LOCALIZATION OF ^{58}Co AND ^{65}Zn-HEMATOPORPHYRIN COMPLEXES IN CANINE LYMPH NODES

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Hematoporphyrins and metallo-hematoporphyrins are known to localize in lymph nodes (*1,2*). However, no attempt has been made to quantitate the fractions of administered hematoporphyrin or metallo-hematoporphyrin which do so.

In this report we present results obtained with two metallo-hematoporphyrins, ^{58}Co and ^{65}Zn-hematoporphyrin complexes. A quantitative study of the tissue distribution of these metallo-hematoporphyrins in a series of dogs and in one human subject is presented.

The possible use of radioactively labeled metallo-hematoporphyrins in selective lymphatic irradiation is discussed.

METHODS

Hematoporphyrin-free base was obtained from K and K Laboratories (Plainview, N.Y.). Carrier-free ^{58}Co-acetate and ^{65}Zn-acetate (5 Ci/gm) were obtained from New England Nuclear Corporation (Boston, Mass.). The ^{58}Co and ^{65}Zn-hematoporphyrin complexes were prepared according to the method of Taylor (*3*).

In one series of studies carrier-free ^{58}Co-acetate was added to an acetate buffer, pH 6, and administered intravenously to two normal dogs. In a second series of studies ^{58}Co-hematoporphyrin was dissolved in 0.05 *N* sodium hydroxide and administered intravenously to seven normal dogs and to one human patient with carcinoma of the colon. In addition ^{65}Zn-hematoporphyrin was dissolved in 0.05 *N* sodium hydroxide and administered intravenously to one normal dog. The amount of hematoporphyrin administered varied from 10 to 50 mg. The amount of metallo-hematoporphyrin administered was negligible because carrier-free ^{58}Co and high specific-activity ^{65}Zn were used in the preparation of the metallo-hematoporphyrins.

In each experiment plasma samples were obtained every 10 min for the first hour of the study and daily thereafter for 5 days. Sample radioactivity was determined using a well scintillation counter. The initial distribution volume of the intravenously administered metallo-hematoporphyrin was calculated by dividing the activity administered by the zero-time extrapolate of the single exponential function fit to the plasma radioactivity clearance curve during the first hour of the study.

In two dogs plasma samples were obtained on Day 1 after the intravenous administration of the ^{58}Co-hematoporphyrin. In the same dogs, plasma samples were obtained before the administration of radioactivity; part of this plasma was incubated with ^{58}Co-acetate and the other part with ^{58}Co-hematoporphyrin. A 16-hr paper electrophoresis was performed on all these plasma samples using B-2 Veronal buffer, pH 8.6 (Spinco). After the electrophoresis the distribution of the radioactivity in the paper was determined by dividing the paper into sections and counting each section in a well scintillation counter.

The loss of radioactivity from the entire body of two dogs and of one human subject given ^{58}Co-hematoporphyrin was determined using the whole-body counter.

At the end of periods varying from 1 to 18 days, the dogs were killed, their tissues weighed and the concentration of radioactivity determined. In one human patient specimens of mesenteric lymph node, fat and muscle were obtained during an exploratory laparotomy 5 days after the intravenous administration of ^{58}Co-hematoporphyrin. On the day before surgery, a needle bone marrow biopsy was obtained from the iliac area. The human tissue samples were weighed and the radioactivity counted using a well scintillation counter.

RESULTS

Figure 1 shows the disappearance of radioactivity from the plasma in a dog (#1) given ^{58}Co-acetate intravenously. The abscissa represents time after the

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intravenous administration of ^{58}Co-acetate and the ordinate the counts per minute per milliliter of plasma. There is an initial rapid disappearance of ^{58}Co from the plasma (half-time $\simeq$ 4 min). Thereafter, there is a change in the slope of the curve with the appearance of a slower second exponential component (half-time $\simeq$ 200 min).

Table 1 shows the tissue distribution of ^{58}Co in the same dog (#1) and in another normal dog (#2). Both dogs were killed on the third day following the intravenous administration of ^{58}Co-acetate. In Table 1 the radioactivity is expressed as the fraction of the administered dose $\times$ 10^{-6} per gram wet weight and as the ratio of activity per unit weight of the given organ to the activity per unit weight of muscle. The organ containing the highest concentration of radioactivity in these studies is the renal cortex, while the organ with the next highest radioactivity concentration is the liver. The radioisotopic concentration in lymph nodes is low and comparable to that found in tissues other than kidneys and liver. In these animals little of the administered radioactivity is retained in the body, the major portion having been excreted in the urine.

