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.

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Absorption of Heparin from the Intestine.* (24974)

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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.

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

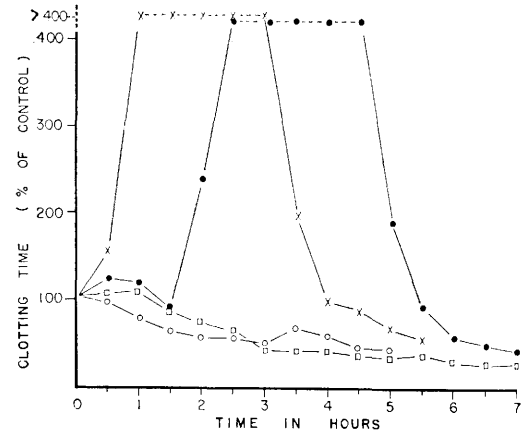

FIG. 1. Influence of acid buffer and saline on absorption of heparin from duodenal loop of dogs. Each curve represents a single separate dog. ● = 200 mg heparin in 20 ml saline instilled at 0 time followed at 1½ hr by instillation of 20 ml buffer, pH 4. × = 200 mg heparin in 20 ml buffer, pH 4, instilled at 0 time. ○ = 20 ml of buffer, pH 4, instilled at 0 time, at 3 hr loop washed with 200 ml saline, at 3¼ hr 200 mg heparin in 20 ml saline instilled. □ = 200 mg heparin in 20 ml saline instilled at 0 time. Control clotting times in all dogs were between 12 and 16 min.

Rather, coagulation time gradually became shorter during this time.

Fig. 1 and Table I show the effect of altering pH of diluent on absorption of a standard dose of 200 mg of sodium heparin. In presence of acid buffer of pH 4.0, a significant systemic anticoagulant effect was obtained. Instillation of buffer only, 2 to 3 hours after heparin in physiological saline had been instilled in the intestine, also resulted in significant systemic anticoagulant effect. Instillation and then removal of only the acid buffer followed by instillation of heparin in physiological saline did not result in a systemic anticoagulant effect. Instillation into the intestinal loop of heparin in alkaline buffer (pH 8.0) did not result in a systemic anticoagulant effect.

pH and concentration of heparin in luminal contents at end of experiments in which acid buffer was used was determined. Four to 5 hours after heparin in acid buffer (resulting solution pH 4.0) had been instilled, the luminal contents had a pH of 6.0 to 6.5 and 40 to 80% of initially instilled heparin was recovered from the luminal contents as determined by extraction and metachromatic activity. Four to 7 hours after heparin in physiological saline (resulting solution pH 5.2) had been instilled into the intestinal loop, luminal contents showed a pH of 6.0 to 6.5 and 80 to 100% of the initially instilled heparin was recovered.

Gross inspection of intestinal loops at end of experiments showed no evidence of general mucosal inflammation. However, there was a mild local inflammatory response adjacent to area which was traumatized by application of ligatures at each end of loop.

Discussion. Schanker *et al.*,(9,10) and Hogben *et al.*,(11) studied the influence of pKa on absorption of drugs from stomach and intestine of rats and from stomach of humans. Their results indicate that absorption of the several drugs studied was dependent on degree of un-ionization; that is, agents in the ionized state were not absorbed, whereas those in the un-ionized state were absorbed from gastric and intestinal lumen. Most drugs studied were of relatively small molecular weight as compared with heparin. However, they also had evidence which indicated that molecular size may not be an important factor. In general, most drugs studied were readily absorbed except for stronger acids (pKa = <2.9) and stronger bases (pKa = >8.4)

TABLE I. Influence of Buffer at pH 4.0 and Saline on Absorption of Heparin from Duodenal Loop of Dogs. Clotting time of venous blood was recorded to nearest 10% of control. Each dog acted as its own control. Control clotting times were between 12 and 18 min.

	Hr after instillation						
	.5	1.0	1.5	2 to 3	4.0	5.0	60
	Clotting time of blood as % of control						
	After 200 mg sodium heparin in 20 ml buffer, pH 4						
	120	>400	>400	>400	100	80	50
	100	280	320	"	200	90	
	240	>400	>400	"	100		
	200	350	"	"	250	70	40
	120	>400	"	"	110	90	
Avg	160	"	"	"	150	80	50
	After 200 mg sodium heparin in 20 ml saline						
	100	120	100	60–80	50		
	110	120	80	50–80	70		
	90	90	70	"	30	20	
	120	80	80	"	80	50	40
Avg	110	100	80	"	60	40	40

which were absorbed only slowly.

Charles and Todd(12) estimated that the barium heparin contained 5 sulfuric acid residues and 2 carboxyl groups/molecule. They believed that the carboxyl groups were unsubstituted. In the current experiments it is probable that only the carboxyl groups are influenced by lowering the pH to 4.0. The state of ionization of the carboxyl groups therefore, may be significant with respect to absorption of heparin.

Our experiments indicate that there is no significant destruction of heparin in the intestinal loop. Approximately 100% of the heparin initially instilled in the gut can be accounted for by estimating amount absorbed. This estimation is based on a metabolic rate of 0.5 μg/kg/minute during the interval that anticoagulant activity was present and measurement of the free heparin remaining in the gut after a 5 to 7 hour experiment. These results indicate that during the experiment when the initial pH in the lumen of intestine was about 4.0, absorption of approximately 10% of instilled heparin took place. During the experiment, however, pH of the luminal content gradually returned toward neutrality, and absorption of drug then ceased.

Summary and conclusion. Sodium heparin is partially absorbed from the lumen of a washed duodenal loop in the dog following addition of acid buffer of pH 4.

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Fat Deposition in Musculature of the Genetically Dystrophic Chicken.* (24975)

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Asmundson and Julian(1) reported that an inherited muscular dystrophy prevented New Hampshire chickens from raising their wings and rising from a flat surface when laid on their backs. The affected birds were homozygous for a recessive autosomal gene (am), and had wider breasts than is normal in this breed. Our exploratory biochemical determinations suggested that this condition in the chicken is similar to certain forms of muscular dystrophy in man(2) characterized histologically by disarrangement of musculature and by large numbers of fat cells. In biochemical characterization of this condition in the chicken, fat deposition was the first major area of interest. When percentage of crude fat in the wet muscle sample was determined, excess fat deposition was associated with physical manifestations of muscular dystrophy, and was a function of age. Further, a sexual dimorphism was discovered, with females generally de-

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