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Heparin Is Not Metabolized by the Perfused Rat Liver (42428)

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Abstract. The role of the liver in metabolism of heparin was studied using the isolated rat liver perfused *in vitro* for 10 hr. Porcine intestinal heparin (1000 u) was added to the recirculating liver perfusate, and serial heparin measurements were performed on the liver perfusate every 2 hr, as well as on bile samples secreted by the perfused liver. Heparin concentration remained at a constant level throughout the 10 hr of perfusion, and there was no detectable heparin secreted into bile samples. The findings suggest that hepatic metabolism/clearance plays a minimal role in heparin kinetics in plasma. © 1986 Society for Experimental Biology and Medicine.

The pharmacokinetic properties of heparin in human subjects and in various animal models have been extensively described (1–4). The anticoagulant effect is known to have a brief plasma half-life ranging from 0.5 to 1.5 hr (2, 4). The half-life does increase slightly with administration of increasing doses of the drug (3), but the metabolic pathways that contribute to the short-lived plasma effects of heparin are largely unknown. Heparin clearance from plasma is somewhat slower in patients with renal dysfunction, but renal excretion has not been shown to be the primary mechanism by which the drug is cleared from plasma (5). There is prolongation of the half-life of heparin in patients with hepatic cirrhosis, suggesting that the liver plays a significant role in metabolism of heparin (6). Heparin is also bound by various plasma proteins, although the exact role of these heparin–protein complexes on metabolism of heparin is not clear (7, 8).

The isolated rat liver perfused *in vitro* provides a single-compartment model in which heparin in an artificial "plasma" perfusate is influenced primarily by hepatic metabolism and/or clearance by biliary secretion. In addition, the perfused rat liver synthesizes all rat plasma proteins except the gamma globulins (9, 10). The progressive accumulation of hepatic secretory proteins in the liver perfusate affords ample opportunity for observation of the effects of heparin–protein complexes if such complexes contribute significantly to the brief half-life of the drug. In the study described here, the isolated rat liver perfused *in vitro* for 10 hr with a perfusate containing heparin was utilized to investigate the contributions of hepatic metabolism and biliary secretion to the kinetics of heparin.

Methods. All rat liver donors utilized in this study were male Sprague–Dawley rats (Holtzman Co., Madison, Wisc.) weighing 300–350 g. Rats were maintained on standard lab chow (Teklad Diets, Madison, Wisc.) with addition of supplemental vitamin K to drinking water as previously described (11).

Liver perfusion techniques. Perfusion of the isolated rat liver was carried out as previously described (12, 13). Hepatectomy was performed while the animal was anesthetized with diethyl ether administered by a nose cone. At the time of hepatectomy, a polyethylene cannula was inserted into the common bile duct and secreted bile was collected throughout the 10-hr perfusion period.

The rat liver perfusate was composed of 38 ml washed bovine red cells (GIBCO, Grand Island, N.Y.) in 50 ml Krebs–Ringer bicarbonate buffer containing 3 g/dl bovine serum albumin, intestinal heparin (Eikins–Sinn, Inc., Cherry Hill, N.J.) 100 mg glucose, 3000 u penicillin, and 3 mg streptomycin–HCl. In selected perfusions, 1000 u porcine intestinal heparin (Elkins–Sinn, Inc.) was added to the perfusate at the outset. In perfusion studies where no heparin was added, 7 ml of sodium citrate (3.8% solution) was added to the perfusate to prevent formation of thrombi. Sufficient Ringer's solution was added to bring the perfusate volume to 100 ml. The liver perfusate was circulated by a Harvard roller pump (Harvard Apparatus Co., Inc., Millis, Mass.) at a flow rate of 1 ml/g liver tissue/min. In all

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experiments a constant infusion of 18 ml of Ringer's solution containing 500 mg glucose, 320 mg essential amino acids, 5 mg cortisol, 8.8 u insulin, 3000 u penicillin, and 1 mg streptomycin–HCl was added to the perfusate at a rate of 1.5 ml/hr. The pH of the perfusate was maintained constant at 7.40 by an infusion of 1 *M* NaHCO from a Radiometer Autoburette (ABU-12, MKS Co., Cheektowaga, N.Y.) and Titrator (TTT-60) equipped with a combined glass calomel electrode (GK3201-C).