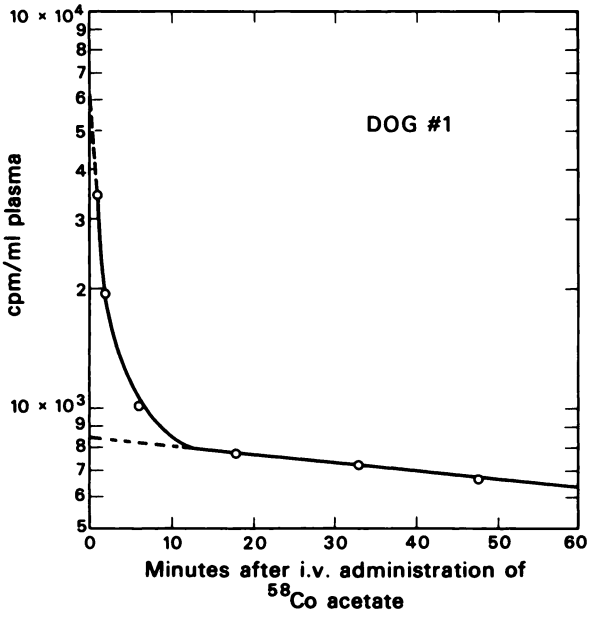

FIG. 1. Disappearance of radioactivity from plasma in dog (#1) given ^{58}Co-acetate intravenously. There is initial rapid disappearance of ^{58}Co from plasma (half-time $\simeq$ 4 min). Thereafter there is change in slope of curve with appearance of slower second exponential (half-time $\simeq$ 200 min).

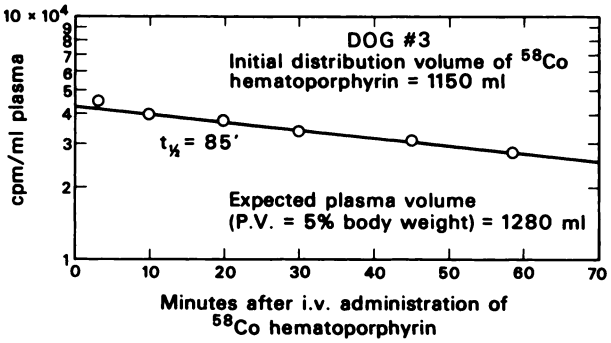

FIG. 2. Disappearance of radioactivity from plasma in dog (#3) given ^{58}Co-hematoporphyrin intravenously. Plasma disappearance of radioactivity during first hour is slow and has single exponential clearance rate (half-time = 85 min).

TABLE 1. FRACTION OF ADMINISTERED ^{58}Co-ACETATE $\times$ 10^{-6} PER GRAM OF WET WEIGHT (3 DAYS AFTER ADMINISTRATION)

	Dog 1	Dog 2	Average	Ratio of average concentration in tissue to average concentration in muscle
Muscle	1.1	3.2	2.1	1.0
Pancreas	3	4.4	3.8	1.8
Lung	5	8	6.5	3.1
Duodenal mucosa	2.5	6.0	4.2	2.0
Spleen	2	3	2.5	1.2
Kidney cortex	12	40	26	12.4
Liver	7	22	14.5	6.9
Femoral bone marrow	1.3	4	2.6	1.2
Mesenteric lymph node	2	3	2.5	1.2
Adrenal	4	4	4	1.9

Figure 2 shows the disappearance of radioactivity from the plasma in a dog (#3) given ^{58}Co-hematoporphyrin. The abscissa represents time after the intravenous administration of ^{58}Co-hematoporphyrin (with only the first hour of the study shown in this figure), and the ordinate represents counts per minute per milliliter of plasma. The plasma disappearance of radioactivity during the first hour is slow and has a single exponential clearance rate (half-time = 85 min). The initial distribution volume of the intravenously administered ^{58}Co-hematoporphyrin is 1,150 ml which agrees closely with the expected plasma volume of 1,200 ml (plasma volume = 5% of body weight). Figure 2 shows the pattern of clearance of radioactivity from plasma in the seven normal dogs and the one human patient given ^{58}Co-hematoporphyrin.

Figure 3 gives the pattern of radioactivity clearance from the plasma in a dog (#3) during a 5-day period following the intravenous injection of ^{58}Co-hematoporphyrin. After about 2 hr there is a diminution in the slope of the curve which eventually corresponds to a half-time of about 52 hr. This study is also representative of three dogs and one human subject followed for 5 days.

