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Metabolism of Tritium-S³⁵-Labeled Heparin

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Potassium heparinate (C 21.5; N 1.7; S 9.84; ash 46.5%; average Mol. Weight 11,000) was labeled with tritium (T) by: 1) exposure to 5 c of T gas, according to Wilzbach; 2) exposure to 1 or 2 c of T gas under electric discharge (800-1000 volts); 3) catalytic exchange in presence of T₂O (1/2 c) and palladium black.

The labeled preparations were purified by repeated precipitations with 95% ethanol containing 10% K acetate, followed by precipitations with cetylpyridinium chloride; by dialysis of the CPC-heparin complex against distilled water for 6 days and by repeated gel filtration. Product 1) had a MW of 7,500, no anticoagulant activity and respectively 11,500 and 314 dpm/mg. (fig. 1). Infrared analysis of the original preparation and of the three products did not show any substantial difference.

Products 2) and 3) were partially desulfamated (0.04 N HCl at 56° C for 4 hours), then resulfamated with pyridine-S³⁵O₃ reagent. These doubly-labeled preparations were purified by passage through anion and cation exchange resins to achieve a constant specific activity for each isotope. Their biological activity being equal or better than that of the original preparation, they were used for metabolic studies.

EXPERIMENTS WITH INTACT ANIMALS. 5-10 mg of doubly-labeled heparin (T cpm/S³⁵ cpm = 1) were injected intravenously into 4-5 kg anesthetized rabbits. Heparin and total acid mucopolysaccharides were isolated respectively from plasma and urine obtained at different intervals of time; their T/S ratio was measured and compared to that of the plasma or urine from which they had been extracted. Circulating radioactivity and anticoagulant activity disappeared at the same rate, returning to original levels within 2-3 hours. The T/S ratio of the plasma samples did not change from the value found for the injection preparation; the T/S ratio of plasma heparin remained unchanged for 2 hours and then increased.

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Measurement of urinary radioactivity indicated that 15% of the dose injected was excreted during the first hour and an additional 5% during the following 24 hours. The T/S ratio of the first urine sample was similar to that of the injected preparation. However, during the following three hours at first more and then less T-labeled material was excreted than S^{35} -labeled material. The T/S ratio of urinary acid mucopolysaccharides, unchanged for the first hour, increased four-five fold within the following 5 hours (fig. 2).

These data indicated that a) there is a considerable retention of heparin in the organism; b) two hours after injection the two isotopes are no longer entirely on the same molecule. The possibility that the kidney might be responsible for the removal of the S^{35} -sulfamic groups was suggested because two hours after injection, the T/S value of plasma heparin was unchanged while the T/S value of urinary mucopolysaccharides was twice higher. In order to explore this possibility, the same experiment was performed in a rabbit with excluded kidneys. The disappearance of circulating radioactivity and anticoagulant activity was not as rapid as that seen in a normal rabbit. The value of the T/S ratio for heparin isolated from plasma at different time intervals did not change from that of the injected heparin. However, the appearance in the blood at the second and third hour after injection of an excess of S^{35} -labeled material indicated that some cleavage of sulfamic acid groups had occurred, even in the absence of the kidneys. (fig. 3)

PERFUSION OF ISOLATED, SURVIVING RAT LIVER. Several liver perfusions were performed using doubly-labeled heparin. Samples of perfusing fluid were taken at different intervals; the circulating heparin was isolated and its biological activity and T/S ratio were measured. Some of the perfused livers were obtained from rats treated with large doses of Vit. A; others were obtained from untreated rats. In either case, the biological activity and the T/S ratio of the heparin isolated did not change from the values obtained for the original preparation. Analyses of

the bile collected during liver perfusion revealed a negligible amount of tritium-labeled material (0.015% dose injected), thus excluding the postulated possibility that the bile constitutes a route of elimination of heparin.

PERFUSION OF ISOLATED, SURVIVING RABBIT KIDNEY. These experiments were performed in order to study to a further extent the role of the kidney in the metabolism of doubly-labeled heparin. Samples of the perfusing fluid and of urine were collected at hourly intervals. The anticoagulant activity of the perfusing fluid did not change over a period of 3 hours. After addition of carrier non-radioactive heparin to each sample of perfusing fluid and urine, the doubly-labeled heparin was isolated and the T/S ratio determined. It was found that each sample of urinary heparin had a T/S ratio higher than that of the corresponding sample of circulating heparin. These data seem to indicate that the kidney may be one of the sites capable of removing the sulfamic acid groups of heparin. --- This research was supported by the Atomic Energy Commission, by grant AM 09397-01 of USPHS and by an Established Investigatorship of the American Heart Association.

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FIGURE I

Preparation	Molecular weight (sedim. equil.)	C	N	S	Ash	Biological activity *	Tritium dpm/ μ g
K heparinate	11,000 $\pm$ 1,000	21.5	1.7	9.84	46.5	100	...
Exposed	7,500 $\pm$ 600	18.9	1.7	10.64	45.8	0	28,600
Discharge	10,500 $\pm$ 700	-	-	-	-	100	11,500
Catalytic	10,500 $\pm$ 500	21.1	1.6	9.86	48.38	100	314

* The biological activity of the original preparation has been arbitrarily made equal to 100.

FIGURE II

FIGURE III