mal variation in size of stomachs may account for this. Inflation of the intragastric balloon with a solution at 38°C produced only a slight decrease in blood flow. However, initiation of local gastric hypothermia with depression of temperature of stomach wall to 15°-16°C resulted in marked reduction of venous outflow. The stomach wall at these temperatures appeared blanched and slightly cyanotic. This occurrence confirms the observation that decrease in blood flow is due to cooling *per se* and not to pressure effect derived from intragastric distention.

Summary. A method has been described for estimating changes in gastric blood flow by direct measurement of gastric venous return through the splenic vein. Blood flow, attending depression of gastric wall temperature to 15-16°C was decreased by a mean value of 66.2% in those animals where some venous collateral was retained and by 68% where total venous isolation was achieved. Flows increased to 48°C. Return of gastric wall temperature to 38°C was followed by restitution of flows to approximately control levels.

1. Wangensteen, O. H., Root, H. D., Jenson, C. B., Imamoglu, K., Salmon, P. A., *Surgery,* 1958, v44, 256.
2. Salmon, P. A., Griffen, W. O., Jr., Root, H. D., Jenson, C. B., Imamoglu, K., Wangensteen, O. H., *Univ. Minn. Med. Bull.,* 1959, v30, 200.

Received April 6, 1959. P.S.E.B.M., 1959, v101.

Failure of Liver to Inactivate Heparin.* (24973)

T. A. LOOMIS
Dept. of Pharmacology, School of Medicine, University of Washington, Seattle

Factors involved in terminating the action of intravenously administered heparin have not been established. The brief duration of anticoagulant action of exogenously administered heparin cannot be accounted for on the basis of excretion of the drug in urine(1). Jaques(2) described a heparin inactivating enzyme (heparinase) which he extracted from liver, but other workers(3) have presented evidence indicating that this is not a true enzyme. This report is concerned with *in vitro* and *in vivo* evidence indicating that liver is not selectively involved in terminating the action of exogenously administered heparin.

Methods. Three types of experiments were conducted using either intact mongrel dogs or dog and rabbit liver preparations. The first of these experiments was of the extirpation type in which 3 dogs were anesthetized with sodium pentobarbital (30 mg/kg), their abdomens opened, and 0.5 mg/kg of heparin injected in a saphenous vein. The anticoagulant effect was determined by Lee-White(4) 3 tube method on jugular vein blood samples. When clotting time had returned to normal, the hepatic artery, common bile duct and portal vein were ligated. The hepatic vein was clamped at its junction with the inferior vena cava and approximately 90% of liver was excised. A second dose of heparin (0.5 mg/kg) was then injected in a saphenous vein and the intensity and duration of anticoagulant action determined. The second type of experiment involved administering heparin by continuous infusion into either a branch of portal vein or a superficial saphenous vein in dogs anesthetized with sodium pentobarbital (30 mg/kg). Duration and rate of disappearance of the anticoagulant action of the drug was determined. The third type of experiment involved preparation of an aqueous extract from approximately 500 g of dog liver and from approximately 500 g of rabbit liver according to method of Cho and Jaques(5). The influence of this extract on the metachromatic action of heparin was studied *in vitro,* also according to the method of above investigators. Heparin used was from Lot. No. 4288A assaying 145.7

* This study supported by Initiative 171 Funds, State of Washington. Assistance of Carolyn S. Spaeth is gratefully acknowledged.

TABLE I. Anticoagulant Effect of Sodium Heparin (0.5 mg/kg) in Control and Partially Hepatectomized Dogs.

	Anticoagulant effect (% of control clotting time)					
	30 min. after inj.		60 min. after inj.		90 min. after inj.	
	Control	Hepatectomized	Control	Hepatectomized	Control	Hepatectomized
	410	380	240	300	110	150
	480	380	320	270	150	120
	400	450	280	240	130	130
Avg	430	400	280	270	130	130

Clotting time determined by Lee-White 3-tube method and recorded to nearest 10%. Each dog served as its own control and clotting time before drug administration was from 12 to 18 min.

U.S.P. units/mg, Eli Lilly and Co.† All doses are reported by weight. Solutions were made in physiological saline from the powder just prior to use.

Results. Table I consists of results obtained following injection of sodium heparin in the external jugular vein in control and subsequently partially hepatectomized animals. The Table shows that hepatectomy did not alter maximum anticoagulant effect or duration of anticoagulant effect resulting from administration of a standard dose of 0.5 mg/kg of sodium heparin.

