Distribution and Excretion of Heparin. (26379)

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The mechanisms responsible for the rapid termination of anticoagulant action of intravenously administered heparin have not been well established. A single average dose of 0.5 to 1.0 mg/kg given to mammals produces almost immediate anticoagulant effect of 2 to 4 hour duration. Increasing the dose increases the intensity of the effect but does not proportionately increase its duration. Excretion of heparin in the urine during the period of systemic anticoagulant effect does not appear to account for termination of effect of the drug. A heparin inactivating enzyme "heparinase" has been described in liver extracts but recent studies indicate that this may not be a true enzyme. Rather, Loomis(1) has obtained *in vivo* evidence which indicates that liver is not selectively involved in terminating the action of heparin. Therefore, although a "normal" blood concentration of endogenous heparin has been described(2,3) it appears that exogenous heparin is either degraded or stored in inactive forms and is subsequently slowly excreted from the body. This study is concerned with some possible mechanisms responsible for terminating the action of administered heparin.

Methods. Sodium heparin S^{35} was biologically synthesized. This consisted of administering $Na_2S^{35}O_4$ (0.5 millicurie, intravenous) once daily on 2 successive days to each of 2 large dogs. Forty-eight hours after the initial dose, the animals were sacrificed, the livers were removed, combined, homogenized with water and extracted exactly according to the method of Eiber and Danishefsky(4). No carrier heparin was added during the extraction procedure. The final product obtained from 1250 g of liver consisted of 132 mg of a light brown powder having 6300 cpm/mg. The product was free of inorganic sulfur and was electrophoretically homogenous. *In vitro* assay showed 72 U.S.P. anticoagulant units per mg and metachromatic assay (with toluidine blue) showed an activity of 85 units per mg. This product hereafter is referred to as heparin S^{35}.

Three small (5 to 6 kg) dogs were anesthetized with sodium pentobarbital (30 mg/kg, intravenous). A section of a femoral vein was exposed and the urinary bladder was cannulated. Each dog was given heparin S^{35} (3 mg/kg, intravenous) and intensity and duration of anticoagulant effect was determined according to a modified Lee-White 3-tube clotting time method(5). Plasma and urine isotope activity was determined on samples obtained at 15 minute intervals for 2 hours by the method described by Walkenstein(6).

Twenty rats were injected with heparin S^{35} (20 mg/kg, intravenous). Groups of 4 of these animals were sacrificed at 2, 6, 12, 18 and 24 hours post injection. Three animals in each group were sacrificed by cervical vertebra crush, and the fourth animal was etherized and prepared for obtaining peritoneal mast cell suspensions.

Samples of plasma, blood cells, kidney, liver, spleen, heart, lungs, small intestine, skin and skeletal muscle were digested in concentrated nitric acid and isotope activity of the product of digestion was measured. The counts were corrected for background activity and converted to mg of heparin S^{35}.

The mast cells in the peritoneal washings were separated by the sucrose differential specific gravity method of Glick *et al.*(7). A suspension containing nearly 100% mast cells was thereby obtained. Radioautographs were made from smears of the cells and identification of heparin S^{35} in the mast cells was accomplished as follows: The sucrose-mast cell suspension from each rat was treated by dilution with 1 ml of a freshly prepared mixture of sodium chloride (.85%) and gelatine (0.01%) in versene phosphate buffer pH 7.4(7). The mixture was centri-

fuged at 1200 rpm for 10 minutes, the supernatant was decanted and the "button" of concentrated mast cells was spotted on one end of a filter paper strip (Whatman No. 1 — ¾ × 7 inches). The strips were arranged for ascending chromatography using 4 parts 25% ethyl alcohol with 1 part 0.1 N NaOH as the mobile phase. The chromatograms were either sprayed with 0.2% toluidine blue for evidence of metachromatic activity or the section of paper at the level of the heparin band was eluted with physiological saline and tested for anticoagulant activity and isotope activity.

Results. Distribution of heparin S^{35} in rat tissues at 2, 6, 12, 18 and 24 hours post injection is shown in Fig. 1. Initially the plasma contained approximately 20 times the radioactivity of the cells and plasma isotope activity decreased to nearly zero in the first 12 hours. Activity in the liver and spleen decreased more slowly so that minimal radioactivity continued to be present for 18 to 24 hours.

Metachromatic material having anticoagulant action *in vitro* which was abolished by protamine was obtained by paper chromatography from the peritoneal mast cells of a single rat. The eluate from the paper showed 25 to 60 cpm. Radioautographs of smears of these cells showed isotope activity in the granular material.

Plasma S^{35} anticoagulant activity and urinary excretion of S^{35} following injected heparin S^{35} (3.0 mg/kg) was determined in 3 dogs. Similar results were obtained in each animal and Fig. 2 shows results obtained in 1 experiment. The figure shows that anticoagulant effect parallels the radioactivity of the sample. Excretion of the isotope in the urine is synchronous with the decrease in blood concentration and anticoagulant activity. This indicates that the short duration of systemic anticoagulant effect is due in part to elimination of the drug from the circulating blood by the kidney. However, only 15 to 20% of injected S^{35} was found in the pooled urine at the time when coagulation time of the blood had returned to normal.