Assays. Samples of 3 ml were withdrawn from the circulating liver perfusate every 2 hr for measurement of heparin concentration as well as protein measurements of antithrombin III, albumin, and fibrinogen.

Heparin was assayed in perfusate plasma samples by the activated partial thromboplastin time (APTT) method (14) and also with an amidolytic assay. Heparin assays on bile samples were only performed by the APTT method. The effects of heparin mixed with bile as the APTT of pooled normal rat plasma was measured are as follows: bile secretions were collected from liver perfusion studies in which citrate, not heparin, was used as anticoagulant. Sufficient heparin was added to each of these bile samples so as to give final concentration of 0, 0.1, 1.0, and 10 u/ml. An aliquot (0.1 ml) of each heparin bile mixture was incubated with 0.4 ml pooled normal rat plasma at 37°C for 60 sec. Samples of 0.2 ml were withdrawn from this mixture and tested by APTT assay, each sample being tested in duplicate. The APTT measurements were then used to construct a heparin "activity curve" for the measurement of heparin in bile samples. Assays for heparin activity in bile samples from "heparinized" liver perfusions were performed by this same APTT method.

Heparin activity was also measured in "plasma samples" of the liver perfusate by two methods. In the first method perfusate samples were adjusted so as to contain 0, 0.1, 1.0, or 10 u/ml heparin. An aliquot (0.1 ml) of this heparinized perfusate sample was then added to 0.4 ml of pooled normal rat plasma and duplicate APTT measurements were made on the mixture. These measurements yielded a "control curve" of heparin activity from which estimates of heparin concentration in liver perfusate samples could be made.

Measurements of heparin concentrations in liver perfusate samples were also made using an amidolytic substrate, S-2222 (Kabivitrum AB, Stockholm, Sweden) by the method of Teien *et al.* (15). In this assay varying concentrations of heparin, 0.1–0.74 u/ml, were incubated with 1 u/ml human AT III, and pooled normal rat plasma for 3 min at 37°C. To this mixture, Factor Xa was added, followed in 30 sec by 200 μl S-2222. The activity of Factor Xa on the chromogen, S-2222, was stopped after 180 sec by addition of glacial acetic acid and absorbance was read at 405 nm. The control curve established in this fashion permitted estimates of heparin concentration in liver perfusate samples when perfusate sample was substituted for pooled normal rat plasma in the assay.

Protein measurements in liver perfusion samples were performed as follows:

Antithrombin III was measured immunologically.

AT III was purified by affinity chromatography of pooled rat plasma on heparin–sepharose (12). An antiserum to purified AT III was raised in rabbits and used in a quantitative immunoelectrophoresis assay by the method of Laurell (16).

Fibrinogen was measured using a monospecific antiserum by the single radial immunodiffusion technique of Mancini *et al.* (17) as modified by Fahey and McKelvey (18).

Albumin was also measured by single radial immunodiffusion using a monospecific antiserum raised in our laboratory (12).

Results. *Heparin measurements.* The effects of increasing concentrations of heparin mixed with bile on APTT measurements of pooled normal rat plasma are shown in Table I. The assay was significantly prolonged by as little as 0.1 u/ml heparin in bile samples, and concentrations as high as 10 u/ml gave greated prolonged cutting times (>180 sec). As can be seen in the table, bile samples from perfusions in which heparin was used as anticoagulant (perfusions A and B) produced no different effects from those perfusions containing citrate instead of heparin (perfusions C and D), indicating that heparin was not cleared from the perfusate into the biliary tract.

Serial samples of liver perfusate "plasma" were also assayed for heparin activity (Table II). From three separate liver perfusions, each

TABLE I

	APTT
NRP + SBB	18.6/18.9
NRP + SBB/bile	19.7/20.0
NRP + SBB/bile heparin 0.1u	38.4/39.1
NRP + SBB/bile/heparin 1.0u	52.0/51.5
NRP + SBB/bile A	18.3/18.4
NRP + SBB/bile B	19.6/19.1
NRP + SBB/bile C	18.3/18.4
NRP + SBB/bile D	19.3/19.2

