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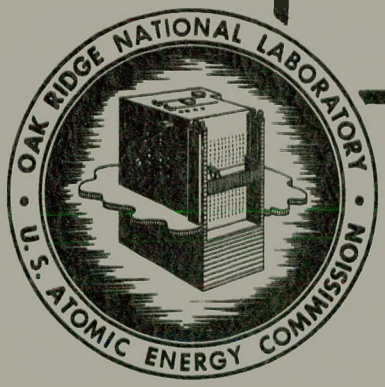
THE DISAPPEARANCE OF ISOTOPICALLY LABELED GOLD COLLOIDS FROM THE CIRCULATION OF THE DOG



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C. W. Sheppard, P. F. Hahn, and Gilbert Jordan

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THE DISAPPEARANCE OF ISOTOPICALLY LABELED GOID COLLOIDS FROM THE CIRCULATION OF THE DOG*

C. W. Sheppard, P. F. Hahn,† and Gilbert Jordan‡

Preliminary experiments have been described (1) in which radioactive colloidal gold was injected into the circulation and its disappearance from the blood was followed. Recent data obtained in rabbits were presented by Barrow et al. (2). Because of the usefulness of radioactively labeled colloids in physiology and radiobiology the results of further experiments of this type, obtained with dogs, are presented.

METHODS

Two untrained female mongrel dogs were used, the first (dog A, weighing 8 kg) in Experiments 1 and 2 and the second (dog B, weighing 7.3 kg) in the remainder. By conducting the experiments in pairs differences in the behavior of different materials were compared within a short time interval in the same dog. In some cases the animals were injected through the saphenous vein, in others by way of the jugular vein or cardiac puncture. Injections were usually completed within 10 seconds. Usually 1 or 2 seconds were required to obtain samples, which were taken from a different vein than that used for injection. Where blood could not be obtained rapidly, the time halfway through the withdrawal period was taken at the time of sampling, and the results were accepted only if rapid changes were not occurring. Occasionally, some 2 to 5 cc of normal saline were injected to keep the sampling needle open. Experiments 1 and 2 were conducted to investigate the effect of heparin on the removal of

* Most of the experimental work was done at Vanderbilt University School of Medicine, Nashville, Tennessee.

† Cancer Research Laboratory, Meharry Medical College, Nashville, Tennessee.

‡ Department of Pediatrics, Jefferson Hillman Hospital, Birmingham, Alabama.

colloidal particles. The animal was first injected with gold, and later heparinized and reinjected.

The gold sols were prepared from gold chloride by reduction with ascorbic acid as described previously (3). The characteristics of gold suspensions are indicated to a considerable extent by their optical properties. The colors of the sols used are given in Table I. By controlling the pH, dispersions of variable properties could be produced. At alkaline pH the color was deep red or reddish purple. Such sols had a weak Tyndall beam which was highly polarized. The Brownian motion was extremely vigorous. After preparation they were centrifuged at about 1000 g for 15 minutes before being used. Electron microscope pictures previously taken on similar colloids indicate particle sizes in the range of 2 - 20 millimicra.

By dispersing in acid solution sols were prepared whose particles were in the range 20 - 200 millimicra. Here transmitted light is pale sky-blue in color. The intense Tyndall effect produces reflected light of a strong brick-dust color. A large fraction of such suspensions settles out in a few hours standing. The Browniam motion is sluggish.

Blood samples for activity determinations were taken in dry oxalate bottles. Where measurement of the radioactivity of whole blood was desired, 2-cc samples of blood were dried in metal ointment boxes and counted directly. Frequently radioactivity was determined by two different methods. In one procedure the covered boxes were placed under a thin brass plate and the gamma-ray activity determined. Usually these samples were allowed to decay and later by the second procedure determinations of the beta-ray activity were obtained by direct counting.

In the study of blood fractions red cell samples were taken after centrifugation and washed with isotonic saline. When white cells were studied the white cell layer was removed after centrifugation and delivered to a new centrifuge

TABLE I

Half-Value Times for Initial Rapid Disappearance

Experiment	Dog	Color of sol	Added protein	Value in minutes
1	A	Deep reddish purple	Gelatin	1.0
2	A	Deep reddish purple	Gelatin	0.9
3	B	Sky blue, brick-dust color by reflected light	Gelatin	2.0
4	B	Deep red	Gelatin	1.2
5	B	Deep red	Gelatin	1.2
6	B	Sky blue, brick-dust color by reflected light	None	1.5

tube and respun. The upper layer of these cells was then delivered to Wintrobe hematocrit tubes and recentrifuged.

