STUDY OF FIBRINOGEN TURNOVER RATES AFTER TOTAL HEPATECTOMY IN DOGS

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The liver is regarded as the site of fibrinogen formation. Evidence for this concept is derived from the investigations of Doyon,[4] Nolf,[20] Meek,[17] Drury and McMaster,[3] Whipple,[26] and others.

Madden and Gould[50] reported that the half-life of dog fibrinogen is approximately 4 days. Hence, total hepatectomy and the subsequent lack of fibrinogen regeneration should result in the gradual disappearance of the circulating fibrinogen. Instead, we found that the circulating fibrinogen disappears in less than 24 hours. Consequently, the rate of disappearance of fibrinogen following total hepatectomy in the dog is considerably faster than expected.

The mechanisms responsible for the rapid defibrination of blood following total hepatectomy in the dog are evaluated here.

TECHNIQUE OF ONE-STAGE TOTAL HEPATECTOMY IN THE DOG

Mongrel dogs with wide costal arches and weighing 10 to 30 kilograms were studied. All animals were anesthetized with Nembutal (60 mg. per 2.5 Kg.). A slow intravenous infusion of 10 percent dextrose containing 500 mg. Achromycin was started and continued throughout the postoperative period at a rate of 4 to 6 drops per minute.

The liver was exposed through a high midline incision extended laterally below the right costal margin. The portal vein was dissected from the confluence of the superior mesenteric vein to its entry into the liver. The hepatic artery was freed close to the liver.

The inferior vena cava was denuded from the entry of the renal veins to the liver. The vein was freed, and the dissection extended along its posterior surface to a point below the diaphragm. The coronary, triangular, and hepatorenal ligaments were divided. An umbilical tape was brought underneath the inferior vena cava and the portal vein. Traction on the tape approximated the two vessels, allowing the application of a vascular clamp. The resultant lips of both vessels were held firmly together for a side-to-side anastomosis, allowing blood to flow through the remaining unobstructed portion of the veins. For a further description of the various steps of the procedure, see Figs. 1 and 2.

The advantage of this technique is that it does not affect homeostasis as extensively as do some of the previously described procedures. No cross-clamping of the inferior vena cava or portal vein is required, a procedure invariably associated with a drop in blood pressure,[22] nor does it require blood replacement or result in air embolism.[16]

The fate of the animals in the posthepatectomy period has been elucidated by Markowitz and co-workers.[16] These authors noted, as we were able to confirm, that hypoglycemic shock develops approximately 6 hours postoperatively. If intravenous glucose is administered the animals survive for

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Table I. *Fibrinogen concentrations in mg. percent after total hepatectomy in dogs not receiving E.A.C.A.*

Dog No.	No. of hours after completion of hepatectomy at which sample was collected												
	1½	3	5	6	7	8	9	10	11	14	16	19	25
H_2		881			637			339					
H_4	233		198			131			103				
H_8	389	378		304			275		194	0	0		
H_9	214		184		131				95		54	7	0

16 to 30 hours, after which terminal coma develops that is not relieved by intravenous infusion of glucose or electrolyte solutions.

METHODS AND MATERIALS

The femoral artery was intubated with a plastic catheter attached to a siliconized No. 15 needle and stopcock. Samples were collected by allowing the blood to flow freely into chilled (0° C.) tubes which were immediately transferred to the laboratory for processing.

Fibrinogen concentrations were determined on samples collected in 10 percent sodium sequestrene by the methods described by Fowell[5] and Kjell Jacobsson.[12] The results are expressed in milligrams percent of fibrinogen.

Plasminogen activator activity was determined by the fibrin plate technique[1] and the euglobulin clot lysis time determination.[10]

Free plasmin activity was determined on heat denatured fibrin plates.[13] Euglobulin samples were used in both the Astrup and Lassen techniques. Results obtained with these methods are expressed in square millimeters of lysis.

