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STUDIES WITH COLLOIDS CONTAINING RADIOISOTOPES OF YTTRIUM, ZIRCONIUM, COLUMBIUM, AND LANTHANUM

II. The Controlled Selective Localization of Radioisotopes of Yttrium, Zirconium, and Columbium in the Bone Marrow, Liver and Spleen

by

Ernest L. Dobson, John W. Gofman, Hardin B. Jones
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STUDIES WITH COLLOIDS CONTAINING RADIOISOTOPES OF YTTRIUM, ZIRCONIUM, COLUMBIUM, AND LANTHANUM.

II. THE CONTROLLED SELECTIVE LOCALIZATION OF RADIOISOTOPES OF YTTRIUM, ZIRCONIUM, AND COLUMBIUM IN THE BONE MARROW, LIVER, AND SPLEEN*

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INTRODUCTION

Several workers have shown that certain colloidally dispersed materials are removed from the blood stream by the liver and spleen. Jones, Wrobel, and Lyons[1] have utilized suspensions of anhydrous chromic phosphate for the selective irradiation of the liver and spleen with P^{32} beta particles. Gersh[2,3] demonstrated that colloidal calcium phosphate is taken up by the liver and spleen. He stressed the failure of bone marrow phagocytes to take up this colloid in rats and dogs (though he referred to possible uptake in the marrow of rabbits under special conditions), and commented on the relative "refractoriness" in general of the bone marrow as compared with liver and spleen with respect to the uptake of colloidal dyes from the blood stream. Some histological data[4] indicate that "Thorotrast" (a colloidal thorium dioxide preparation) is deposited in the bone marrow as well as in the liver and spleen, but no quantitative data as to the relative distribution are available.

In the preceding communication (this journal p._____) the methods for the preparation of colloids incorporating radioisotopes of yttrium, columbium, and zirconium were given. The present studies are concerned with the localization of such colloids primarily in the bone marrow or primarily

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* This work was supported in part by Contract N6-ORI-111-Task Order III, U. S. Navy, by the A. E. C. (NDP 48A Division II), and the Donner Foundation, Inc.

in the spleen and liver, with an analysis of some of the factors which may be responsible for differences in localization.

A. Selective Localization of Radioisotopes of Zirconium, Columbium, and Yttrium in the Liver and Spleen.

Several different colloids have been studied, all of which show the common property of localizing in the liver and spleen of mice, rats, and rabbits. In every case the colloids having this distribution are those of "relatively large" particle size, sedimentible in large part in ordinary centrifuges. These sols are turbid, and produce an intense Tyndall effect on illumination. Further, in general, for the colloids which localize in the liver and spleen, the disappearance rate of intravenously injected colloid from the blood stream is rapid, the blood colloid level decreasing approximately exponentially with time, the half time for blood clearance (and liver and spleen uptake) being in the neighborhood of 30 seconds to 1 minute. Representative distribution data for the three colloids: (1) zirconium oxide peptized in nitric acid, (2) zirconium phosphate peptized in di-sodium acid phosphate, and (3) zirconium oxide in dilute sodium lactate - in the mouse are given in Table 1. The preparative procedure for these colloids is given in the preceding communication.

In the case of all of these colloids the blood-disappearance curves show a small percentage (between 1 and 10%) of more slowly clearing components in addition to that having the $T_{1/2}$ of 30 seconds to 1 minute. The methods of preparation for these sols are such that polydispersity of the sols is expected. Several types of experimental data lend support to the idea that the smaller particles in such polydisperse sols are responsible for the slowly clearing fraction. First, experiments have been performed, using the mouse, where a colloid having ~ 1% of the total activity in the slowly disappearing fraction has been centrifuged to remove the larger particles.