RESULTS

The disappearance of activity from the circulation in Experiment 1 is shown in Fig. 1. About 1 1/2 hours later 1 cc of Upjohn heparin (containing 10 mg sodium salt) was injected. The disappearance of a second injection of gold (after 20 minutes) is indicated in Fig. 2 (Experiment 2).

In Experiments 3 and 4 a large particle sol was first injected (Fig. 3), and about 1 1/2 hours later, small particles (Fig. 4). Unfortunately in Experiment 4 about 20 per cent of the material was lost in the tissue at the site of injection.

Because of the question of tissue retention, an additional study was made (Experiments 5 and 6). In the first stage small particles in gelatin were used; the results are shown in Fig. 5. The second stage using large particles without gelatin was initiated 2 hours and 20 minutes later. The results are shown in Fig. 6. The first point in Figs. 2, 4 and 6 is the residual level from the preceding experiment.

Additional experiments were performed to investigate the portion of activity in the circulation which disappears slowly, and to attempt to determine if a particular fraction of the blood contains the activity. On three occasions samples of blood were obtained after the base line phase of the disappearance curve was reached. These were separated into plasma, red cells, and white cells. In two cases a sufficient amount of white cells was obtained to permit activity measurements. In a third case difficulty was encountered in separating the white cells. The results are shown in Table II. The behavior of a base line sample of blood, when transfused into another dog, is shown in Fig. 7.

Figure 1

Experiment 1; Dog A, without heparin.

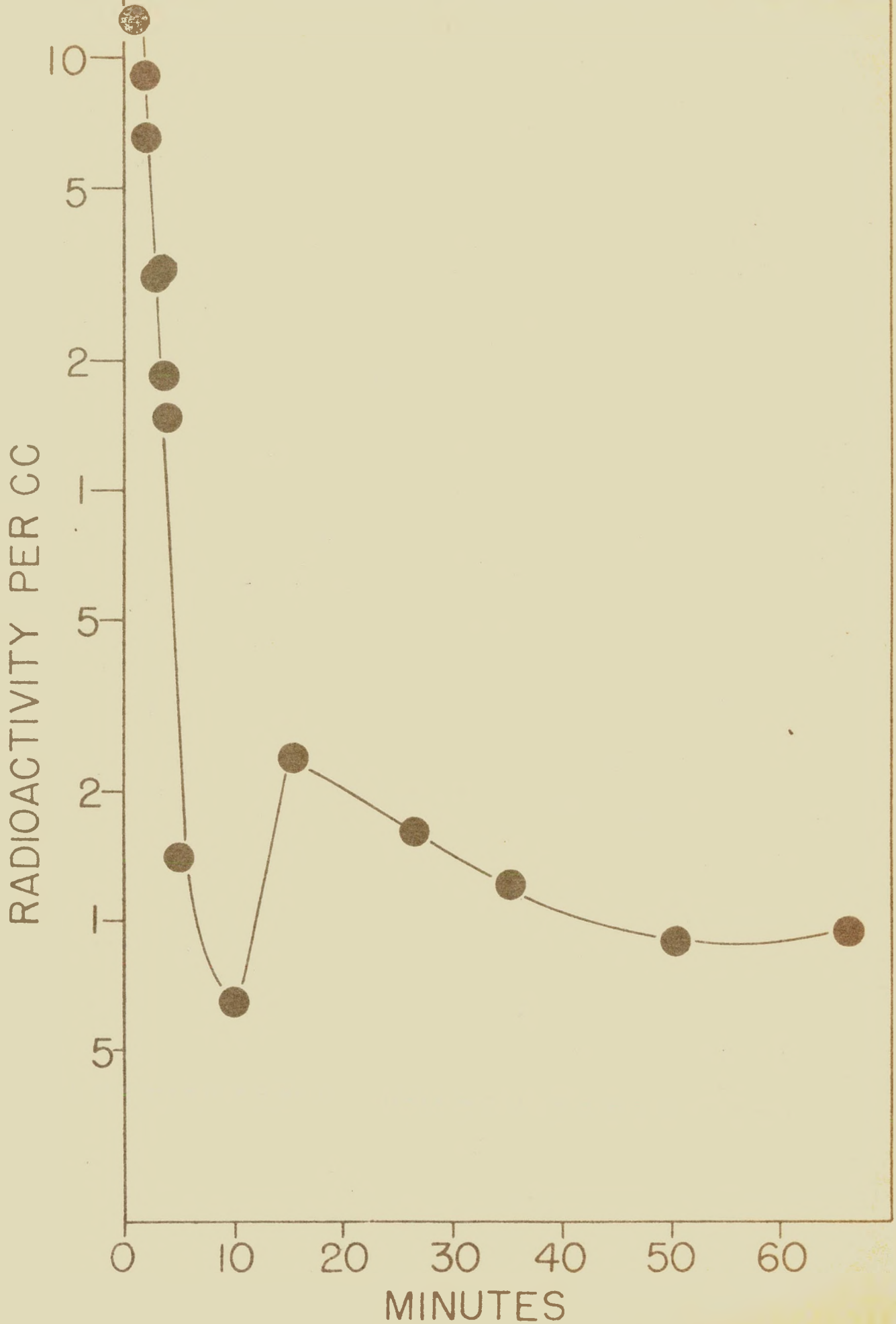

RADIOACTIVITY PER CC
10
5
2
1
5
2
1
5
0
10
20
30
40
50
60
MINUTES

Figure 2

Experiment 2; 1.8 hours after Experiment 1, Dog A, heparinized.

