Preparation and Characterization of Tritiated Heparin. (31616)

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The properties of heparin as an inhibitor of blood coagulation have been known for several years(1). However, the physiology and metabolism of heparin has been only slightly clarified. With the preparation of radioactive heparin the possibility for quantitative metabolic studies of this substance can be undertaken. Eiber and Danishefsky(2) reported on the preparation of heparin labeled with S^{35} by biosynthesis in the dog. With this material they have studied the disposition of heparin after injection by following its disappearance from the circulation(3) and its deposition in various organs(4). More recently Levy and Petracek(5) have prepared heparin-S^{35} by the chemical reaction of pyridine $S^{35}O_3$ or trimethylamine-$S^{35}O_3$ with partially desulfated heparin. Both preparative methods led to biologically active preparations with radioactivity counts of the order of 10^5 dpm/mg. There is still a need for a radioactive heparin labeled with another isotope, *e.g.*, tritium, in positions other than the sulfate moiety which is the most labile bond in the molecule. Recent interest involves the use of graphite-benzalkonium-heparin (GBH) surfaces in artificial organs especially the artificial heart valves(6); an understanding of the mechanism of action of this surface might be facilitated by a stable isotopically labeled heparin molecule.

Tritiation. We used a modification of the Hempel Toepler pump set-up for catalytic tritiation. The apparatus will be described later. Three hundred mg of sodium heparin (157 μ/mg) was suspended in 3 ml of water and 2 ml of diethyleneglycol monoethyl ether. One hundred milligrams of 30% palladium on carbon were then added. The resultant suspension was frozen in a liquid nitrogen bath, evacuated, and the vessel filled with hydrogen. The catalyst was saturated with hydrogen by mixing with a magnetic stirrer for one hour at atmospheric pressure and temperature. Five curies of tritium gas were pumped into the 10 ml reaction vessel after freezing and evacuation. The reaction mixture was then adjusted to room temperature and atmospheric pressure with hydrogen. The reaction for the catalytic exchange tritiation was 48 hours with constant mixing. After 48 hours the catalyst was separated from the reaction mixture by filtration. Labile tritium was removed by distilling five 250 ml portions of water from the product. The crude product weighed 275 mg and assayed 137 μC/mg by scintillation counting.

Purification and characterization. The sample was purified by dissolving it in isotonic sodium chloride and precipitating with cetylpyridinium chloride (CPC), 3 mg per mg of heparin; the resultant complex was redissolved in 2 M sodium chloride and the heparin precipitated with absolute alcohol, 2 volumes. The heparin was redissolved in 0.5 M sodium chloride and the entire purification procedure was repeated. The slightly colored product weighed 150 mg, had a specific radioactivity of 8.2 μC/mg and a biological potency in the USP assay of 124 μ/mg.

The radioactive powder diluted with cold starting material was subjected to paper electrophoresis under conditions previously described(7). The paper strip was scanned for radioactivity and stained with toluidine blue for location of metachomasia. As shown in Fig. 1, the metachromasia and the radioactivity coincide in a single peak, indicating that the two properties reside in the same electrophoretic species. The mobility of this area is in agreement with the value for heparin.

Another sample was analyzed in the Spinco ultracentrifuge and a single peak of $S_{20,w}$ of 2.3S was observed. The good agreement between the tritiated product and the starting material indicates that no depolymerization has occurred.

Discussion. The use of catalyzed tritium exchange reactions has been reported by Yavorsky and Gorini(8) who used borontrifluorophosphoric acid as the catalyst; Garnett

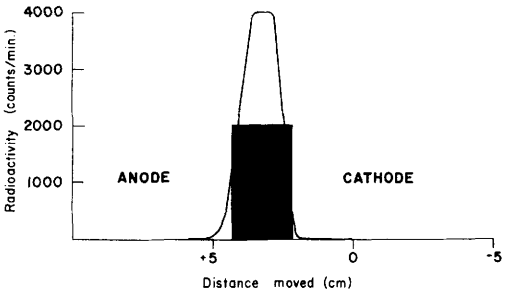

FIG. 1. Typical radiochromatogram scan of H^3 heparin electrophoretic pattern. Location of metachromasia peak obtained with toluidine blue shown as darkened bar.

(9) reported on a catalytic exchange method using tritium oxide and transition metals as catalysts. We have found that palladium on carbon catalyzes the tritium exchange reaction to the extent that specific activities of some compounds are high enough to permit their use as tracers in biological experiments. Tritiated heparin is an excellent example of this type of reaction, the final product having 18.2×10^6 dpm/mg, which allows for accurate measurement of submicrogram quanities.

Radioactive heparin prepared by this method has been used by Kramer(10) in studies on the stability of the GBH surfaces. The remarkable stability of this compound was shown by dissolution in saline at 24°C, in a tightly sealed container. *In vacuo* distillation of the solution 2-1/2 months thereafter demonstrated that only 5.2% of the contained activity was present as ^3H_2O; the remaining material was still precipitable with protamine sulfate, indication that 90% of the tritium was still organically bound.

The 50% yield of tritiated heparin indicates that the tritiation process brings about considerable degradation. The elimination of these breakdown products is apparent in the first precipitation with CPC, where the bulk of the radioactivity remains in the supernatant. It is possible that a single purification would suffice since the radioactivity in the supernatant following the second precipitation was minimal.

The availability of tritiated heparin should lead to further investigations on the metabolic fate of this interesting biopolymer. It is expected, but has not been demonstrated, that the tritium label is more stable than the S^{35} label on heparin.

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Production of Rubella Complement Fixing Antigen in BHK-21 Cells.* (31617)

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Serological diagnosis of rubella infection has been complicated in the past by the lack of a simple, quick, and accurate method of measuring the development of rubella antibodies. The generally applicable technique of fresh cell sedimentation (cell packing) for concentrating viral complement fixing (CF) antigens demonstrated recently was used for the concentration of rubella CF antigen (1,2).

Sever *et al*(2) have shown the usefulness of the complement fixation test for the demonstration of rubella antibody. In this publi-

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