than 100 g were excluded. Urine dialysis was performed against polyvinylpyrrolidone (PVP). Liquid samples were assayed in duplicate in a well-type scintillation counter. Stool activity was measured with a scintillation probe, positioned 15 cm from top of specimen container. Adequate counts were collected to assure an accuracy of greater than 10% for assay of stools and better than 5% for all other samples.

Results. 1. *Blood Zn^{65}.* Mean Zn^{65} concentrations (% dose/liter) of whole blood, erythrocytes, and plasma as a function of time are indicated in Fig. 1. The whole blood curve falls rapidly during the first 2 hrs (not indicated in figure) following injection, rises to its maximum on the eleventh day, and thereafter decreases slowly. Red cell concentration pattern is similar to that of whole

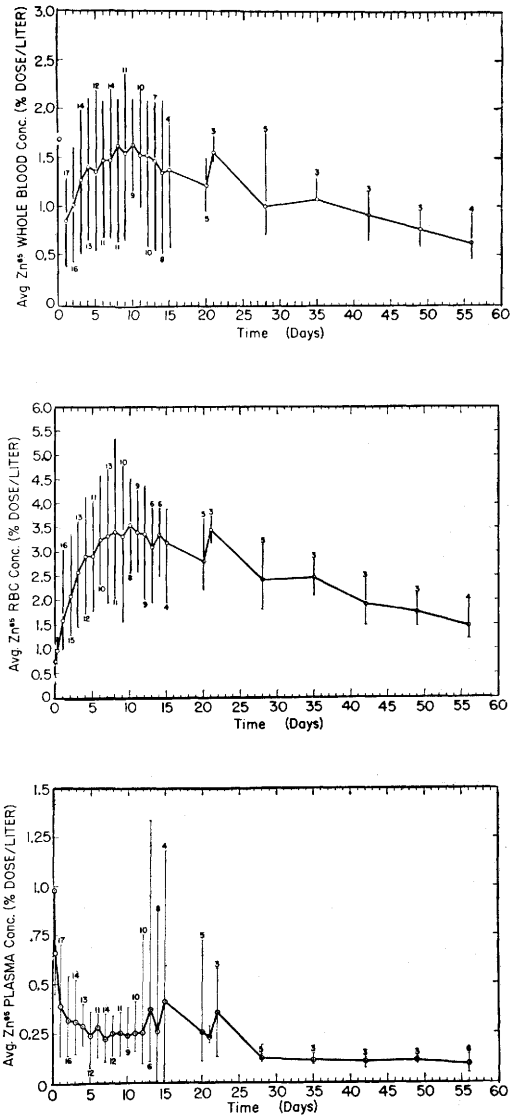

FIG. 1. Variation of Zn^{65} concentration of whole blood, RBC, and plasma with time. Individual points represent mean values of samples obtained from number of patients indicated at end of vertical lines extending from each point. Length of vertical line denotes range (max and min values).

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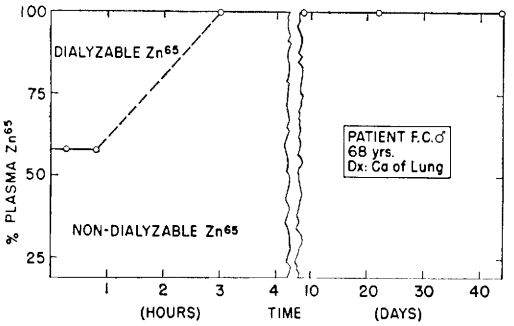

FIG. 2. Results of 24 hr dialysis of plasma samples obtained at various times following Zn^{65} administration, demonstrating binding of radiozinc to plasma proteins.

blood. Plasma curve falls continuously, although the range is considerable for several of the points. Semi-logarithmic plots of individual whole blood, red cell and plasma concentrations against time suggest that they can be resolved into exponential components. Zn^{65} erythrocyte concentration grows at a constant rapid rate for the first 4 days and at a second, slower rate thereafter until the eleventh day.

Plasma dialysis was performed on numerous samples obtained from 4 patients (Fig. 2) with essentially the same results. Up to one hour after injection, approximately one half of radiozinc freely traverses the membrane; by 3 hrs and until 40 days, virtually all is in a non-dialyzable form. Complete binding of radiozinc to plasma proteins at 3 hrs coincides with levelling of plasma concentration.