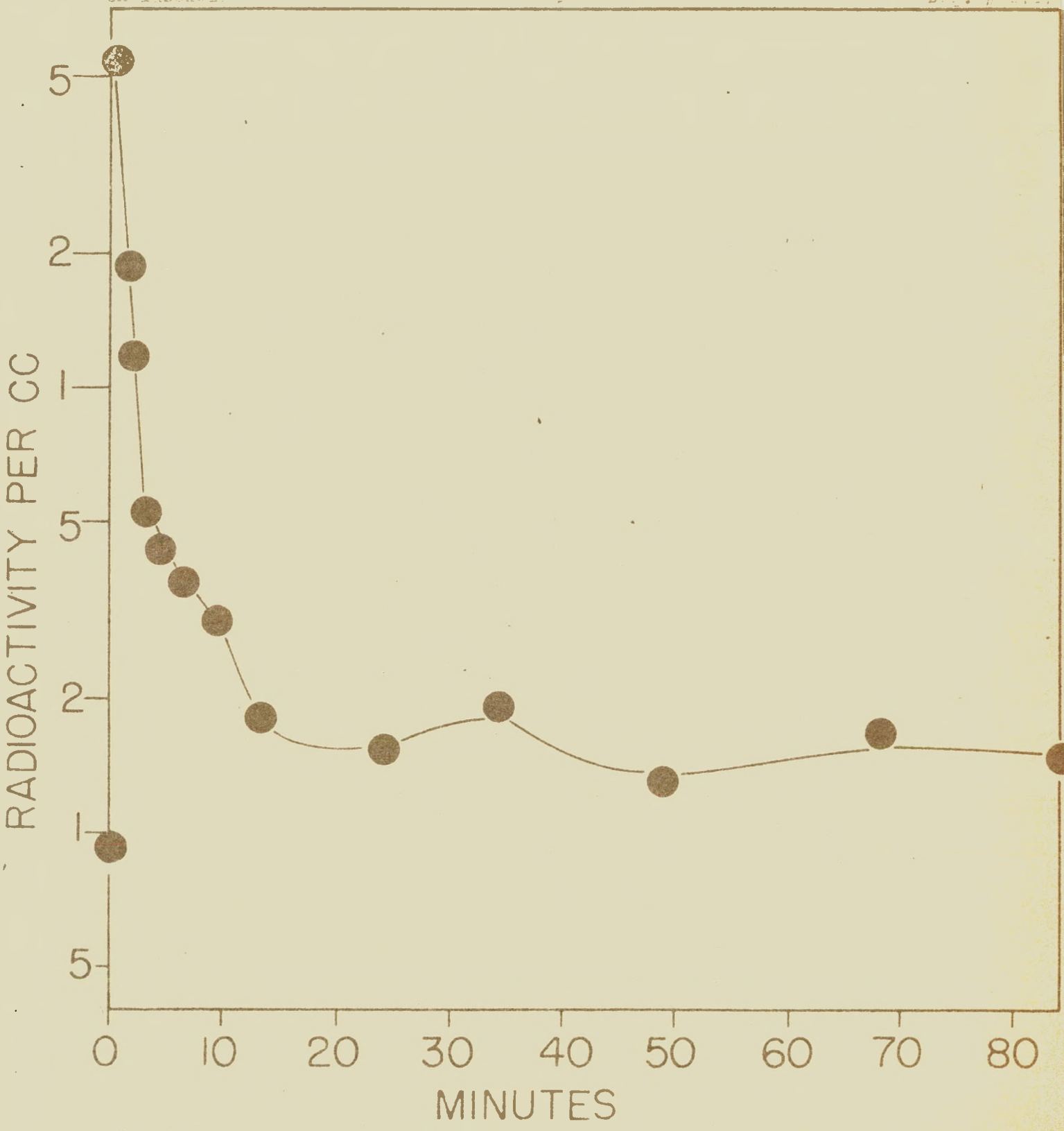


Figure 3

Experiment 3; Dog B, large particles.

