PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 69–74 (1980)

The Biodistribution of Exogenous [^{35}S]Heparin in the Dog (40826)

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Heparin has been used clinically as an inhibitor of blood coagulation for over 40 years. For the immediate treatment of thromboembolic disorders, it is usually administered by intermittent iv injections or by continuous infusion.

Numerous studies have been made on the distribution and excretion of heparin administered as a single injection (1–3). However, there is little information for heparin administered by continuous infusion.

Modern studies on heparin have been considerably aided by the introduction of a sulfur-35 label into the aminosulfate moiety of heparin. This paper considers the relationship between the ^{35}S radiolabel and anticoagulant activity in the blood of the dog, the fluid distribution kinetics of iv heparin variously administered, and the biodistribution patterns in dog tissues.

Materials and methods. Acute and chronic experiments were performed on 10 male mongrel dogs weighing 10–16 kg. The animals were anesthetized with 2.5% sodium pentothal during the acute phases. An iv drip of 5% dextrose and water was administered to maintain urine output. Porcine mucosa heparin, labeled with $^{35}SO_4$ by the method of Levy and Petraeck (4) was obtained from Amersham/Searle in batch lots. Anticoagulant activity varied from 100 to 120 IU/mg. Radioactivity varied from 37 to 80 μCi/mg. Less than 3% of the administered radioactivity was due to inorganic radiosulfate. The [^{35}S]heparin was administered by constant infusion pump through the venous catheter. Blood samples (9 vol) were drawn via arterial cannula into (1 vol) 7.6% sodium citrate. The bladder was catheterized for collecting urine samples.

The Lee–White whole blood clotting time (5), Quick one-stage prothrombin time (5), or the kaolin-activated partial thromboplastin (APTT) time was employed to test for hypocoagulation of the blood. Plasma, urine, and tissue samples were counted for ^{35}S activity in a Beckman LS-150 liquid scintillation counter. Liquid scintillation materials were obtained from Amersham/Searle, Arlington Heights, Illinois. Plasma was digested in NCS solubilizer, a quarternary ammonium base in toluene, for 30 min and a PPO–POPOP–toluene scintillation solution was then added. Tissues were digested in NCS at 56°C for 48 hr. Because of the extreme quenching, a portion of the digest was added to an NCS–PPO–POPOP mixture for counting. Urine was added directly to PCS, a xylene-surfactant-based scintillation solution. All counts per minute were converted to disintegrations per minute after correction for dilution, decay, and quenching.

Total ^{35}S activity in the plasma at each sampling period was calculated by multiplying the disintegrations per minute by plasma volume. The blood volume was assumed to be 9.4% body weight (6). It was assumed that fat muscle mass represented 40% of the body weight and lean muscle mass 30% of the body weight (7). Total skin mass was calculated using the formula CHW, where C = circumference of the thorax, H = crown to rump length and W = weight of 1 unit^2 of skin.

Initial experiments were designed to investigate the relationship between ^{35}S-activity and anticoagulant activity in the blood as well as the compartmental distribution pattern of [^{35}S]heparin. In these experiments, a single iv dose or one followed by continuous infusion of radiolabeled heparin was used. The continuous infusion was begun 1 hr after the initial heparinization. Four animals received an initial dose of 8–24 mg of [^{35}S]heparin and were infused over a 3- to 27-hr period (Table 1). One animal (Dog

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TABLE I. [^{35}S]HEPARIN DOSE AND INFUSION RATES—INITIAL SERIES

Dog No.	Weight (kg)	Initial heparin dose (mg)	Average infusion rates (mg/hr)	Average infusion rates (ml/hr)	Infusion period (hr)	Duration of study (hr)
101	8.6	8.0				3
102	8.6	16.0	3.98	3.06	3	48
103	14.5	23.7	7.01	0.70	27	96
104	14.1	23.7	17.23	1.46	7	63
105	11.4	23.7	20.30	1.72	25	91

101) received a single dose only. In a subsequent group of five experiments, the heparin infusion was started 8–17 min after initial heparinization. The animals of this group were sacrificed after 2–6 hr by exsanguination and tissue samples immediately collected. The intestines were opened, rinsed, and carefully freed of their contents. The amount of [^{35}S]heparin administered was increased in each succeeding experiment of the second group to see what effect, if any, this would have on the distribution data (Table II). The extravascular component was estimated by calculation (100% − amount in urine and plasma).