An example of hemolysis results, as studied in 6 cases, is given in Table I. Almost all radioactivity is found in the supernate. Dialysis of this solution demonstrates that Zn^{65} becomes protein-bound within the red cell.

TABLE I. Effect of Hemolysis on RBC Zn^{65} Concentration. (Patient H. N. ♂, 67 yr; bronchogenic Ca.)

Time from dose (days)	RBC Zn^{65} conc. (% dose/l)	Zn^{65} activity (% dose) after hemolysis	
		Supernate	Sediment
1	1.76	1.87	.056
2	2.14	2.27	.086
3	2.74	2.91	.065
4	3.21	3.11	.078
5	3.45	3.40	.096
6	3.16	3.46	.100
7	3.58	4.06	.068
9	3.80	3.22	.067
10	3.98	3.96	.071
11	3.95	3.95	.052
12	3.99	4.04	.061
23	3.35	3.43	.050

2. Urinary and stool Zn^{65}. Maximum urinary excretion rate (0.2 to 1.3% of dose per day) occurred during the first 2 days following injection. After the third day, daily urinary excretion remained quite constant, 0.2 to 0.6% of dose. Cumulative urinary and stool excretion for first and second weeks following Zn^{65} administration is given in Table II. During first week from 1.8 to 6.5% of dose appeared in the urine; by the end of second week, urinary output totalled between 3.3 and 11% of dose. Daily samples of urine from 2 patients were dialyzed and assayed with consistent results. Urinary Zn^{65} was almost entirely dialyzable (Table III), indicating that excretion of zinc occurs as a non-protein compound. Zn^{65} stool levels fluctuated widely from day to day. Total stool excretion of the first week ranged from 3.4 to 20% of dose. The patient with 20% fecal excretion had diarrhea throughout most of the collection period.

TABLE II. Cumulative Zn^{65} Urinary and Fecal Excretion (% Dose).

Sex & age	Diagnosis	Urine		Stool	
		1st wk	2nd wk	1st wk	2nd wk
♂ 52	Ca sigmoid	2.58	4.43		
♂ 76	" prostate	4.82	7.23		
♀ 51	" breast	2.13	3.30		
♂ 34	" lung	6.46	10.9	3.40	
♀ 70	Met. ca breast	1.79		8.87	
♀ 54	*Idem*	2.52		20.3	
♂ 65	Ca lung	4.40	6.50	4.56	7.60
♂ 49	Ca esophagus	2.11	3.80	3.43	7.42

TABLE III. Inorganic Fraction of Urinary Zn^{65}. (Patient G. S. ♂, 49 yr; Ca esophagus.)

Time from dose (days)	% dialyzable Zn^{65}	Time from dose (days)	% dialyzable Zn^{65}
1	97.1	8	98.9
2	95.3	9	98.5
3	95.3	10	95.5
4	95.9	11	99.2
5	96.8	12	98.7
6	97.4	13	99.3
7	97.7	14	99.3

Discussion. The slope of the whole blood graph reflects the rapid disappearance of isotope from plasma during the initial 24 hrs; thereafter it largely mirrors rate of uptake by the red cells. The plasma curve and red cell curve are similar to those published by Ross (7). Plasma Zn^{65} becomes non-dialyzable very shortly after injection, indicating that it is protein-bound. Since certain amino acids (11,12) and imidazole groups(13) generally combine avidly with zinc, it is not surprising that the radioisotope is bound rapidly to the great excess of plasma proteins.

The gradual increase of Zn^{65} red cell concentration tempts one to speculate that this signifies incorporation into young red cells. That the ascending phase of red cell concentration curve is apparently governed by 2 exponential functions, suggests as possibilities that radiozinc is picked up by erythrocytes only at a particular stage of maturation or that circulating red cells take up the isotope at a different rate than precursors in the bone marrow. Site of binding within the red cell is uncertain. While it was stated(14) that Zn^{65} does not exchange *in vitro* with the zinc of erythrocyte carbonic anhydrase, this has not been tested *in vivo*. It remains a possible binding site as does lactic dehydrogenase, another zinc metalloenzyme in the red cell(15). Hemoglobin itself, as indicated in a recent study(16), or some other protein without enzymatic activity may also be able to bind the radiozinc.