TABLE 2. FRACTION OF ADMINISTERED ^{58}Co-HEMATOPORPHYRIN $\times$ 10^{-6} PER GRAM WET WEIGHT

	Dogs							Human	Average for all dogs	Ratio of average concentration in tissue to average concentration in muscle
	3	4	5	6	7	8	9			
Time (days)	1	3	3	4	4	6	18	5		
Muscle	5	20	13	2	6	13	6	1	9.2	1
Pancreas	12	32	40	15	16	15	20	—	21.4	2.3
Lung	33	70	80	40	60	40	60	—	54.7	5.9
Duodenal mucosa	40	90	110	50	45	60	120	—	73.5	8.0
Spleen	60	130	200	80	80	72	360	—	140	15.2
Kidney cortex	340	1,800	1,900	900	330	800	600	—	987	107.3
Liver	850	760	1,000	320	48	1,600	1,500	—	908	98.6
Femoral bone marrow	60	220	250	85	570	300	180	11*	163	17.7
Mesenteric lymph node	250	2,220	1,200	500	600	1,900	2,000	62	1,230	133.6
Adrenal	170	400	40	160	140	—	480	—	290	31
Kidney medulla	30	—	—	—	—	—	54	—	42	4.6
Brain	6	—	—	—	—	—	3	—	4.5	0.5
Bone	6	—	—	—	—	—	7	—	6.5	0.7
Fat	12	—	—	—	—	—	20	2	16	1.9

* Bone marrow obtained from iliac crest.

Table 2 gives the tissue distribution in seven dogs and one human patient given ^{58}Co-hematoporphyrin intravenously. The radioisotopic concentration in tissue is expressed as a fraction of the administered dose $\times$ 10^{-6} per gram of wet weight and as the ratio of activity per unit weight of the given organ to the activity per unit weight of muscle. The dogs were killed at intervals varying from 1 to 18 days after the administration of the isotope. The dogs varied in body weight from 32 to 40 lb and the human subject weighed 160 lb. The results reported under mesenteric lymph nodes are representative of other nodes obtained from the cervical, inguinal and paratracheal areas. Although the absolute concentration of radioactivity in different tissues varied widely from dog to dog, there appeared to be little change in relative distribution of radioactivity in the various tissues from the 1st to 18th day following i.v. administration of ^{58}Co-hematoporphyrin. From the averaged relative distribution of radioactivity in different tissues for all dogs studied expressed as the ratio of averaged activity per gram of the tissue in question to the activity per gram of muscle (last column Table 2), it can be readily appreciated that the highest average relative concentration of activity occurred in the mesenteric lymph nodes, followed by kidney and liver. Additionally, the ratio of activity in lymph node is, on the average, 7.5 times greater than in bone marrow and 16.7 times higher than in duodenal mucosa.

In the human subject the relative distribution of radioactivity in bone marrow, lymph node, muscle and fat is similar to that exhibited by the dogs. The concentration of radioactivity per gram of lymph node is approximately six times greater than that obtained in bone marrow.

Figure 4 shows the relationship of plasma radioactivity to migration zones corresponding to various electrophoretically determined plasma-protein con-

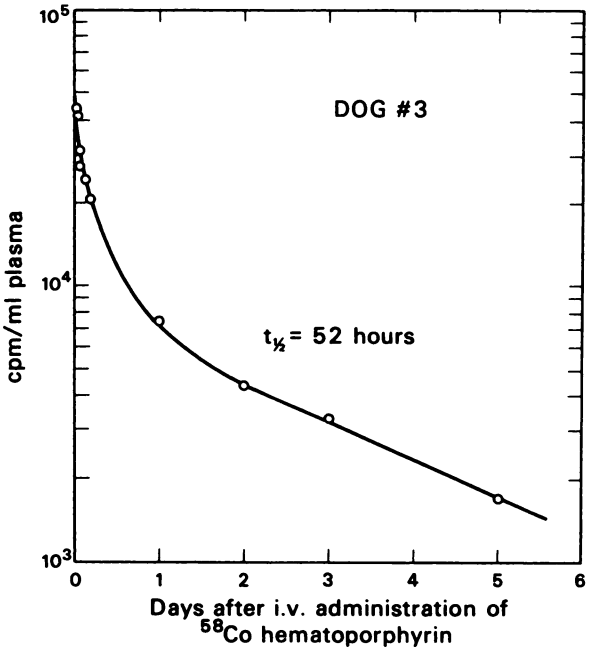

FIG. 3. Pattern of radioactivity clearance from plasma in dog (#3) during 5-day period following intravenous injection of ^{58}Co-hematoporphyrin. After 2 hr there is diminution in slope of curve which eventually corresponds to half-time of 52 hr.