Results. Figure 1 shows representative results for the relationship between the radiolabel and anticoagulant activity in the blood of animals receiving a single iv dose of heparin. In general, for a single dose as well as for constant infusion, there was a direct relationship between ^{35}S disintegrations per minute and anticoagulant activity. This was true for all three clotting time assays employed.

In some dogs, after 1–2 hr anticoagulant activity was no longer present but radioactivity was noted. This suggested that some of the circulating material contained the label but was not exerting an anticoagulant effect.

Compartmental ^{35}S distribution. Compartmental distribution of the heparin radiolabel in the dogs' plasma and urine was determined from the ^{35}S-activity.

In Figs. 2 and 3 are shown representative results. Initial urine excretion of the radiolabel was rapid with 20–25% of the dose excreted in the first hour. The excretion rates approximated 1–2%/hr thereafter. Total excretion in the urine averaged 42% (range 23–65%). In animals which received increasing amounts of heparin, there appeared to be no correlation between urine excretion rate and the amount of the [^{35}S]heparin infused after the first hour (Table III).

After a single iv injection, plasma activity decreased in exponential fashion. As much as 66% was lost within the first 15 min after administration. Plasma activity fell to 4% of the total dose administered within the first 24 hr and persisted at about the 1% level thereafter.

When iv injection was followed by infusion, the infusion rates employed could not keep up with the rate of heparin loss from the plasma. The activity still decreased in

TABLE II. [^{35}S]HEPARIN DOSE AND INFUSION RATES—SECOND SERIES

Dog No.	Weight kg	Heparin dose (mg) Initial	Heparin dose (mg) Total	Average infusion rates (mg/hr)	Average infusion rates (ml/hr)	Infusion period (hr)	Duration of study (hr)
201	14.0	8.0	8.0				6
202	16.3	10.0	16.5	4.19	23.28	2	2
203	15.9	25.0	39.5	10.25	4.06	2	2
204	16.4	37.5	142.5	65.55	8.73	4	4
205	15.4	37.5	139.5	43.80	5.82	4	4

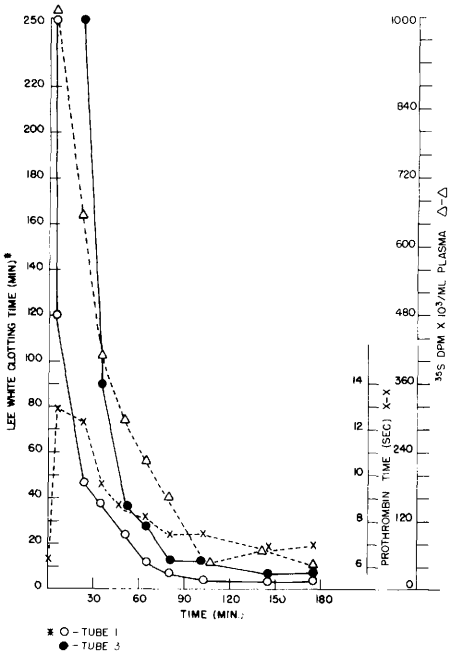

FIG. 1. Relationship Between ^{35}S activity And [^{35}S]heparin anticoagulant activity in blood.

an exponential fashion. At equilibrium, plasma activity was as high as 15% of the total dose administered.