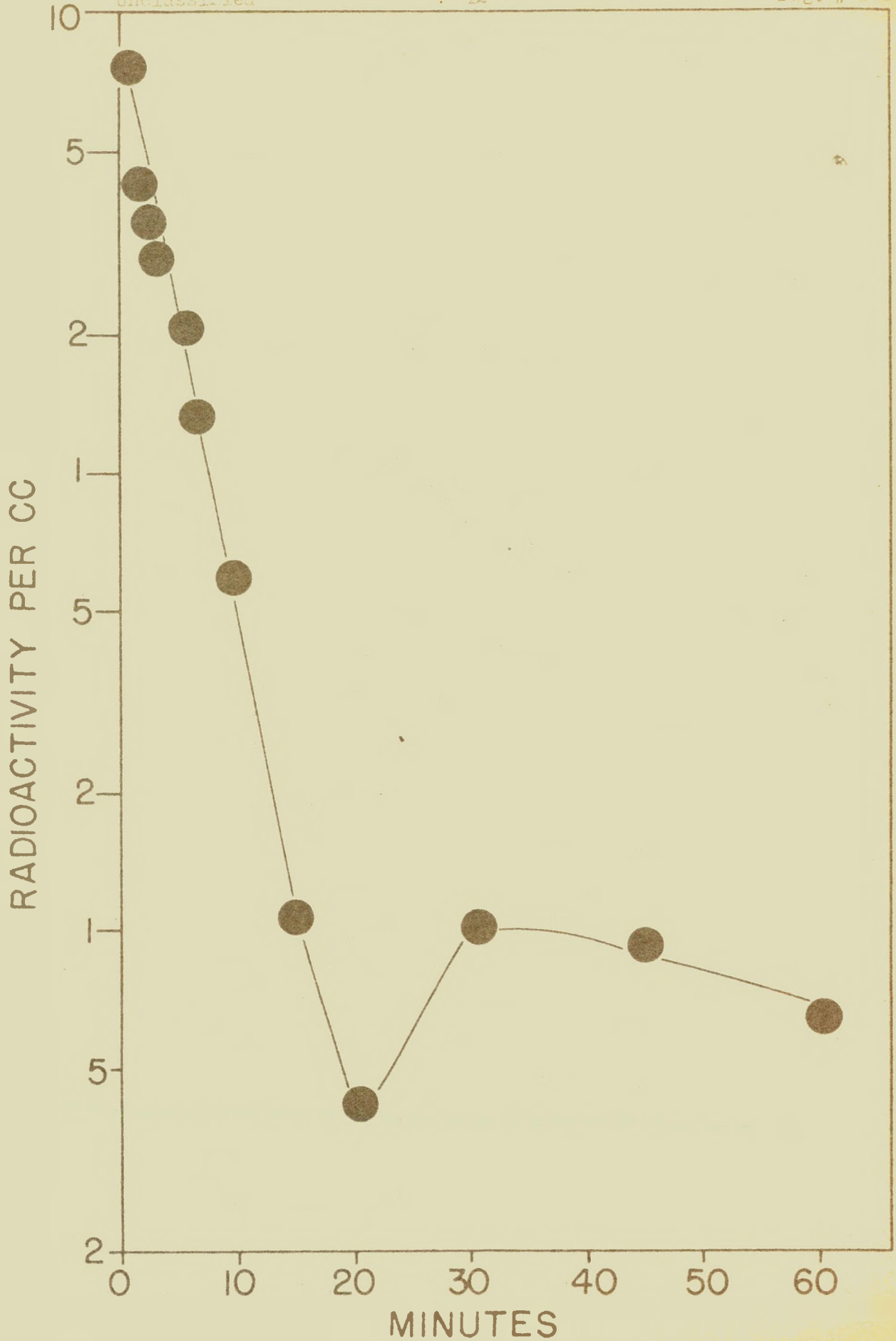

10
5
2
1
5
2
1
5
2
RADIOACTIVITY PER CC
0
10
20
30
40
50
60
MINUTES

Figure 4

Experiment 4; Dog B, small particles - 1.5 hours after Experiment 3. Part of the material was lost into the tissue at the injection site.