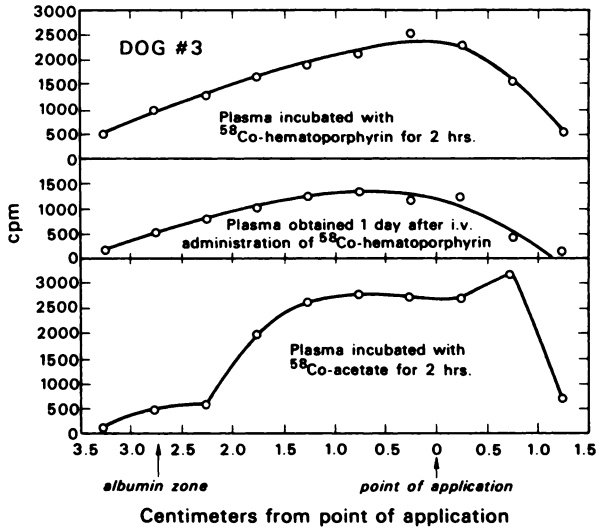

FIG. 4. Relationship of plasma radioactivity to migration zones corresponding to various electrophoretically determined plasma protein constituents. Different distribution pattern results with use of ^{58}Co-hematoporphyrin compared to ^{58}Co-acetate; following *in vivo* administration of ^{58}Co-hematoporphyrin distribution of radioactivity in plasma is similar to plasma incubated *in vivo* with ^{58}Co-hematoporphyrin.

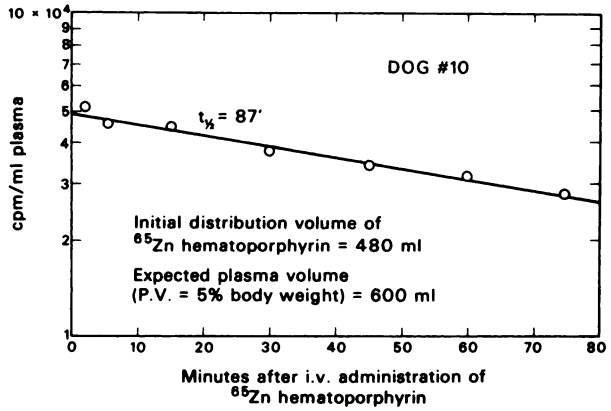

FIG. 5. Disappearance of radioactivity from plasma in dog (#10) given ^{65}Zn-hematoporphyrin intravenously. During first hour of study disappearance of radioactivity from plasma is slow and follows single exponential function (half-time $\simeq$ 87 min).

stituents. The top figure gives results obtained when plasma is incubated with ^{58}Co-hematoporphyrin for 2 hr at 37°C while the lower figure shows results obtained when plasma is similarly incubated with ^{58}Co-acetate. The middle figure gives results obtained from dog plasma 1 day after i.v. administration of ^{58}Co-hematoporphyrin. It can be seen that a different distribution pattern results with the use of ^{58}Co-hematoporphyrin compared to ^{58}Co-acetate and that following *in vivo* administration of ^{58}Co-hematoporphyrin the distribution of radioactivity in the plasma is similar to the pattern obtained when plasma is incubated *in vitro* with ^{58}Co-hematoporphyrin.

Figure 5 shows the disappearance of radioactivity from the plasma in a dog (#10) given ^{65}Zn-hematoporphyrin i.v. During the first hour of the study the disappearance of radioactivity from the plasma is slow and follows a single exponential function (half-time = 87 min) similar to that obtained with ^{58}Co-hematoporphyrin.

Table 3 gives the relative distribution of radioactivity in the tissues of this dog (#10) 3 days after administration of ^{65}Zn-hematoporphyrin. The concentration of radioactivity in this animal's lymph nodes is 8.6 times greater than the duodenal mucosa and 5 times greater than bone marrow.

