Heparin in Blood.* (23093)

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The problem as to whether heparin is present in blood as a normal constituent has been investigated by a number of workers(1-9). The results of these studies are conflicting because even if heparin is present in blood, its concentration is very small. Isolation of heparin from normal blood and unequivocal identification could consequently not be accomplished by ordinary means. In a recent report(10), we have shown that exogenous inorganic sulfate can act as a precursor for the sulfate ester groups of heparin. Thus, when sodium sulfate containing S-35 is injected into dogs, the heparin which is subsequently isolated from the liver is radioactive. On the basis of this finding, it was expected that if heparin is a normal constituent of blood it should be susceptible to identification on the basis of its radioactivity in animals which had previously been treated with radioactive sulfate. The present report describes the procedures and the results obtained.

Methods. Dogs weighing 16 to 19 kg were injected intraperitoneally with 12 ml of an aqueous solution of $Na_2S^{35}O_4$ (10 mg) containing 1.3 x 10^9 c.p.m. and blood was drawn from different animals after 26 and 48 hours. Subsequent to addition of sodium heparin (Lederle) as a carrier, 100 ml of blood were extracted with 100 ml 0.5 N NaOH and 14 ml saturated ammonium sulfate at 50° for 1.5 hours. The solution was then heated to 75°, centrifuged and washed twice with 50 ml 0.06 N NaOH and 5 ml saturated ammonium sulfate. The supernatant and washings were combined and concentrated at room temperature to 50 cc. Acidification with H_2SO_4 to pH 2 yielded a precipitate which was collected, washed with dilute H_2SO_4, extracted with ethanol and dried. The subsequent steps involving digestion with trypsin and reprecipitation from different solvents were similar to those described for isolation of labeled heparin from liver(10). The barium acid salt which was finally obtained was recrystallized 3 times and similar values for radioactivity were obtained after each recrystallization. Eighteen ml blood samples were also extracted by the procedure of Monkhouse and Jacques(8) subsequent to addition of 100 mg of carrier heparin. The aqueous solution, after extraction with phenol, was dialyzed, passed through Dowex 2 anion exchange resin, purified and recrystallized in a manner similar to the first procedure. Radioactivity measurements were made on 1.2 to 1.6 mg aliquots with a thin window gas flow counter on plates having an area of 8 sq. cm. It was found that these conditions gave infinitely thin samples so that no absorption corrections were necessary.

TABLE I. Radioactivity of Heparin Isolated after Addition of Carrier.

Time (hr)	Recrystallization	Total count	mg recovered	Cpm/100 mg
26	1	2475	45.9	5392
	2	2068	38.8	5381
	3	2020	37.6	5372
48	1	2205	48.6	4537
	2	1903	42.0	4531
	3	1359	30.0	4530

Results. Table I shows the results obtained by the first method. It can be seen that a significant amount of radioactivity can be detected in the heparin which is isolated from blood. If labeled inorganic sulfate had been present in the original blood, it was completely eliminated in the process of isolation and purification. That this is so has been shown in studies with liver(10) and in control experiments with blood to which $Na_2S^{35}O_4$ and heparin were added.

Recrystallization of the barium acid salt did not reduce specific activity appreciably (Table I), further indicating the radioactive purity of the heparin. The material had an anticoagulant activity of over 100 U.S.P. units per milligram and was inhibited by pro-

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tamine as would be expected for heparin. Analysis showed it to contain 23.52% barium and 19.2% glucosamine. Radioactivity of the blood heparin 48 hrs. after administration of the radioactive sulfate is less than 24 hours. This has also been observed when heparin was isolated from liver. The reason for this is that the time of maximum incorporation of the label is about 30 hours after sulfate injection.

Extraction of blood heparin by the second procedure gave material of much lower specific activity. Furthermore, the amount of radioactivity obtained could not be duplicated from one experiment to the other. Data obtained by this method were, therefore, not considered sufficiently reliable for tabulation.

Discussion. The activity due to radioactive heparin in 100 ml blood would be in the order of 5300 c.p.m., 26 hours subsequent to administration of the radioactive heparin. The liver heparin isolated at this time has a specific activity of 10^4 c.p.m./mg.

Since maximum incorporation into liver heparin is not reached until after 26 hours, it would be expected that its activity in the blood would be lower at this time, assuming that heparin is formed in the liver. The conclusion might consequently be made that normal blood contains at least 0.5 mg of heparin/100 ml.

The second extraction procedure (*i.e.* Monkhouse & Jacques(8)) yielded heparin with less than half the specific activity obtained by the first method. It would, consequently, appear that the phenol procedure does not completely extract heparin from the complex in which it exists in the blood. To release heparin from this complex, digestion with proteolytic enzymes seems to be essential. This may also be the reason why Charles and Scott(3) and the others who have used their method found heparin in blood, while Monkhouse and Jacques(8) did not. Further studies to verify this conclusion are being conducted.

Summary. The presence of heparin in normal blood is demonstrated by the "carrier" technic, using radioactive sulfate as precursor. It appears to be found in blood in a combined form so that preliminary decomposition of the complex is required before it can be isolated.

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