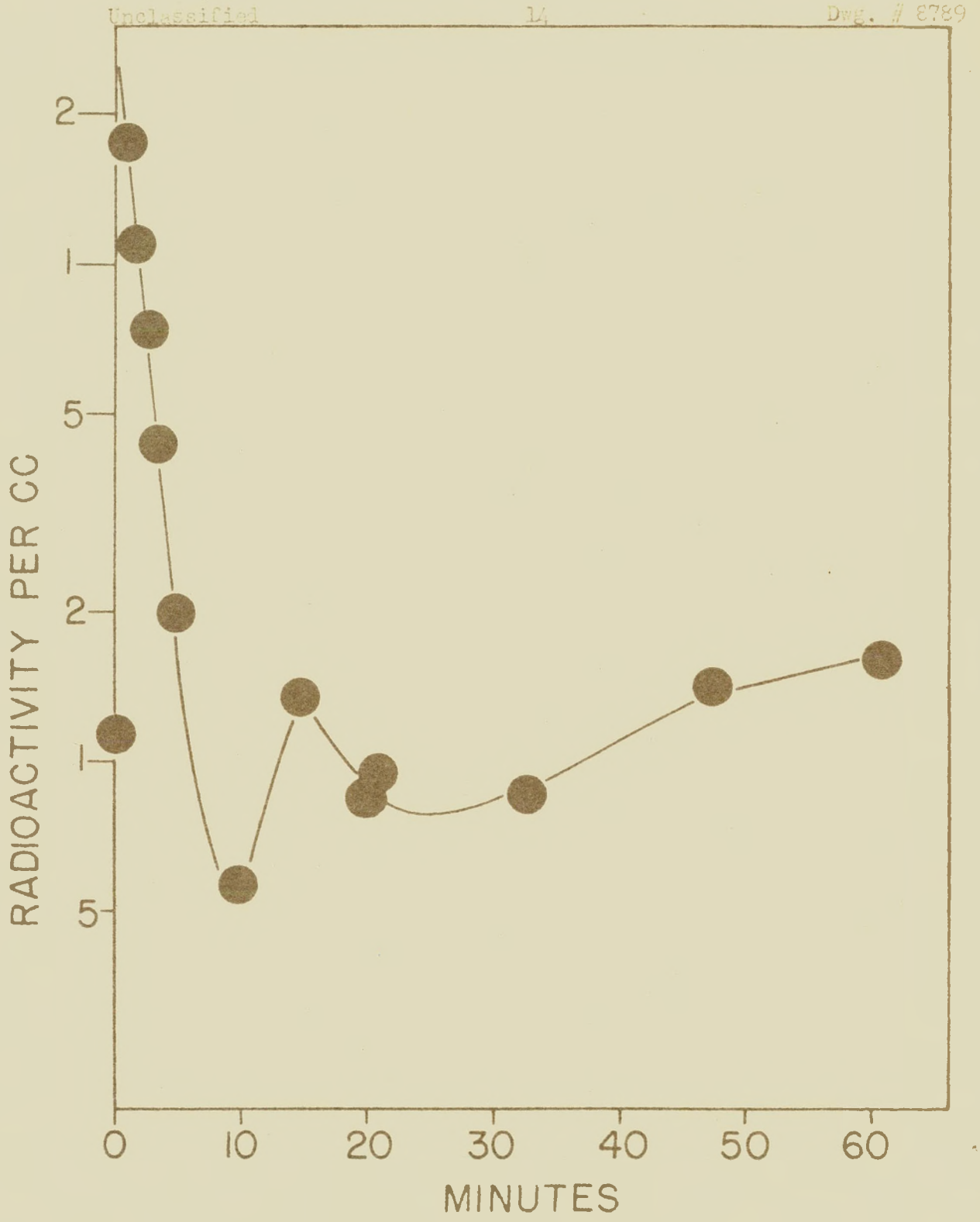

Unclassified
14
Dwg. # 8789
RADIOACTIVITY PER CC
2
1
5
2
1
5
0
10
20
30
40
50
60
MINUTES

Figure 5

Experiment 5; Dog B_9 small particles in gelatin.