There was significant extravascular distribution of ^{35}S activity for the first 2 days or so. Thereafter, this was apparently mobilized and eliminated. Extravascular

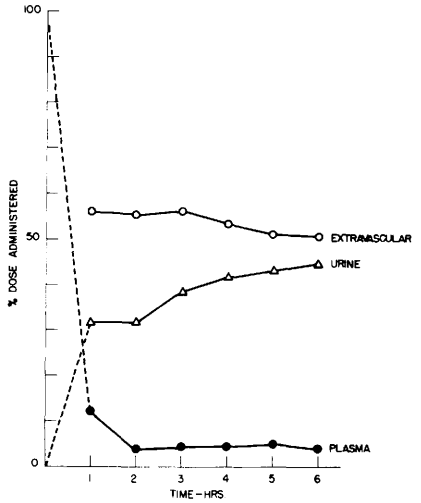

FIG. 2. Compartmental [^{35}S]heparin distribution following a single intravenous dose.

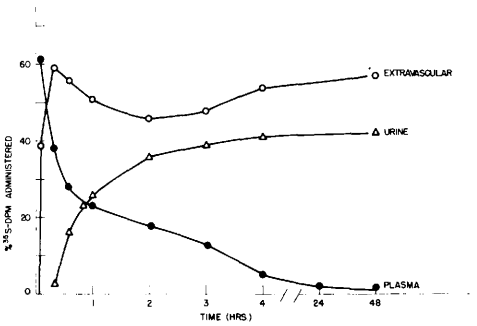

FIG. 3. Compartmental [^{35}S]heparin distribution following an initial dose and continuous infusion.

distribution at equilibrium was variable but substantial in all animals, ranging from 24 to 64% of the administered dose.

Tissue ^{35}S Distribution. In Table IV, the relative distribution of the radiolabel in various selected organs is shown. In Table V, the distribution of the radiolabel is detailed in terms of the percentage of dose administered per 100 g tissue. From 15 to 55% of the total dose administered was recovered in the tissues assayed. No relationship to the total dose administered was apparent. Liver, skin, and muscle consistently showed high concentrations of ^{35}S activity (Table IV). Considered on a gram basis, liver, kidney, and spleen, in that oder, showed the highest amounts of radioactivity (Table IV). This was true whether the radiolabeled heparin was given in a single dose or given in a single initial dose followed by constant infusion.

Relative concentrations of the retained radiolabel in the tissues and plasma of each animal were calculated. Fig. 4 shows the data for one of the animals which received some form of infusion after the initial ad-

TABLE III. URINE EXCRETION RATES[a]

Dog No.	Total heparin dose (mg)	Excretion rate (%/hr)
201	8.0	3.0
202	16.0	11.0
203	39.5	7.3
204	142.5	15.4
205	139.5	3.0

[a] Starting 1 hr. after the initial heparin dose was given.

TABLE IV. RELATIVE ^{35}S DISTRIBUTION IN NECROPSY TISSUES

	Percentage dose administered/organ				
	201[a]	202	203	204	205
Liver	8.07	35.88	4.78	14.66	18.75
Skin	11.68	3.78	3.30	13.17	5.66
Muscle	6.00	7.77	4.86	8.69	2.89
Kidney	0.38	1.85	1.01	2.87	1.38
Spleen	0.47	0.57	0.18	0.28	0.46
Lungs	0.46	0.81	0.49	0.89	0.45
Large intestine	0.02	1.90	0.66	0.31	1.62
Small intestine	0.09	0.01	0.19	3.62	2.07
Heart	0.19	0.36	0.23	0.51	0.36
Aorta	0.019	0.013	0.006	0.010	0.009
Vena cava	—	—	0.002	0.001	0.009
Brain	0.001	0.014	0.005	0.021	0.011

[a] Dog No.

ministration of heparin. The calculations were based upon the average retained dose per gram dog. The horizontal line at the 100% level represents the concentration of ^{35}S per gram dog if the radiolabel had been evenly distributed throughout the animal.