Table 1

Distribution of Intravenously Injected Zirconium Colloids of Relatively Large Particle Sizes in Mice (Zr^{95} and Cb^{95} radioisotopes incorporated into colloid)

	Zirconium oxide in dilute HNO_3 (1)	Zirconium phosphate in dilute Na_2HPO_4 (2)	Zirconium oxide in dilute sodium lactate (3)
Organ	% injected dose	% injected dose	% injected dose
Liver plus spleen*	97.3	90.8	95.0
Lungs	0.6	0.9	included with carcass
Blood	0.4	1.8	0.7
Entire remainder of carcass including bones	1.7	6.5	4.5

(1) Mouse sacrificed 6 minutes after injection.
(2) Mouse sacrificed 3 minutes after injection.
(3) Mouse sacrificed 5 minutes after injection.

* Data on many animals for spleen alone show uptakes varying between 1% to 4% of injected dose.

The supernatant colloid was then injected and was shown to have a slowly disappearing fraction of ~ 8%. These data appear to support the idea that the smaller particles constitute the slowly disappearing fraction. Further experiments with a colloid of zirconium-oxide suspended in sodium lactate were done in the rabbit. Figure 1 gives the blood disappearance curve. This particular preparation has an appreciable percentage of slowly clearing components in addition to the major fraction of very rapid blood clearance rate.

At 2 hours after injection (point A), when all the rapidly disappearing fraction had been cleared, serum was collected and the rabbit sacrificed. The serum containing the slowly disappearing component was injected into a

second rabbit. The second rabbit was sacrificed after its blood had been cleared, and the tissue distribution of the colloid was determined. The data are given in Table 2.

Table 2

	Distribution of zirconium colloid in initial rabbit (including rapidly clearing fraction)	Distribution of zirconium activity in animal injected with serum from initial animal
Organ	% Injected dose in entire organ	% Injected dose in entire organ
Bone plus marrow	9.3	43.0
Liver	85.0	23.0
Spleen	3.2	3.0
Muscle	1.3	17.0
Lungs	1.0	---
Kidney	0.3	14.4

It is seen that the liver received the major fraction of the rapidly clearing colloid, whereas the bone plus bone marrow received the major fraction of the slowly clearing colloid component. Thus in all the experiments tabulated (and in several others for similar colloids), where the colloidal particles are relatively large in size, sedimentible in large part even with ordinary centrifuges, producing sols that are turbid and demonstrate an intense Tyndall effect, we have found the blood clearance rate to be very rapid ($T_{1/2}$ = 30 seconds - 1 minute) and the site of uptake of the colloid to be the liver and spleen primarily. From the magnitude of the blood clearance rate it is certain that much of the colloid is cleared in a single passage through the liver, so that for these colloids the rate of blood flow through the liver relative to that through the bone marrow probably plays a major role in securing localization of the colloid in the liver plus spleen rather than in

the bone marrow. For chromic phosphate suspensions, also, it is now known that there is association of relatively large particle size, rapid blood clearance, and liver and spleen localization just as with the above-described zirconium colloids. The mechanism responsible for the rapid uptake by the liver and spleen of relatively large particles is not yet clear, nor has the significance of factors other than particle size alone been evaluated in determining blood clearance rate and site of uptake of the colloids.

B. Selective Localization of Radioisotopes of Zirconium, Columbium, and Yttrium Primarily in the Bone Marrow, and Secondarily in the Spleen and Liver.

As described in the preceding communication, anionic colloids of zirconium and yttrium may be prepared of much smaller particle size than those just shown to deposit primarily in the liver plus spleen. These colloids are of the zirconium-hydroxy-lactate and the yttrium-hydroxy-citrate type. In contrast to the rapid blood clearance of the "relatively large size" aggregates, these latter colloids of intermediate particle size are cleared much more slowly in the rabbit, rat, cat, and mouse, showing exponential decay from the blood stream with half-times from 30 to 80 minutes (see Figure 2), the colloid remaining quantitatively in the plasma until cleared. In the rabbit, cat and rat, the slowly disappearing colloids localize primarily in the bone marrow, with spleen and liver receiving the next highest quantity (all expressed on specific activity basis).* The distribution data in the rabbit for the zirconium-hydroxy-lactate colloid are given in Table 2 and for yttrium-hydroxy-citrate colloid in Table 3. In the case of zirconium and yttrium colloids which localize primarily in the bone marrow we find associated the features 1) particle size much smaller than those colloids

* In the mouse the liver and spleen take up the major fraction of the slowly disappearing colloids, although the bone marrow uptake is greater than for the "large particle" colloids.

localizing primarily in the spleen and liver (less pronounced Tyndall effect, non-centrifugibility with ordinary centrifuges), 2) slow disappearance from the blood stream ($T_{1/2}$ = 30 to 80 minutes contrasted with 30 seconds to 1 minute for "liver and spleen" colloids).