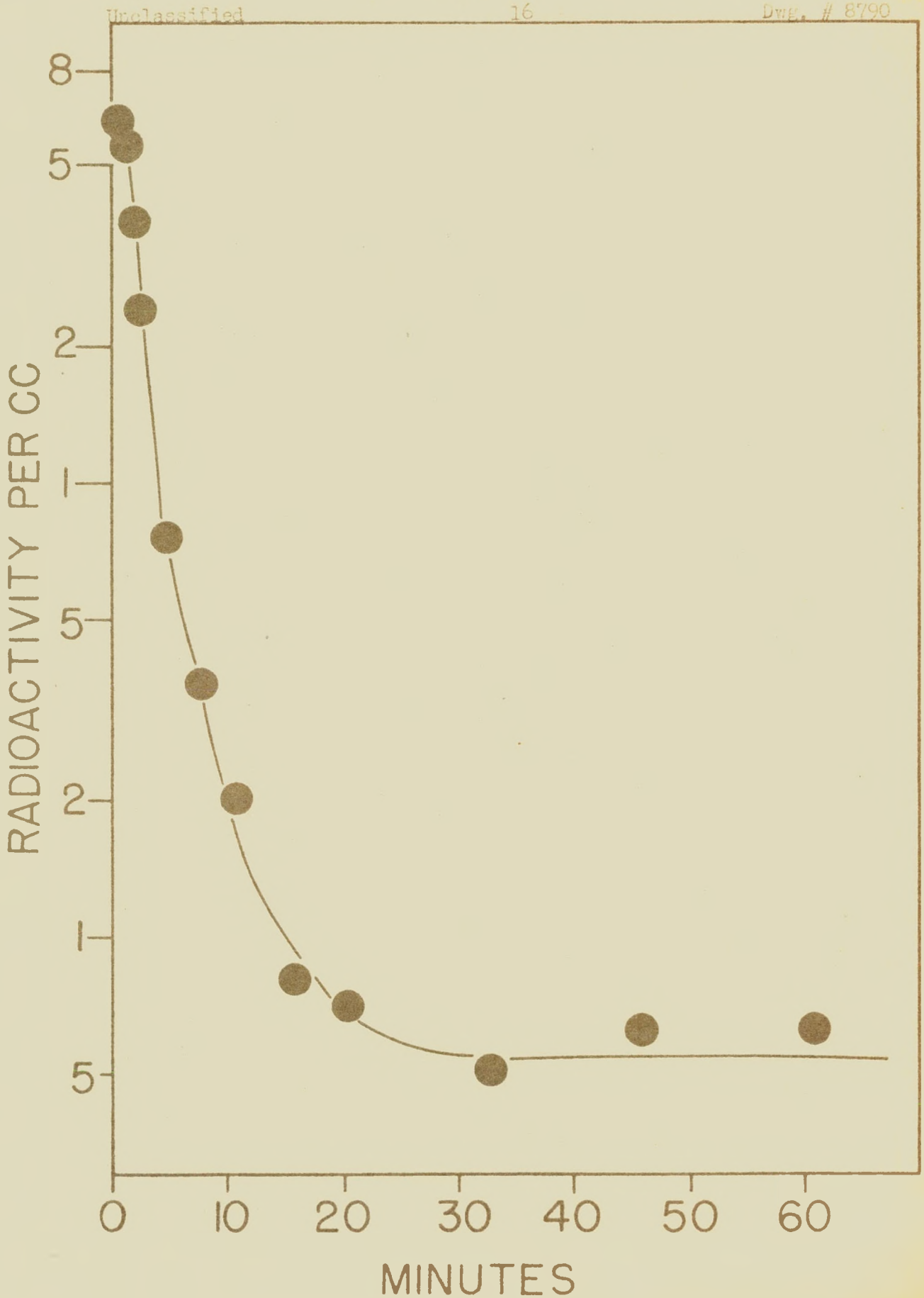

8
5
2
1
5
2
1
5
RADIOACTIVITY PER CC
0
10
20
30
40
50
60
MINUTES

Figure 6

Experiment 6; Dog B, large particles without gelatin 2.3 hours after Experiment 5.