Plasminogen concentrations were determined by the method described by P. Norman.[21] Results of these determinations are expressed in optical dentistry units (O.D.U.).

Thrombin time determinations were performed as described by Biggs and MacFarlane.[2] Five units of bovine thrombin (Parke, Davis & Company) were used in each thrombin time determination. The results of these tests are expressed in seconds. Prolonged thrombin times reveal the presence of antithrombin activity.

A thromboelastogram continuously measures and records the rate and kinetics of fibrin formation in a clotting blood sample. It also records the firmness of the clot at any time during and after its formation. Blood samples (0.35 ml.) without anticoagulant were transferred to the cup of the thromboelastograph and recordings were subsequently made.[18]

Epsilon amino caproic acid (E.A.C.A.), an antifibrinolytic agent, was used in five dogs. It was noted that E.A.C.A. in doses tolerated by nonhepatectomized animals (4 to 6 Gm. intravenously) would kill the hepatectomized animal. Autopsies performed upon these animals revealed subendocardial hemorrhages similar to the ones observed in normal dogs receiving toxic doses of this drug. E.A.C.A. was administered during the post-

Table II. *Fibrinogen concentrations in mg. percent after total hepatectomy in dogs receiving E.A.C.A.*

Dog No.	No. of hours after completion of hepatectomy at which sample was collected								
	3	6	8	9	11	12	16	22	25
H_5	256	0							
H_6	550	346		474		286			
H_7	509	494			661		629	0	0
H_{10}	794	695	675			0	0		
H_{11}	265	212		158					