As was shown in the preceding communication it is possible to prepare particles of different chemical composition (but of the same size range) by altering the final solution pH at a particular metal ion/complexing ion ratio in the yttrium hydroxide-citrate system. With quantitative measure of particle sizes of these types, it is planned to try to gain some insight into the possible effect of variations in chemical composition (at the same particle size) on blood stream disappearance rate and site of uptake of colloids, and thus perhaps obtain a clearer idea of the mechanism operative in selective localization in one element or another of the reticulo-endothelial system. From the present data it is not possible to determine whether or not polymerization of the slowly disappearing colloids is a necessary preliminary to reticulo-endothelial deposition, or whether the particles remain in the serum at the original size and are slowly removed at this size.

Table 3

Distribution of Intravenously Injected Yttrium-Hydroxy-Citrate Colloid in the Tissues of the Rabbit ("Intermediate" particle size)

(Half-time for Blood Stream Clearance = 70 minutes)
(Animal sacrificed 18 hours after injection)

(Y^{91} tracer -- 1.0 cc. injection)

Organ	% of Injected Dose in entire organ	% of Injected Dose / per gram of tissue (specific activity)
Bone and Marrow	35.0	0.07
Bone (rib)*	--	0.21
Bone (femur)*	--	0.11
Marrow (rib)	--	0.45
Marrow (femur)	--	0.71
Spleen	1.0	0.67
Liver	45.0	0.22
Kidneys	1.2	0.056
Urine (first 18 hrs. after injection)	3.6	--
Gastrointestinal tract	2.4	0.013
Heart	0.12	0.057
Lymph nodes	--	0.01
Muscle	0.54	0.0003
Testes	0.05	0.006
Brain	0.02	0.002
Lungs	0.53	0.02

* It is difficult mechanically to remove all the marrow, so that bone specific activities always tend to be falsely high when most of the colloid is in the marrow.

/ The specific activity is the really significant value for calculation of irradiation dose received by a tissue following localization.

Table 4

Distribution of Intravenously Injected Zirconium*-Hydroxy-Lactate Colloid in the Tissues of the Rabbit

(Half-time for Blood Stream Clearance = 70 minutes)
(Animal sacrificed 24 hours after injection)

Organ	% of Injected Dose in entire organ	% of Injected Dose per gram of tissue (specific activity)
Bone and Marrow	44.0	0.11
Bone (femur)	--	0.03
Marrow (femur)	--	0.84
Spleen	1.3	0.65
Liver	37.0	0.28
Kidneys	2.7	0.15
Heart	0.09	0.045
Lungs	0.04	0.022
Urine (first 4 hrs. after injection)	3.2	--
Muscle	1.2	0.0007
Lymph nodes (~ 60% of total)	0.5	--
Gastrointestinal tract	3.0	0.018
Thymus	0.025	--
Pituitary	0.001	--
Adrenals	0.002	--
Brain	0.01	0.0014
Eyes	0.1	0.014

* A mixture of Zr^{95} and Cb^{95} radioisotopes was used in preparation of this colloid. The distribution data differed in no significant details from that for Zr^{89}, a positron emitter, the yttrium daughter of which is stable indicating that the Cb^{95} radioisotope shares the metabolic fate of the zirconium colloid.

C. Retention of Yttrium and Zirconium Colloids in Tissue Following Initial Uptake.

For several applications of such colloids in selective irradiation it is necessary to know to what extent the colloids remain fixed in tissues once deposited.