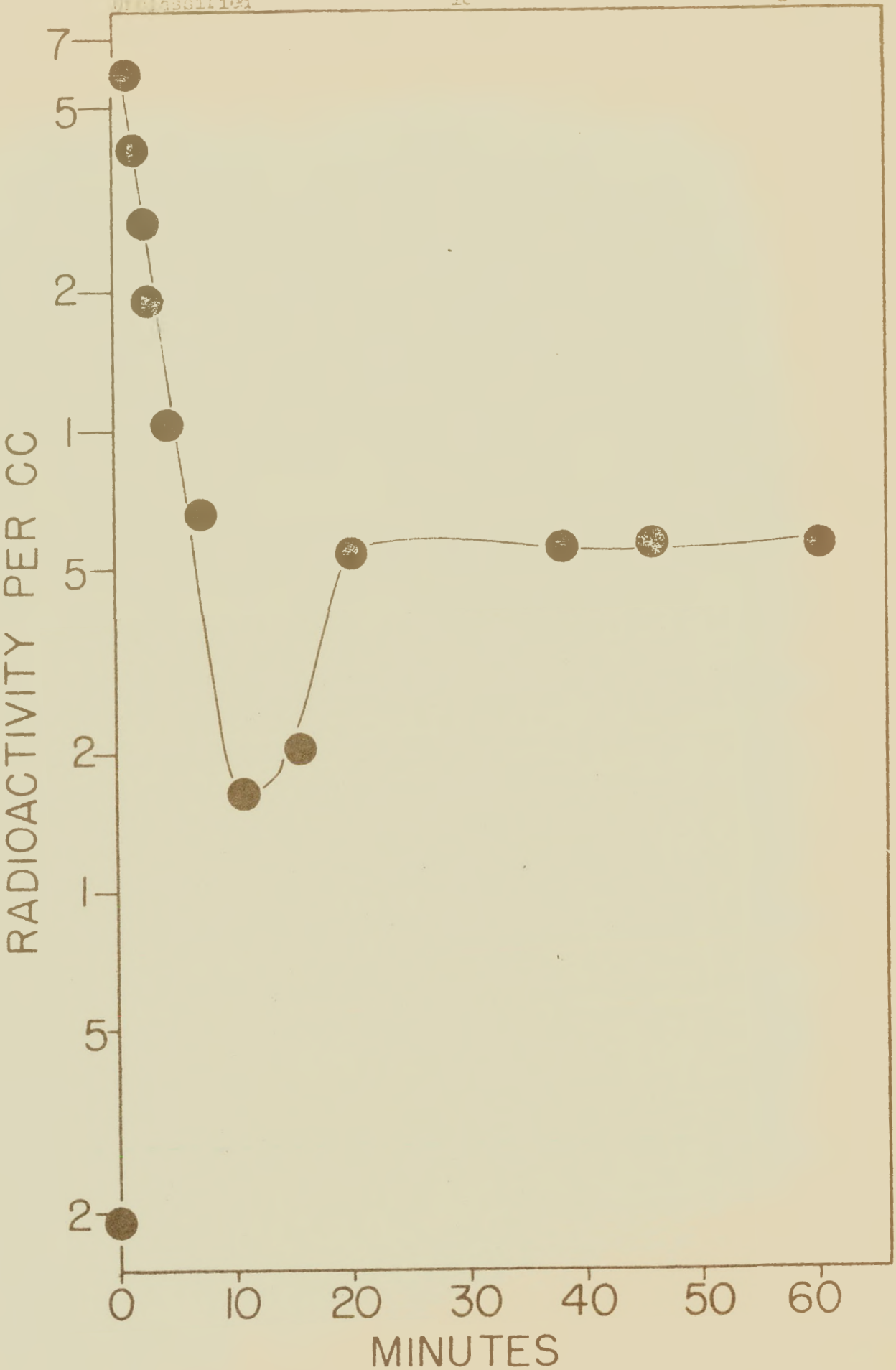

RADIOACTIVITY PER CC
7
5
2
1
5
2
1
5
2
0
10
20
30
40
50
60
MINUTES

TABLE II

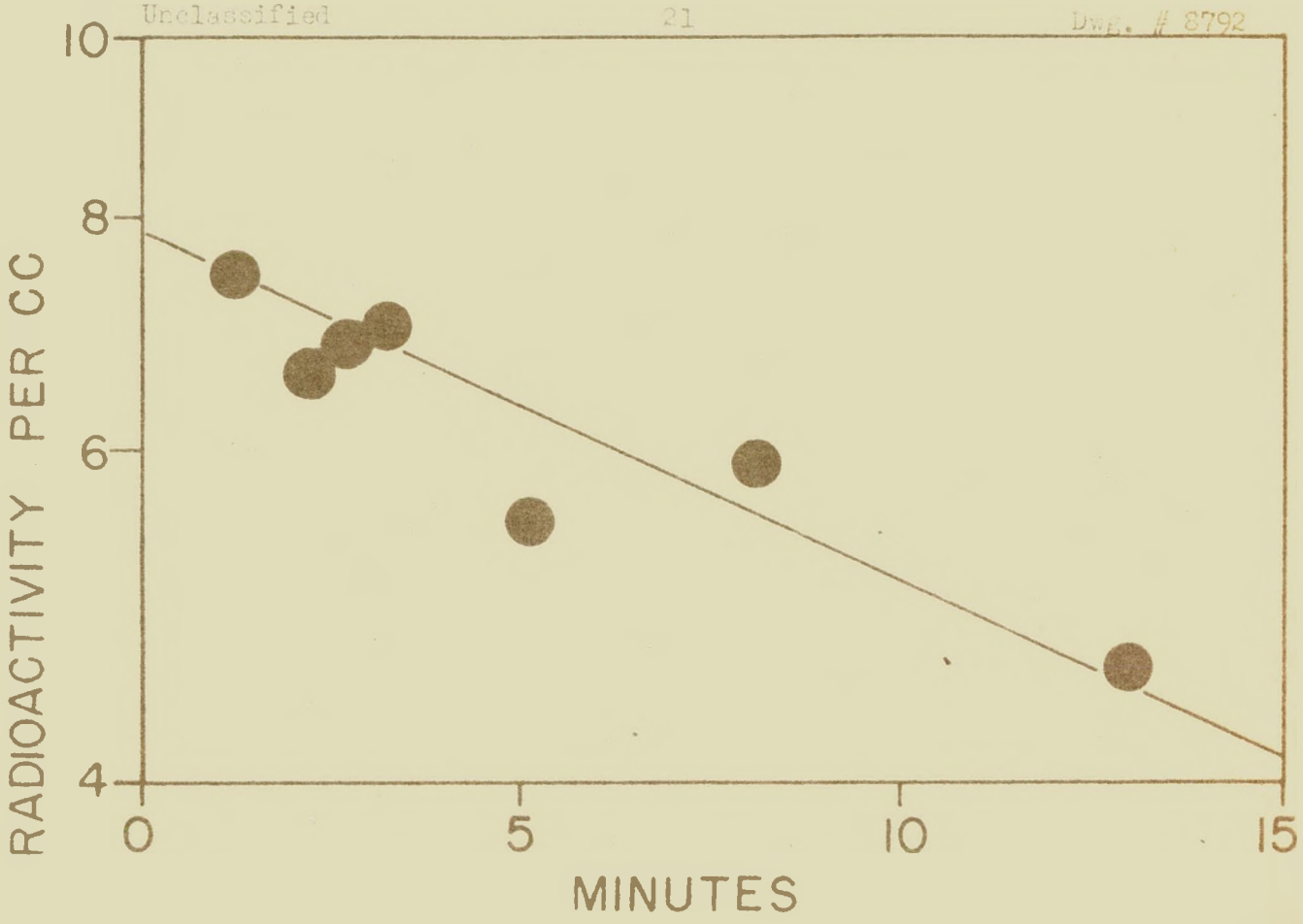
Distribution of Residual Activity in Blood