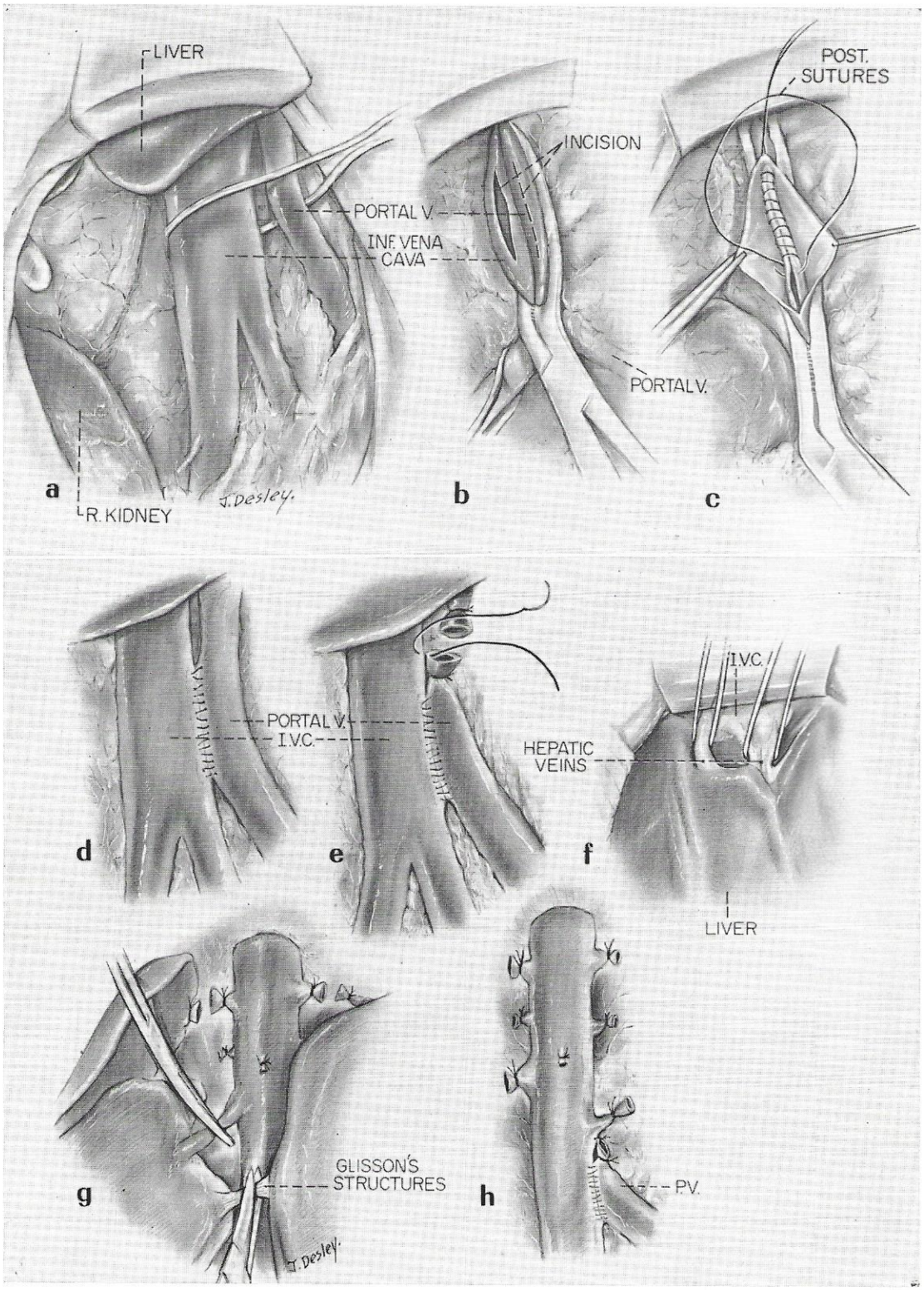

Fig. 1. *For legend see opposite page.*

operative period in a continuous drip of 0.5 Gm. per hour.

RESULTS

Blood samples obtained from these dogs revealed a rapid decline in fibrinogen concentrations in the posthepatectomy period (Table I).

Possible mechanisms responsible for the rapid defibrination of the blood following hepatectomy were evaluated. Considerable increase in plasminogen activator and free plasmin activity was noted. This increase in activity was found to be associated with a steady decline of the plasminogen content of the blood (Table III). These findings indicate marked activation of the plasminogen-plasmin system. Simultaneously, the generation of antithrombin activity (as reflected in prolongation of the thrombin time) was observed (Table IV).

The possibility that the noted rapid defibrination resulted from increased plasmin activity had to be considered. Therefore, a number of hepatectomized animals were treated with E.A.C.A. These animals showed changes in fibrinogen concentrations similar to those noted in nontreated hepatectomized dogs (Table II).

In view of the fact that the E.A.C.A.–treated hepatectomized dogs show no significant increase in antithrombin activity

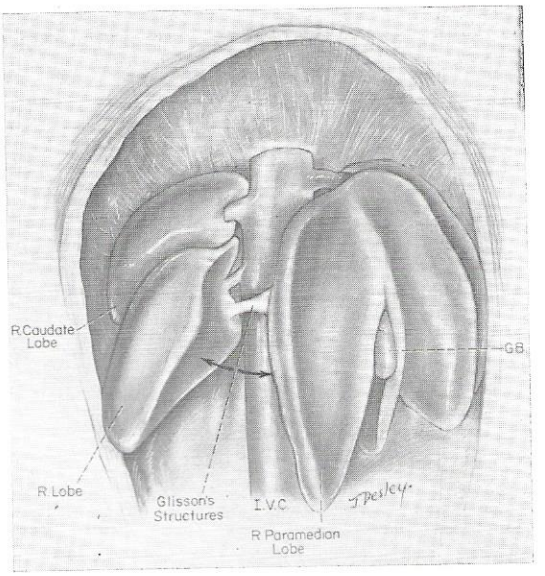

Fig. 2. Exposure of the intrahepatic portion of the inferior vena cava. The inferior vena cava is approached through the bridge of liver tissue which connects the right lobe with the right paramedian lobe. The liver edges do not bleed because the portal vein and hepatic artery have previously been divided at the porta hepatis.