For the zirconium phosphate colloids (large particle type) in the mouse, analysis shows that there is at 10 days after injection 96.5% of injected zirconium in liver plus spleen, 0.3% in the lungs, and 3.3% in the entire remainder of the carcass. Comparison of these data with those for the same colloid in Table 1 shows that no significant loss of activity or change in distribution occurs for this colloid over this time interval.

Similar data for the yttrium-hydroxy-citrate and zirconium-hydroxy-lactate "intermediate particle size" colloids are given in Table 4.

Excretion data in the rabbit show negligible losses of yttrium colloid via the urine after the first day, while 1/2 to 1% of the injected dose is lost daily via the feces.

Except for a possible trend toward slow loss of activity from the liver, it is seen that the bulk of the yttrium and zirconium colloids tend to remain localized at the site of deposition, at least for the periods of time given in Table 4.

Table 4

Extent of Retention of Yttrium-Hydroxy-Citrate and Zirconium-Hydroxy-Lactate Colloids in Tissues of Deposition in the Rabbit*

	% Injected dose still present in entire organ					
	Yttrium-Hydroxy-Citrate Colloid		Zirconium-Hydroxy-Lactate Colloid			
Days after injection	2/3	11	1/6	1	10	22
Organ						
Bone and Marrow	35.0	53.0	44.0	46.0	35.0	34.0
Spleen	1.0	0.4	1.3	2.9	1.8	1.1
Liver	45.0	29.0	37.0	31.0	24.0	22.0
Kidneys	1.2	0.7	2.7	2.0	3.1	2.3
Lungs	0.5	0.3	0.4	0.9	0.6	0.3

* Data in this table were obtained by injecting separate rabbits with the colloids and sacrificing the animals at the stated time intervals after injection.

D. Experimental and Therapeutic Applications.

For experimental work requiring specific irradiation of the liver, spleen, or bone marrow, or combinations of these organs, the colloids described are useful. With the range of short and long-lived radioisotopes of yttrium, zirconium, and columbium available and with the evidence presented that colloids incorporating such isotopes may be retained in tissues of deposition for at least several weeks, it is possible to achieve continuous specific irradiation of tissue at any desired intensity level either for short or long periods of time.

In certain neoplastic diseases of the hematopoietic system, as the leukemias, myelomas, or other neoplasms involving the liver or bone marrow, and in polycythemia, the therapeutic use of yttrium or zirconium colloids

seems feasible. With these colloids irradiation is localized in three tissues while minimizing the irradiation of other tissues. With a range of half-lives of beta-emitting isotopes from 17.0 hours to 65 days, one may have a great latitude in choosing duration and intensity of therapeutic irradiation. A clinical evaluation of these colloids, with incorporated radioisotopes, is now being made both with respect to tissue distribution in humans and with respect to therapeutic efficacy in polycythemia, the leukemias and certain other selected diseases. While no great differences from other forms of radiation therapy are anticipated, it is possible that some improvement in the management of these diseases may result.

SUMMARY

1. Colloids of zirconium of relatively large particle size show rapid disappearance from the blood stream ($T_{1/2}$ = ca. 30 seconds to 1 minute) and are deposited mainly in the liver and spleen.

2. Colloids of smaller particle size, both of zirconium and yttrium, dissapear much more slowly from the blood stream ($T_{1/2}$ = 30 to 80 minutes) and are deposited primarily in the bone marrow and spleen; secondarily in the liver - the liver specific activity being approximately 1/3 that of marrow.

3. Both types of colloids, once deposited in these organs, show no significant change in distribution pattern, at least over a period of 2 to 4 weeks, and only slow excretion from the body.

4. The distribution of the colloids and the availability of the isotopes of a wide range of nuclear properties render them suitable for experimental studies requiring specific irradiation and for therapeutic utilization in certain diseases.