Fig. 1 shows anticoagulant effect of repeated injections into the saphenous vein or of infusion of heparin into the saphenous or

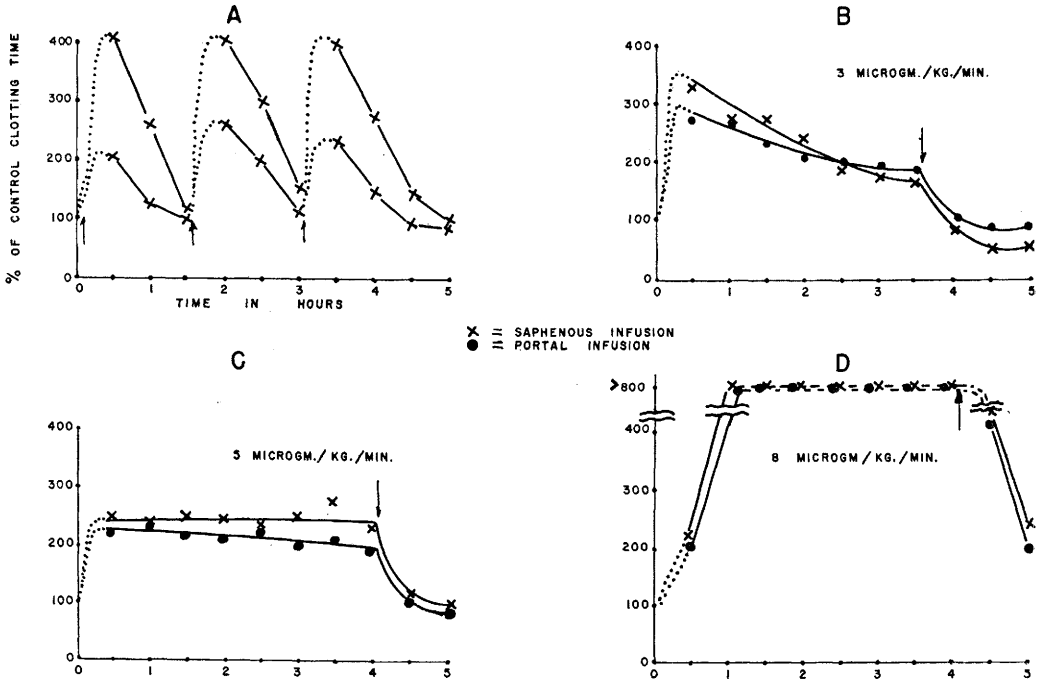

FIG. 1. In Graph A, repeated inj. of 0.25 mg/kg (lower curve) or 0.50 mg/kg (upper curve) sodium heparin are indicated by arrows. In Graphs B, C, and D, infusion of sodium heparin was started 15 min. after priming dose of 0.25 mg/kg of sodium heparin. Infusion rate in all experiments was 0.7 ± 0.05 ml of the solution of sodium heparin in physiological saline/min. as delivered by Merck constant infusion pump. Arrows in Graphs B, C, and D indicate time when infusion solution was stopped. Each curve in Graphs A, B, and C represents mean effect on 4 dogs. Each curve in Graph D represents mean effect on 3 dogs.

† Heparin was generously supplied by Eli Lilly and Co., Indianapolis.

portal vein in 30 intact anesthetized dogs. Fig. 1, Graph A, shows response to 3 separate injections of 0.25 mg/kg and 0.5 mg/kg of sodium heparin. Intensity of response was greater to the larger dose but duration of anticoagulant effect was not different between the 2 doses. Fig. 1, Graphs B, C, and D, show the effect of infusion of sodium heparin at rates of 3, 5, and 8 μg/kg/minute following a priming dose of 0.25 mg/kg *via* portal and saphenous vein routes. Anticoagulant effect was determined on jugular venous blood samples. Figure 1 demonstrates that rate of metabolism of heparin is approximately 5 μg/kg/minute as far as anticoagulant effect is concerned when the anticoagulant effect is between 2 and 3 times normal. Furthermore, the anticoagulant effect following portal infusion was not different from the effect following saphenous infusion. A deviation in infusion rate of -2 or $+3$ μg/kg/minute from the 5 μg/kg/minute rate respectively produces a decrease and an increase in anticoagulant action. The curves also indicate that clotting times returned to normal within 30 to 45 mintues after infusion was discontinued.

The *in vitro* effect on metachromatic action of heparin as produced by liver extracts prepared according to method of Cho and Jaques (5) was confirmed. Although these workers had indicated that dog liver does not contain an extractable active material, both dog and rabbit liver used in present experiments showed an effect of decreasing metachromatic activity of heparin. The procedures described by Cho and Jaques were followed both for preparation of liver extracts and for testing of liver extracts on heparin metachromasia. The parallelism between metachromatic activity and antithrombin activity of heparin as described by those authors was also confirmed.

Although original liver extracts (2 preparations each from dog and rabbit) contained some metachromatic activity, control tests showed that it was not significantly altered by the procedure used for testing effect of liver extracts on heparin metachromasia. When liver extracts were incubated at 37° with heparin at pH of 4.0 or 6.0 (MacIlvaine's buffer) and aliquots were removed at 0, 15, and 30 minutes, there was a respective loss of 0 to 10%, 25 to 50%, and 60 to 80%, of metachromatic activity due to heparin. These results are qualitatively similar to those reported by Cho and Jaques although the present liver extracts showed quantitatively greater activity than did preparations prepared by those investigators. However, when pH was raised to 9.0 by addition of sodium hydroxide after incubation and before tests for metachromatic activity were performed, from 80 to 100% of initial metachromatic activity was recovered. These results are similar to those reported by Schuytema and Cushing(3) and indicate that the action of liver extracts on heparin is due to a binding phenomenon and that active heparin can be eluted by appropriate pH adjustment.