During the division of this tissue bridge the bundle containing the right Glissonian structures (right portal vein, right hepatic artery, and right hepatic duct), which crosses anterior to the inferior vena cava, is visualized. The structures contained in this bundle are clamped, divided, and ligated. In this way a clear view of the intrahepatic inferior vena cava is obtained which greatly facilitates the dissection of the segmental hepatic veins.

Fig. 1. Technique of one-stage total hepatectomy in the dog:

a, Dissection of veins. After dissection of the portal vein and inferior vena cava, an umbilical tape is placed around the veins. Traction on the tape approximates the vessels, facilitating the proper placement of a vascular clamp.

b and *c,* Technique of portacaval anastomosis. An elipse of tissue is cut from the edge of the inferior vena cava, the lip of the portal vein is incised along the indicated line, and a side-to-side anastomosis is made.

d, Completed anastomosis. The length of the anastomosis ranges from 20 to 35 mm., depending upon the size of the dog's veins.

e, Division of portal vein. Care is taken to reinforce the corner of the anastomosis by suturing the stump of the portal vein to the side of the inferior vena cava. Subsequently, the lesser omentum, including the hepatic artery and the common duct, is divided close to the liver.

f, Dissection of major hepatic veins. The hepatic veins are dissected at the site of entry into the inferior vena cava just below the diaphragm. Long venous pedicles are obtained by carrying the dissection around these vessels a short distance into the liver.[6]

g, Exposure of inferior vena cava. The intrahepatic portion of the inferior vena cava is approached as indicated in Fig. 2. The hepatic veins, thus exposed, are carefully freed, ligated, and divided.

h, Resection completed. Structures which remain after the resection in right subdiaphragmatic space are the inferior vena cava and the portacaval shunt. The vascular pedicles seen along the inferior vena cava are remnants of ligated hepatic veins.

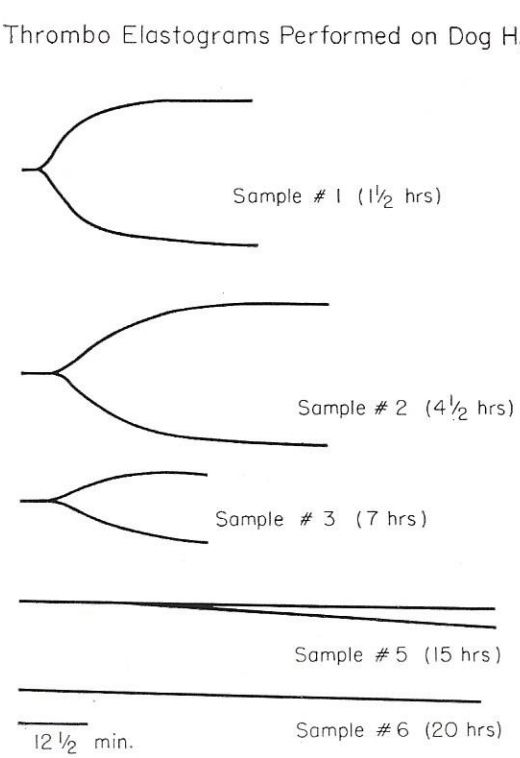

Fig. 3. Thromboelastograms. Thromboelastograms of blood samples taken at 1½, 4, 7, 15, and 20 hours after total hepatectomy. These recordings of the in vitro clotting process demonstrate the development of prolongation of the clotting time as well as a decrease in the firmness of the clot. The coagulation defect responsible for the observed changes contributes to the development of a bleeding tendency in the dog.

(Table V), it is assumed that the observed antithrombin release results from enhanced fibrinolytic activity and probably represents formation of antithrombin IV.*

Because rapid defibrination of blood was also noted in E.A.C.A.–treated animals it seems unlikely that defibrination resulted from increased fibrinolytic activity. A more logical explanation for the observed changes is that defibrination results from intravascular clotting.