Note. The effects of saline-barbital buffer (SBB) and bile, with or without heparin, on the APTT measurement of pooled normal rat plasma (NRP) is shown in the upper four lines of the table. As little as 0.1 u/ml of heparin caused significant prolongation of the APTT (line 4). Bile from perfusion studies where citrate, not heparin, was used for anticoagulation (bile A and B) are compared to bile samples from perfusion studies where heparin was added to the liver perfusate (bile C and D).

of 10 hr duration, samples were taken for assay at 0, 1, 2, 4, 6, 8, and 10 hr of perfusion. Measurements made by the amidolytic assay showed 10.2 ± 1.8 u/ml of activity at the outset and 9.8 ± 0.4 u/ml after 10 hr. Using the APTT method, 10.0 ± 0.4 u/ml were detected at the outset and 10.5 ± 0.6 u/ml after 10 hr. It is apparent from the table that results from the two methods correlated very well, and there was no significant variation in heparin activity measurable over the 10-hr perfusion period.

Protein measurements. Net biosynthesis of AT III, fibrinogen, and albumin by the isolated rat liver perfused for 10 hr is shown in Figs.

TABLE II. HEPARIN ACTIVITY (u) IN LIVER PERFUSATE

Hour sample taken	Samples measured by	
	Amidolytic assay	APTT method
0	10.2 ± 1.8	10.0 ± 1.4
1	10.9 ± 0.8	9.6 ± 1.2
2	10.2 ± 0.3	10.3 ± 0.8
4	10.1 ± 0.4	9.7 ± 1.4
6	10.4 ± 0.4	9.9 ± 1.2
8	9.1 ± 0.6	8.9 ± 1.8
10	9.8 ± 0.2	10.5 ± 0.6

Note. Measurement of units of heparin activity in samples from three perfusion studies, drawn at intervals as shown, was performed by (a) amidolytic assay and (b) APTT assay. Shown are mean activity measurements ± SD of the three perfusion samples tested by each method.

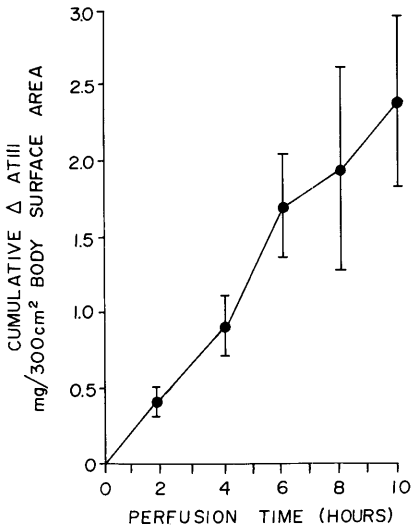

FIG. 1. Net cumulative synthesis of antithrombin III in three perfusions where 1000 u heparin was added directly to the liver perfusate at the outset. Vertical bars show SEM.

1–3, respectively. In each case, synthesis of the proteins continued throughout the 10-hr perfusion period. Cumulative synthesis of AT III reached 2.4 ± .6 mg/300 m^2 body surface area of the rat liver donor in 10 hr, fibrinogen, 20.2 ± 1.6 mg/300 cm^2, and albumin, 56.2 ± 8.6 mg/300 cm^2.

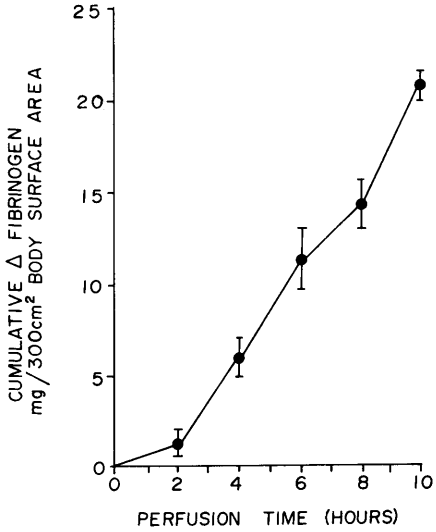

FIG. 2. Net synthesis of fibrinogen in three perfusion studies where heparin was used as an anticoagulant.