Discussion. Eiber and Danishefsky(4) es-

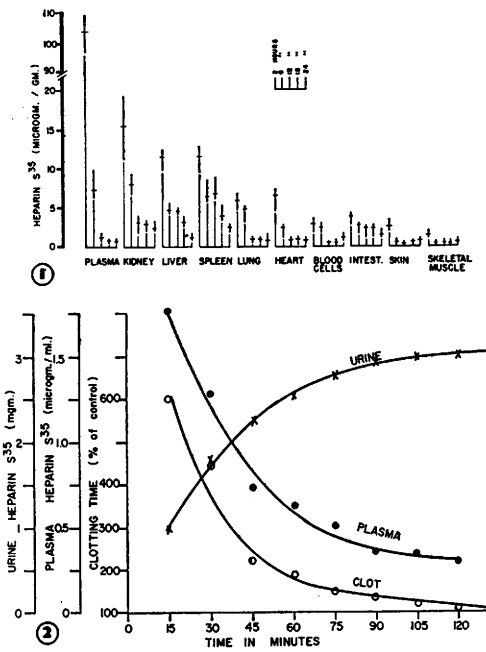

FIG. 1. Distribution of intrav. administered heparin-S^{35} (20 mg/kg) in rats at 2, 6, 12, 18 and 24 hr post-inj. Each column indicates avg and range of results obtained in 4 rats. Weight of heparin-S^{35} indicated on ordinate calculated on basis of 6,300 cpm/mg.

FIG. 2. Whole blood clotting time, plasma isotope concentration and excretion of isotope in urine at 15 min. intervals following intrav. inj. of heparin-S^{35} (3 mg/kg) in a 6 kg dog. Weights of heparin-S^{35} indicated on ordinates calculated on basis of 6,300 cpm/mg. Excretion of isotope in urine is represented on a cumulative basis.

tablished that when labeled inorganic sulfate is injected into dogs, it is incorporated into liver heparin. They concluded that heparin is synthesized in the intact animal from glucose and inorganic sulfate and showed the product to have a half life of about 3½ days. These workers have also indicated that circulating heparin may exist in part as a complex and that total anticoagulant activity can be determined only after proteolytic digestion(3).

The current study indicates that heparin S^{35} does not appear in significant amounts in the blood cells and that moderate doses of heparin are rapidly cleared from the plasma. Since intensity and duration of anticoagulant effect parallel isotope concentration in plasma, administered heparin appears not to be bound or complexed in inactive form in the

circulating blood. Urinary excretion accounts for only approximately 20% of injected heparin and therefore does not account for the rapid elimination of anticoagulant from the blood. Liver and spleen appear to retain isotope activity after anticoagulant and isotope activity have been eliminated from the plasma.

Mast cells are believed to be a site of synthesis or storage of heparin in the intact animal. Spolter and Marx(8) have shown that isolated mouse mastocytoma is capable of synthesizing heparin from inorganic sulfate. These workers have also shown that phosphoadenosine-phosphosulfate serves as the sulfate donor for synthesis of heparin by the mouse mastocytoma. The intense metachromatic activity of mast cell granules is believed to be due to presence of mucopolysaccharides having heparin-like activity. The current experiments indicating that injected heparin S^{35} appears in the granules of peritoneal mast cells of the rat suggest that the mast cells may function as a storage depot for administered heparin.

Summary. Heparin S^{35} was extracted from the liver of dogs administered inorganic $Na_2S^{35}O_4$. Distribution of this heparin was determined in rats. The mast cells were found to take up injected heparin S^{35}, and radioactivity persisted in liver and spleen after virtual disappearance from plasma. Anticoagulant activity was found to parallel isotope activity of the plasma of dogs injected with heparin S^{35}. Urinary excretion in dogs accounted for only about 20% of the injected isotope at a time when plasma anticoagulant activity had returned to normal.

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Effect of Thyroxine on Enzymes of the Urea Cycle in Regenerating Rat Liver.* (26380)

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Studies in this laboratory have shown(1,2) that treatment of *R. catesbeiana* tadpoles with low concentrations of L-thyroxine (2.6×10^{-8}M) resulted in a marked increase in the specific activity of liver carbamyl phosphate synthetase. This increase has been shown to be due to *de novo* synthesis of the enzyme(3). An increase in specific activity has also been observed for 2 other urea cycle enzymes, namely ornithine transcarbamylase and arginase, during thyroxine-induced metamorphosis (unpublished studies).

In view of these findings, it seemed desirable to determine whether the thyroid hormone played a similar role in mammalian tissues. For this purpose, the effect of thyroidectomy on the activity of carbamyl phosphate synthetase, ornithine transcarbamylase and arginase in regenerating rat liver was investigated.

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