For the group as a whole, liver and kidney contained more than five times the average dose per gram dog (pgd). Spleen and plasma contained two to five times the average retained dose pgd. Tissues of medium concentration included the large intestine, small intestine, and lung and contained for the most part concentrations of one to two times the average dose pgd. Muscle, aorta, vena cava, heart, and skin were consistently low in activity and in general had less than the average retained dose pgd. The brain was consistently lowest in activity and almost invariably contained less than 5% of the average retained dose pgd.

Although the tissues and organs were carefully treated to exclude excess blood contamination, it was impossible to exclude all blood. To determine the degree of error in tissue radioactivity introduced by its blood content, the amount of blood contained in each organ was calculated where possible for two animals. The values of tissue blood content were those published by Altman and Dittmer (6). The relative tissue concentrations were reevaluated after the tissue ^{35}S disintegrations per minute was corrected for any contribution from the blood ^{35}S activity. There was no significant contribution from the blood remaining in

TABLE V. RELATIVE ^{35}S DISTRIBUTION IN NECROPSY TISSUES

	Percentage dose administered/100 g tissue				
	201[a]	202	203	204	205
Liver	2.67	9.27	1.09	2.72	5.21
Skin	0.73	0.38	0.17	0.36	0.23
Muscle	0.10	0.15	0.06	0.17	0.06
Kidney	0.57	2.78	1.32	3.28	2.15
Spleen	1.60	1.40	0.37	0.54	0.19
Lungs	0.53	0.50	0.37	0.52	0.31
Large intestine	0.05	0.80	0.86	0.36	0.58
Small intestine	0.02	0.50	0.40	0.63	0.61
Heart	0.19	0.29	0.15	0.41	0.32
Aorta	0.13	0.33	0.17	0.16	0.26
Vena cava	—	—	0.13	0.06	0.41
Brain	0.00	0.02	0.01	0.02	0.01

[a] Dog No.

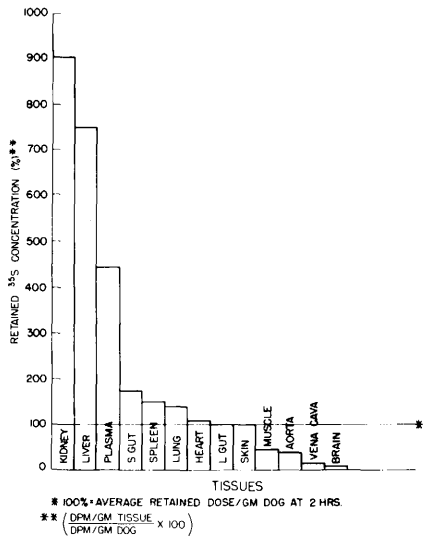

FIG. 4. Relative tissue concentration of retained radioactivity.

the tissues with the exception of the brain and the spleen. The values for spleen were reduced to a minor extent. It was found that all of the radiolabel of the brain could be accounted for by its blood content.

Discussion. Ten dogs were given radiolabeled heparin intravenously. The relationship between circulating ^{35}S activity and tests of coagulation was noted. The distribution and excretion of exogenous [^{35}S]heparin and/or its metabolic products was determined from the radioactive concentration of blood, urine, and tissue samples. A parallel relationship between the decreasing anticoagulant activity and the decreasing ^{35}S activity in the blood was observed for the first hour after a single injection of radiolabeled heparin. The relationship between the levels of radioactivity and hypocoagulability remained essentially constant with the infusion of [^{35}S]heparin. These close relationships suggested that the ^{35}S was a part of the intact heparin molecule or at least the part of the molecule responsible for the anticoagulant effect. The lack of correlation between ^{35}S activity and anticoagulant activity in the blood of a few animals several hours after a single dose was administered may be due to the presence of inorganic radiosulfate and highly degraded heparin molecules. While published data appear lacking for the dog, evidence for such metabolites in the blood of rats has been presented (8, 9).

The urine represented a variable but significant compartment of radiolabel distribution. Similar observations in animals given single injections of heparin have been reported by others (10, 11). Total urinary excretion of the radiolabel varied from an average value of 40% after a few hours up to a high value of 90% at 96 hr. Similar findings have been reported by others (1).