Our Zn^{65} urinary excretion data are in accord with published findings concerning stable zinc. They confirm the observation that urinary excretion of zinc is independent of urine volume(17) and that it occurs at a fairly constant rate after equilibration in plasma. It is apparent from the dialysis experiments that Zn^{65} is not excreted in a protein-bound form, a point about which there has been controversy.

Fecal excretion of Zn^{65} varied considerably in the patients studied. The cumulative stool values are consistent with those reported in dogs(1,4), in whom injected zinc was excreted largely through the gastrointestinal tract(18). Increased quantities of stainable zinc were found in small intestinal epithelium following its intravenous injection(19), and it was suggested that fecal excretion might depend on tissue saturation. Although it has been stated(20) that the average hospital diet contains sufficient amounts of zinc, it is possible that patients with chronic wasting illnesses have depleted body stores. The single case of very high fecal excretion in a patient with diarrhea suggests that besides poor intake, increased fecal losses might contribute to zinc depletion.

Summary. Distribution and concentration of Zn^{65} in whole blood, plasma and red cells were studied following intravenous infusion. Analyses of concentration curves suggest that they can be resolved into exponential components. Plasma Zn^{65} becomes protein-bound by 3 hrs following its injection and remains non-dialyzable at least up to 40 days. Red cell Zn^{65} becomes protein-bound and cannot be removed by dialysis from the hemoglobin solution. Excretion of Zn^{65} in the urine occurs presumably as an inorganic compound. Fecal excretion of Zn^{65} is variable and may depend on tissue saturation. The possibility exists that prolonged diarrhea may lead to zinc depletion.

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Coenzyme Q_{10} and Succinate-Tetrazolium Reductase Activity of Proliferative Lesions of Liver.* (25848)

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Histochemical studies of the succinic dehydrogenase system using tetrazolium salt reduction technics have shown a decrease of succinate-tetrazolium reductase activity in a number of benign and malignant proliferative lesions(1,2,3). Recent work employing the tetrazolium salt, 2 - (p - iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), has indicated that a quinone, presumably coenzyme Q_{10} (CoQ_{10}), serves as an intermediate electron transport agent in the succinate-INT reductase reaction and that content of quinone in normal tissues is below that required for maximum rate of activity of this reaction(4). In the present investigation quantitative histochemical determinations of succinate-INT reductase activity of sections of normal liver, regenerating liver and the Novikoff hepatoma have been carried out in presence of added CoQ_{10} and also in presence of a second quinone, 2-methyl-1,4-naphthoquinone (menadione). These experiments were performed to determine the individual roles of the primary dehydrogenase and quinone in decreases in reductase activity which have been observed. Parallel studies have also been carried out on a second reductase system, alpha-glycerophosphate-INT reductase, in which a quinone electron transport agent takes part in a manner similar to that of the succinate-INT reductase system(4).

Materials and methods. Quantitative procedures have been described(4). Frozen and dried sections 16 μ thick, with a dry weight of approximately 0.5 mg were incubated in a reaction mixture containing sodium succinate or sodium alpha-glycerophosphate, 0.05 M; NaCl, 0.11 M; KCl, 0.003 M; $MgSO_4$, 0.001 M; Na_2HPO_4, 0.03 M; acetone 5%; and INT, 0.4 mg/ml; pH was adjusted to 7.4. Sections were incubated at 37° rather than 28° as in the former investigation because of inclusion in this study of tissues with very low activity. At the higher temperature the enhancing effect of the quinones is relatively less compared to total activity of the section than at the lower temperature. An incubation period of 15 minutes was used for determination of succinate-INT reductase activity and 30 minutes for alpha-glycerophosphate-INT reductase activity. As in the earlier work, addition of CoQ_{10} is made by coating the compound on the coverslip and placing the tissue section in direct contact with the dried material. A considerably greater enhancement of enzyme activity has been found with this method than by placing the quinone directly into reaction mixture. For this procedure, 0.02 ml of a solution of CoQ_{10}, 1 mg/ml, in equal volumes of ethyl ether and acetone was placed on a 22 mm square coverslip and the solvent allowed to evaporate at room tempera-

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