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BLOOD STREAM DISAPPEARANCE FOR "RELATIVELY LARGE PARTICLE SIZE" COLLOID IN THE RABBIT

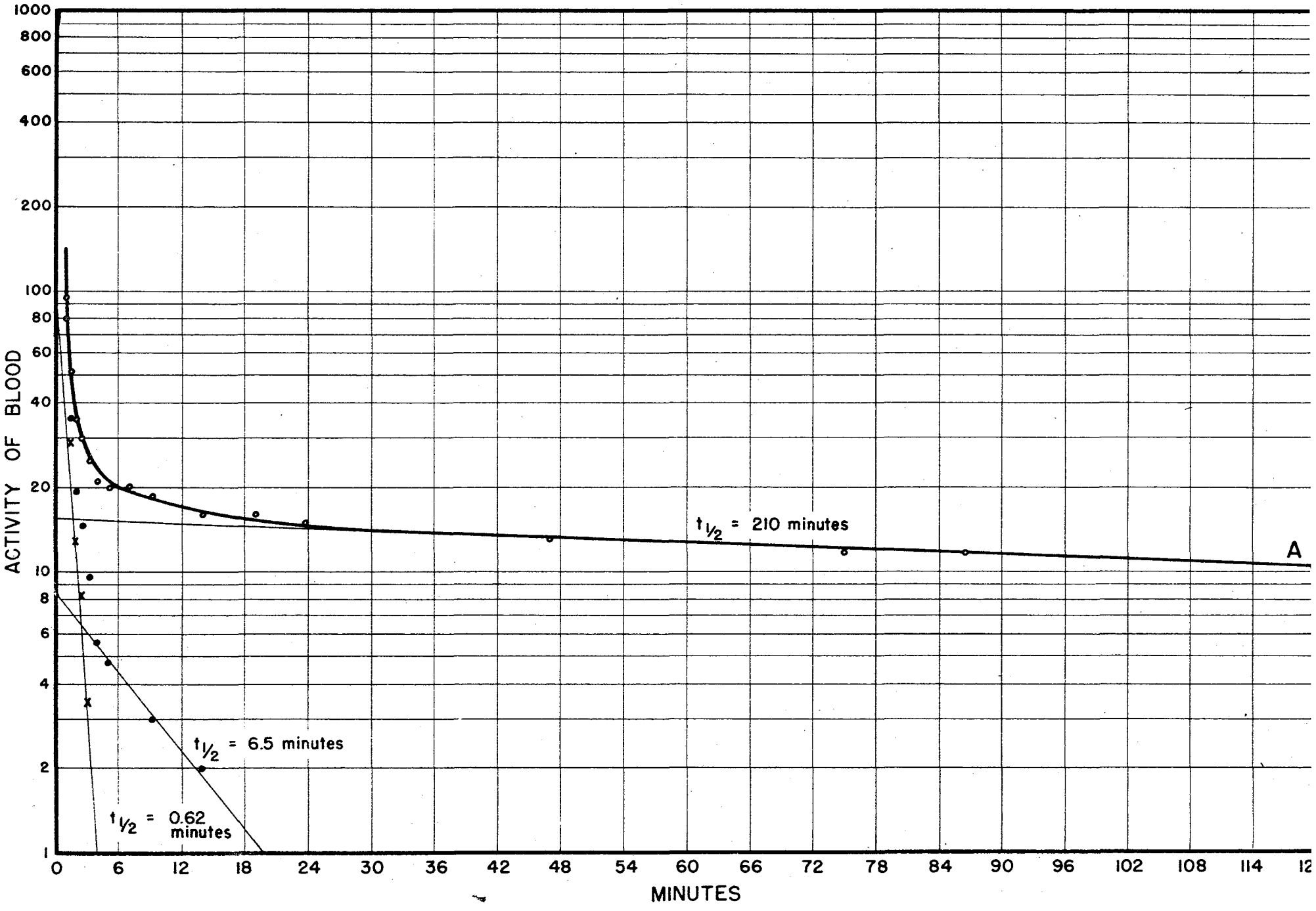

FIGURE 1.