Discussion. The strong electro-negative property of heparins is presumed important for their anticoagulant action. All heparin-like substances studied are similarly electro-negative and are usually sulfated or sulfonated compounds. Binding of heparin with cationic dyes such as toluidine blue and azure A or with protamine abolishes the anticoagulant action of the drug. Action of drug both as anticoagulant and antilipemic agent is believed to involve binding or reaction of heparin with plasma or tissue co-factors resulting in active antithrombin, antithromboplastin, and antilipemic complexes. Eiber and Danischevsky(6), using heparin tagged with S^{35}, obtained evidence which indicated that short duration of action of heparin is neither the result of excretion of the drug from body nor of selective deposition in any specific type of tissue which they tested (liver, lung, spleen, kidney). It therefore seems possible that the action of heparins is terminated by non-selective deposition in inactive and probably bound forms following which the drug is only slowly excreted in the urine in inactive forms.

Cho and Jaques(5) recognized that heparin may be absorbed on protein in tissue extracts and thus removed non-enzymatically. The present experiments demonstrate that our liver extracts by the method of those investigators are active in binding heparin. Furthermore, *in vivo* experiments reported here indicate lack of a highly active mechanism in

liver for terminating heparin anticoagulant action in the dog. It is possible that surgical procedure altered susceptibility of animal to the anticoagulant effect of heparin. This possibility has not been adequately tested, because currently available chemical methods for determining blood heparin concentrations at levels we used have not been found sensitive enough.

Summary. Evidence obtained by 2 methods in the *intact* dog indicate that liver is not involved in terminating the anticoagulant action of exogenously administered heparin. Liver extracts reported to contain a heparin inactivating enzyme were found to influence heparin only by a binding mechanism.

1. Best, C. H., *Annal. N. Y. Acad. Sci.,* 1948, v49, 501.
2. Jaques, L. B., *J. Biol. Chem.,* 1940, v133, 445.
3. Schuytema, E. C., Cushing, I. B., *Fed. Proc.,* 1958, v17, 307.
4. Lee, R. I., White, P. D., *Am. J. Med. Sci.,* 1913, v145, 495.
5. Cho, M. H., Jaques, L. B., *Canad. J. Biochem. Physiol.,* 1956, v34, 799.
6. Eiber, H. E., Danischevsky, I., *A.M.A. Arch Int. Med.,* 1958, v102, 189.

Received April 6, 1959. P.S.E.B.M., 1959, v101.

Absorption of Heparin from the Intestine.* (24974)

T. A. LOOMIS
Dept. of Pharmacology, School of Medicine, University of Washington, Seattle

Oral administration of heparin does not result in systemic anticoagulant effects of this drug, and anticoagulant activity can be recovered in the feces(1). Although there is some evidence(2) that sublingual wafers of sodium heparin will result in a systemic anticoagulant effect this work has not been confirmed. Fuller(3) has indicated that potassium heparin administered sublingually will decrease intensity of post alimentary hyperlipemia without producing an effect on clotting time; however, in similar studies Engelberg(4) found no significant sublingual absorption in 18 of 21 patients. This report describes experiments which show that small amounts of heparin† can be absorbed from intestinal lumen of the dog and that this is dependent at least in part on the pH of heparin solution.

Methods. Sixteen adult mongrel dogs, 10 to 15 kg were used. Each animal was anesthetized with sodium pentobarbital (25 mg/kg intravenously). The abdomen was opened in midline, and 10 to 12 inch segment of duodenum located about 12 inches distal to pylorus was clamped, ligated and sectioned on both ends, leaving mesenteric blood supply intact. The duodenal loop was irrigated with approximately 200 ml of physiological saline. Test buffer solutions or buffer plus sodium heparin solutions were instilled into the loop. The loop was then replaced in the abdomen, and abdominal incision was closed with clamps. Systemic anticoagulant effect was determined by the Lee-White(5) 3-tube method on femoral or external jugular venous blood before and at ½ hour intervals following instillation of solutions into the intestinal loop. Analyses for residual heparin in the loop were performed according to the phenol extraction method of Monkhouse and Jaques(6) as applied to metachromatic assay (7). Undiluted MacIlvaine's (citric acid-disodium phosphate) buffer(8) was used for buffer solutions. Heparin sodium, Lot No. 4288A, potency - 145. 7 U.S.P. units/mg, Eli Lilly and Co.† was used and solutions prepared from this powder just before use.

Results. Sodium heparin in doses of 100 or 200 mg in 20 ml of physiological saline (pH 5.2) instilled into intestinal loop in 4 dogs did not result in a significant increase in coagulation time during the following 8 hours.

* This study was supported by Initiative 171 Funds, State of Washington.

† Heparin was generously supplied by Eli Lilly and Co., Indianapolis.