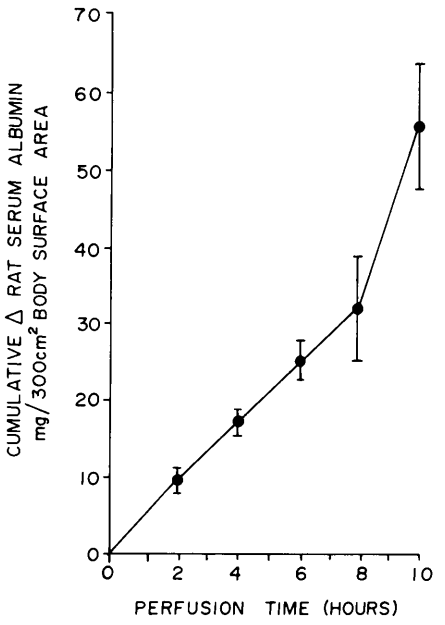

FIG. 3. Net synthesis of albumin in three perfusion studies where heparin was used as an anticoagulant.

Discussion. The observations described in this report indicate that the liver by itself plays a minimal role in the metabolism of heparin in the rat. Over a 10-hr perfusion period there was no significant fluctuation of heparin concentration in perfusate plasma as measured by APTT techniques or by a chromogenic substrate. As would be expected from the perfusate heparin measurements, there was no detectable heparin in bile samples secreted during the same 10-hr perfusion period.

Many studies have described protein synthesis and secretion by the perfused rat liver, and the measurements of cumulative production of AT III, fibrinogen, and albumin reported here indicate that the production capacity of the perfused organ remained intact and functional. Whether there was significant binding of heparin by any of the hepatic secretory proteins synthesized during the perfusion period cannot be answered from the data reported here; certainly if such binding took place, it did not have significant impact on heparin kinetics.

1. Estes JW, Poulin PF. Pharmacokinetics of heparin. Thrombos Diathes Haemorrh **33**:26–37, 1974.
2. Estes JW. Kinetics of the anticoagulant effect of heparin. J Amer Med Assoc **212**:1492–1496, 1970.
3. Estes JW, Pelikan EW, Kruger-Thiemer E. A retrospective study of the pharmacokinetics of heparin. Clin Pharmacol Ther **10**:329–337, 1969.
4. Estes JW. Clinical pharmacokinetics of heparin. Clin Pharmacokin **5**:204–220, 1980.
5. Danishefsky I, Eiber HB. Studies on the metabolism of heparin. Arch Biochem Biophys **85**:53–61, 1959.
6. Teien AN. Heparin elimination in patients with liver cirrhosis. Thrombos Haemostas **38**:701–706, 1977.
7. Marciniak E. Binding of heparin *in vitro* and *in vivo* to plasma proteins. J Lab Clin Med **84**:344–356, 1974.
8. Takeda Y, Kobayashi N. Studies of heparin affinity to antithrombin III and other proteins *in vitro* and *in vivo*. Thrombos Haemostas **38**:685–695, 1977.
9. Miller LL, Bale WF. Synthesis of all plasma protein fractions except gamma globulins by the liver. J Exp Med **99**:125–132, 1954.
10. Miller LL, Hanavan HR, Titthasiri N, Chowdhury A. Dominant role of the liver in the biosynthesis of the plasma proteins with special reference to the plasma mucoproteins (seromucoid) ceruloplasmin and fibrinogen. Chemistry **44**:17–26, 1964.
11. Owens MR, Cimino CD. The inhibitory effects of sodium salicylate on synthesis of Factor VII by the perfused rat liver. Thrombos Res **18**:839–845, 1980.
12. Owens MR, Miller LL. Net biosynthesis of antithrombin III by the isolated rat liver perfused for 12–24 hours. Biochim Biophys Acta **627**:30–39, 1980.
13. Owens MR, Cimino CD. Synthesis of fibronectin by the isolated perfused rat liver. J Lab Clin Med **105**: 368–373, 1985.
14. Triplett DA, Harms CS, Koepke JA. The effect of heparin on the activated partial thromboplastin time. Amer J Clin Pathol **70**:556–559, 1978.
15. Teien AN, Lie M. Evaluation of amidolytic heparin assay method: Increased sensitivity by adding purified antithrombin III. Thromb Res **10**:399–410, 1977.
16. Laurell CB. Electroimmunoassay. Scand J Clin Lab Invest **29**:21–37, 1972.
17. Mancini G, Corbenara AD, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Int J Immunochem **2**:235–254, 1965.
18. Fahey JL, McKelvey EM. Quantitative determination of serum immunoglobulins in antibody organ plates. J Immunol **98**:84–90, 1965.

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