FIG. 2. BLOOD STREAM DISAPPEARANCE FOR INTERMEDIATE PARTICLE SIZE COLLOID IN THE RABBIT

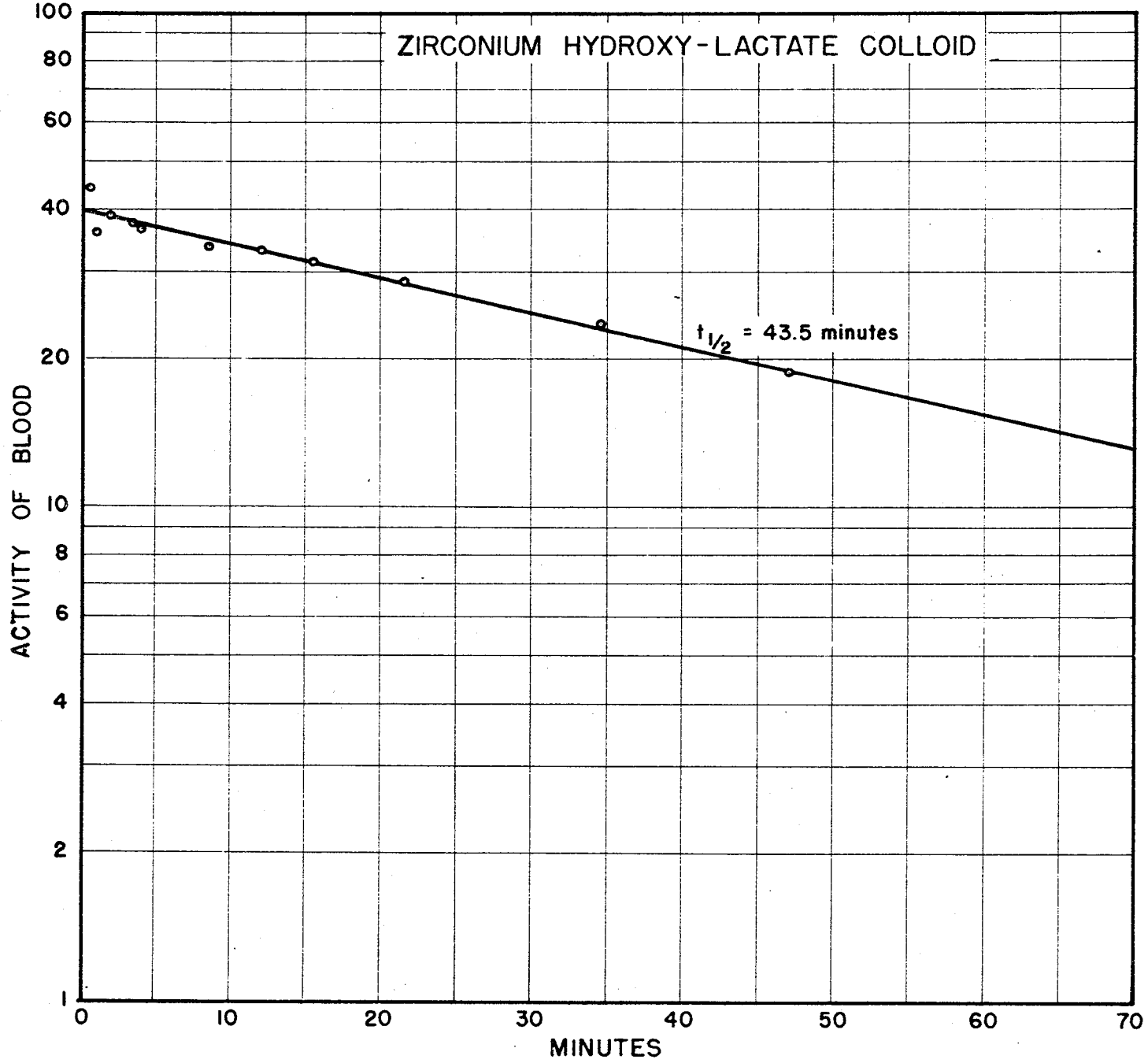

FIG. 3. BLOOD STREAM DISAPPEARANCE FOR INTERMEDIATE PARTICLE SIZE COLLOID IN THE RABBIT