DISCUSSION

Totally hepatectomized dogs, besides losing the ability to manufacture a great number of materials, fail to remove substances from the portal vein blood which are derived from the bowel, pancreas, and spleen. Some of these substances, such as trypsin (from the pancreas), endotoxin (from the bowel), and blood thromboplastin (from the spleen),

*Antithrombin IV activity, first observed by Niewiarowski and Kowalski[19] during conditions of enhanced fibrinolytic activity, is related to the release of fibrin-breakdown products into the blood. These products of proteolysis exert a marked anticoagulant effect. This effect is measured by determining the degree of prolongation of the clotting time after addition of small, known amounts of thrombin to plasma. The mechanism of action of antithrombin IV has recently been elucidated by Latallo and associates.[14] It should be noted that antithrombin IV activity is not neutralized by polybrene or protamine. However, adequate pretreatment with E.A.C.A. will prevent its appearance.

Table III. *Plasminogen activator and plasmin activity, and plasminogen concentrations after total hepatectomy*

		No. of hours after removal of liver at which sample was obtained									
		1½	*4*	*6*	*7*	*9*	*10*	*11*	*15*	*16*	*19*	*25*
Dog H₃												
Plasminogen activator and plasmin activity	A	298	273	207		295		237	307	420		
	L	57	50	91		100		49	56	90		
	E	110	116	45		15		13	15	N.C.		
Plasminogen concentration		0.313	0.284	0.246		0.233		0.223	0.197	0.177		
Dog H₉												
Plasminogen activator and plasmin activity	A	20	123		173		216		200		200	
	L	0	36		64		60					
	E	95	190		135		188		115		15	
Plasminogen concentration in O.D.U.		0.229	0.226		0.227		0.225		0.213		0.186	0.165

A = mM.² of lysis on Astrup fibrin plate after incubation for 24 hrs. at 37° C.
L = mM.² of lysis on Lassen fibrin plate after incubation for 24 hrs. at 37° C.
E = euglobulin clot lysis time in minutes.
N.C. = no clot formed.

Table IV. *Thrombin times in minutes in dogs not receiving E.A.C.A.*

Dog. No.	Sample No.							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
H_4	6	9	15	>180				
H_8	9	9	12	14	21	27	>60*	>60*
H_9	6	10	18	>180*	>180*	>180*		

*Fibrinogen concentration less than 100 mg.%.

Table V. *Thrombin times in minutes in dogs receiving E.A.C.A.*

Dog No.	Sample No.							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
H_7	4	4	5	6				
H_{10}	4	4	4	5	7	8	> 180*	
H_{11}	4	4	4	6	>180*			

*Fibrinogen concentration less than 100 mg.%.

have definite clot-inducing properties. It is known that some of these substances are taken up by the Kupffer cells, e.g., thromboplastin in the rat.[22] It is conceivable that failure to remove these substances results in a hypercoagulability state.

It is significant that defibrination is markedly accelerated a few hours before the death of the experimental animal. This finding suggests that the shock, which Markowitz and co-workers[16] and subsequently Starzl and associates[23] observed at this time in hepatectomized dogs, might act as a contributory factor toward the final defibrination of blood.

It has been shown in dogs that fibrinogen concentrations decline during various types of shock: trypsin shock[25]; endotoxin shock[8, 9, 11]; and hemorrhagic shock.[11, 25] It has also been demonstrated that the decline in fibrinogen concentrations noted during shock of varied etiology probably results from enhanced intravascular clotting.[8, 9, 11, 25]

SUMMARY

The findings presented suggest that the presence of the liver is required for the preservation of blood fluidity. It is conceivable that the liver, with its extensive reticuloendothelial system, serving as a trap between bowel and systemic circulation, removes coagulants from the portal vein blood.[7] The rapid defibrination observed after total hepatectomy indicates that the mechanism described is an important liver function.

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