In general, extravascular retention of the radiolabeled material averaged around 60%. In some cases, it was as high as 90%. This degree of extravascular retention was more or less stable for the first 2–3 hr in most animals.

Plasma retention of the radiolabel decreased rapidly during the first hour. This, in part, was due to rapid excretion into the urine and in part to the rapid movement into the extravascular compartment.

Information on the biodistribution of the radiolabel was obtained on tissues taken at the end of the experiments. There was no remarkable difference between the biodistribution patterns of animals receiving a single dose and those receiving a single dose plus infusion. In animals receiving some type of constant or repeated infusion of [^{35}S]heparin, the expected increase in plasma and kidneys was found. Plasma represented the initial compartment of distribution and the kidneys the entrance to a second major compartment of distribution: urine.

An attempt was made to rationalize the mass of data for tissue distribution of the radiolabel by distinguishing five arbitrary categories of concentration: maximum, high, average, low, minimum. The classification was not perfect, but the generalizations held for over 90% of the cases. The tissue group of maximum concentration included the liver and kidney. To find the liver in this category was not surprising and is consistent with reports in the literature (8, 9). The high concentrations in the kidney could be due to the storage of heparin for subsequent excretion or degradation.

The storage of metachromatic heparin granules in the cells of the proximal convoluted tubules has been reported after re-

peated injections of heparin in rabbits (12). However, Day *et al.* (8) from studies on rats, have reported that the kidney appears to rapidly degrade exogenous [^{35}S]heparin. This was based on changes in the recovery of the label associated with sulfomucopolysaccharide and nonsulfomucopolysaccharide fractions from the kidney.

The second category of tissue (high concentration) included spleen and plasma. The fact that spleen is in this high category further supports the concept that the reticuloendothelial system plays a significant role in retention and possible metabolism of heparin (3, 9). In fact, this may be the major or even possibly the sole reason for its presence in the liver, since the liver contains the prime tissue mass of the reticuloendothelial system. Plasma, the other representative of the second category, was the first compartment which the radioheparin encountered. Plasma probably represents a transit medium between various compartments of distribution.

The third category (medium concentration) appeared to include the intestines and lungs. These may function as temporary storage sites (3, 13).

The fourth category (low concentration) includes skin, aorta and vena cava—tissues which contain large amounts of connective tissue. Heart and striated muscle are also in this category. Heart muscle and skeletal muscle are highly cellular tissues compared to the other three. This suggests that heparin and/or its metabolites do not penetrate the intracellular fluid compartment in these tissues and do not have a high affinity for collagen-type tissues such as the great vessels and skin in the dog.

The fifth category—minimal concentration—is represented by one organ only: the brain. The low concentrations consistently found suggest selective exclusion from this tissue and probably reflects the functioning of an intact blood-brain barrier.

Summary. Following the intravenous administration of [^{35}S]heparin in the dog, a direct relationship between plasma ^{35}S activity and anticoagulant activity was observed suggesting that the radiolabel remained part of the intact heparin molecule.

An initial rapid loss of [^{35}S]heparin from the intravascular compartment was observed. This was due to a rapid extravascular distribution and renal excretion.

The quantity of ^{35}S radiolabel excreted into the urine during the first hour accounted for as much as 25% of the dose administered. There appeared to be no correlation between the amount of [^{35}S]heparin infused and urine excretion rate.

Extravascular retention of the radiolabel was variable but substantial in all animals, averaging 60% of the dose.

The retained radiolabel in necropsy tissues showed widely ranging concentrations. The highest amounts were consistently found in the liver. The lowest amounts were consistently found in the brain. No remarkable differences were found between animals receiving a single dose of heparin or additional heparin by infusion. Five arbitrary categories of concentration were distinguished and the tissues were classified accordingly.

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Received October 9, 1979. P.S.E.B.M. 1980, Vol. 1980.