Figure 6 shows the disappearance of radioactivity from the entire body of a human subject (JD) given ^{58}Co-hematoporphyrin. The abscissa represents time in days and the ordinate percent activity remaining in the body. For the first 4 weeks the dis-

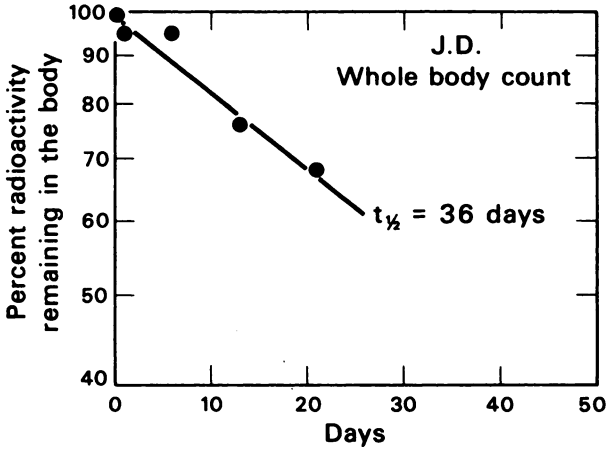

FIG. 6. Disappearance of radioactivity from entire body of human subject, JD, given ^{58}Co-hematoporphyrin intravenously. For first 4 weeks disappearance of radioactivity follows simple exponential with half-time of 36 days.

TABLE 3. FRACTION OF ADMINISTERED ^{65}Zn-HEMATOPORPHYRIN $\times$ 10^{-6} PER GRAM OF WET WEIGHT OF TISSUE (3 DAYS AFTER ADMINISTRATION)

	Dog 10	Ratio of concentration in tissue to concentration in muscle
Muscle	16	1.0
Pancreas	32	2.0
Lung	30	1.8
Duodenal mucosa	96	6.0
Spleen	160	10.0
Kidney cortex	800	50.0
Kidney medulla	16	1.0
Liver	1,600	100.0
Femoral bone marrow	160	10.0
Mesenteric lymph node	830	51.9
Adrenal	600	37.5

appearance of radioactivity follows a single exponential with a half-time of 36 days. The results of this study are similar to that obtained in two normal dogs. The loss of activity occurs both from the urine and stools in a ratio of 3-to-2, urine-to-stools.

DISCUSSION

Cobalt chelated with porphyrins *in vitro* appears to be very stable; the metal dissociates from the porphyrin only in the presence of concentrated sulfuric acid (*4*). The present work suggests that the ^{58}Co-hematoporphyrin complex is stable *in vivo* as is shown by the kinetic studies and tissue-distribution studies.

The clearance of radioactivity from the plasma in dogs injected intravenously with ^{58}Co-hematoporphyrin is described by at least two exponential terms, an initial one with a half-time of about 85 min and a second one appearing about the second hour of the study with a half-time of about 52 hr. The presence of the second exponential component raises two possibilities:

1. that the ^{58}Co is bound to more than one form of porphyrin and
2. that there is a feedback of ^{58}Co-hematoporphyrin from tissues into the plasma.

These two possibilities cannot be resolved from the present experiments.

The loss of radioactivity from the body of the human and dogs given ^{58}Co-hematoporphyrin as determined by the whole-body counter showed a loss of about 2% per day (half-time $\simeq$ 36 days). In man this loss during the first 7 days occurred through the urine and fecal routes in a ratio of 3-to-2.

After the intravenous administration of ^{58}Co-hematoporphyrin to dogs, the lymph nodes, kidney and liver showed the highest uptake of radioactivity. However, two of the most radiosensitive organs, the duodenal mucosal cells and the bone marrow cells showed much less concentration of radioactivity with an average lymph node-to-duodenal mucosal cell ratio in dogs of 16-to-7 and an averaged lymph node-to-bone marrow ratio of 7.5-to-1. These findings coupled with the marked vulnerability of the lymph cells to irradiation raises the possibility of selective lymph node irradiation, using appropriate radioisotopic metallo-hematoporphyrins. The feasibility of such a procedure would depend on: (1) use of a radioactive metal that is strongly chelated by hematoporphyrin; (2) use of a radioactive metal that decays predominantly by beta or weak gamma emission since strong gamma emitting isotopes deposit a significant amount of their energy far from the site of their localization.

We are presently studying various radioisotopic metallo-hematoporphyrins as possible agents for selective lymphatic irradiation for possible use in therapy of lymphatic neoplasms and in suppression of the homograft rejection response.

SUMMARY

After the intravenous administration of ^{58}Co-hematoporphyrin to a series of dogs, the lymph nodes, kidneys and liver showed the highest uptake of radioactivity. Radiosensitive tissues such as the duodenal mucosa and the bone marrow showed much less concentration of radioactivity. These findings, coupled with the marked vulnerability of the lymph cells to irradiation, raises the possibility of selective lymph node irradiation using appropriate radioisotopic metallo-hematoporphyrins.

ACKNOWLEDGMENT

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