(All values are in counts per minute per cc)

Red cells	Buffy coat	Plasma	Remarks
28	470	0-75	Plasma irregular. Poor separation of white cells.
75	9,700	4,500	Buffy coat greatly diluted with red cells.
235	350,000	11,000	Good separation.

Figure 7

Disappearance of the activity of transfused blood.


10
8
6
4
RADIOACTIVITY PER CC
0
5
10
15
MINUTES

DISCUSSION

Table I shows the half-value times for the initial disappearance phase of the curves. In all cases this is exceedingly brief. The transition to a very slowly declining base line occurs rather abruptly, and often this portion of the curve shows fluctuations. If circulating blood at this point is transfused into another animal (Fig. 7) the decline is slow. Although there is still some activity in plasma (Table II), the high concentration in the buffy coat fraction is of particular interest. No significant difference is seen in the initial decline rate of colloids of varying particle size. However, some unknown factor is involved which is still uncontrolled since in Experiment 3 the decline rate is about half as great as in its companion experiment. It should be emphasized that these results apply only to canine experiments using gold colloids of a particular range of particle sizes. Other more subtle factors which might influence the disappearance still await investigation.

In the single comparison no significant difference is found in the initial rate of disappearance for the normal and heparinized animals. If this portion of the curve truly represents the uptake of particles by the liver the results in the dog are contrary to an early observation that in the mouse heparin inhibits the uptake of colloidal gold by macrophages (4). More recently Rigdon and Schrantz failed to observe an effect of heparin on India ink phagocytosis in rabbits (5).

It is suggested by Schubert et al. (6) that certain colloids may be cleared from the blood by a single passage through the liver. Although many details of the process of colloid removal remain to be investigated, this hypothesis may be tested on the initial phase of our experimental curves. It is assumed that the particles are uniformly mixed with the circulating blood, and are removed only by passage through the liver. The spleen, although active, will have a much smaller effect because of its small size. Under these conditions the concentration of

particles C will vary exponentially with time. The half-value time will be:

$$T_{1/2} = 0.69\ B/k\ Q$$

where B is the blood volume, Q the rate of blood supply to the liver, and k the fraction removed on a single passage through the organ. Solving for Q and inserting for the other values, B = 500 cc, k = 1, and $T_{1/2}$ = 1.5 minutes, we obtain Q = 230 cc/minute. This is an entirely reasonable value for the sum of the blood flow through the portal vein and hepatic artery (7). The result suggests a possible method for studying the liver circulation but the ultimate conclusion on this point must await further study.

SUMMARY

Immediately following injection of radioactively tagged colloidal gold particles in the dog the circulatory level drops exponentially with time, the half-value time being about 1 1/2 minutes. A very low residual level is reached in about 10 - 20 minutes showing occasional fluctuations. A high concentration of activity is then found in the buffy coat fraction, and if blood is withdrawn and transfused into a second animal the initial rapid rate of decline is not observed. The possibility is explored that the rate of the initial disappearance might be quantitatively related to the total blood flow rate to the liver.

We wish to acknowledge the assistance of Mr. W. W. Woodbury who recorded the time during some of the experiments.

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