Significance of the Rate of Decrease in Fibrinogen Level after Total Hepatectomy in Dogs *

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OUR INTEREST in the natural decay rate of fibrinogen, that is, its rate of disappearance barring further production, grew out of earlier experiments on hemorrhagic shock wherein a consistent fall in fibrinogen levels was observed.[8] To explain such a drop one must consider four possible mechanisms: 1) hemodilution, 2) destruction by fibrinolysins, 3) decreased production and 4) increase in consumption by intravascular coagulation. In our experiments on shock, dilution by fibrinogen-poor fluid entering the intravascular space had been adjusted by considering plasma protein levels an index of such change. Fibrinolytic activity was not always demonstrable and, even when present, was of such low activity as to have little effect on fibrinogen levels.[9] Increase in intravascular coagulation seemed the best explanation for the fall in fibrinogen level and for observed changes in other clotting factors. However the profound effects of shock on hepatic circulation [17, 18] make it necessary to consider depression in production of fibrinogen as a plausible alternative. Rapid disappearance of fibrinogen observed after total hepatectomy [4, 5, 14, 16] suggests that absent or markedly impaired production alone could account for the magnitude of fall seen in our experiments. On the other hand, by fibrinogen tagging [1, 12] the biologic half-life of fibrinogen in dogs is estimated at two and a half days. Such a slow rate of decrease would suggest that lack of production is not the cause of the fibrinogen fall observed. However, both methods of estimating the "natural" decay rate of fibrinogen have limitations and the wide discrepancy between them has been difficult to resolve. Experience with hepatectomy reported herein not only appears to close this gap but explains the apparent discrepancy. In addition, the contention that disseminated intravascular coagulation plays a prominent role in the pathogenesis of hemorrhagic shock in dogs is reinforced.

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Methods

Principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. Twenty-eight healthy dogs of mixed breed and sex were divided into the following experimental groups: Group I—hepatectomy alone (17 dogs); Group II—hepatectomy with postoperative heparinization (7 dogs); Group III—evisceration (4 dogs).

Group I. Total hepatectomy was carried out under Nembutal anesthesia through a midline abdominal incision as described by Starzl.[19] Using a small tangential DeBakey clamp to grasp both portal vein and inferior vena cava simultaneously, a side-to-side portocaval shunt was performed without portal occlusion. Ligamentous attachments of the liver were then divided and three vascular clamps were placed in rapid

succession across the portal triad and across the inferior vena cava, above and below the entrance of the hepatic veins. During a brief (10–15 minute) occlusion the hepatic lobes were removed and veins were ligated. After flow had been restored in the inferior vena cava, portal structures were individually ligated.

Group II. Hepatectomy was carried out as described. Two hours after removal of the liver, heparinization was begun with an initial intravenous dose of 1 mg./Kg. Half of this dose was repeated at 2-hour intervals. Clotting time determinations and heparin assays confirmed the adequacy of this dose schedule.

Group III. Evisceration was performed by first dividing the esophagus and rectum between umbilical tape ligatures and then freeing abdominal viscera so that only vascular attachments remained. Vascular clamps were placed in rapid succession across the celiac axis, superior and inferior mesenteric arteries, porta hepatis and inferior vena cava, above and below the liver. The liver was quickly separated from the vena cava by ligation of hepatic veins and removed by dividing the porta hepatis above its clamp. After restoring vena cava flow, remaining viscera were removed by dividing and ligating arterial pedicles. In this manner evisceration could be performed with only a 10 to 15-minute period of inferior vena cava occlusion.

Avoidance of portal occlusion, abrupt circulatory isolation of viscera to be removed and the brief vena cava occlusion avoids pooling and minimizes release of vasoactive, thrombogenic or fibrinolytic substances from the liver or intestines into the systemic circulation, thus obviating spurious effects on the fibrinogen level. For similar reasons blood transfusions or other means of support which might prolong survival were avoided with the exception of intravenous infusion of 5 per cent dextrose in water at 20 to 30 drops/minute.

At the time of removal of the liver and at 1 to 3-hour intervals thereafter plasma fibrinogen,[20] plasma protein, hematocrit and urine output were measured in all dogs. To explain the unexpected secondary phase of accelerated decrease in fibrinogen level in some early experiments, the following measurements were made in a few later hepatectomies: pressure, pH, pO_2 pCO_2 in the aorta and inferior vena cava and platelets, glass and silicone clotting times, and prothrombin times from arterial blood. These secondary measurements were not all done on any one dog to avoid modifying the disappearance rate of fibrinogen by further loss of blood in multiple samples.

Fibrinogen Values. Fibrinogen values were plotted against time in six different ways: original determination in mg%, difference between this and the baseline value in mg%, and as a per cent of the baseline measurement. Then, because progressive hemodilution of varying degree was observed in all experiments, each value was adjusted for hemodilution by multiplying the initial value by the ratio between baseline and concomitant plasma protein value. Hemodilution can be accounted for by continuous intravenous dextrose infusion with increasing oliguria and by migration of fibrinogen-poor fluid from extravascular spaces to replace blood lost by sampling and oozing. The adjusted value for fibrinogen expressed as per cent of the original level was finally chosen because of greater uniformity and because there was a more meaningful comparison within and between groups of dogs, and with fibrinogen-tagging experiments. Basic trends, however, were alike regardless of the form of expression used, varying only in degree.

Results

Fibrinogen levels plotted against time showed that in all untreated hepatectomy dogs (Group I), there was a gradual initial decrease. In 11 of these untreated 17 dogs, however marked acceleration in rate of disappearance began between the 5th

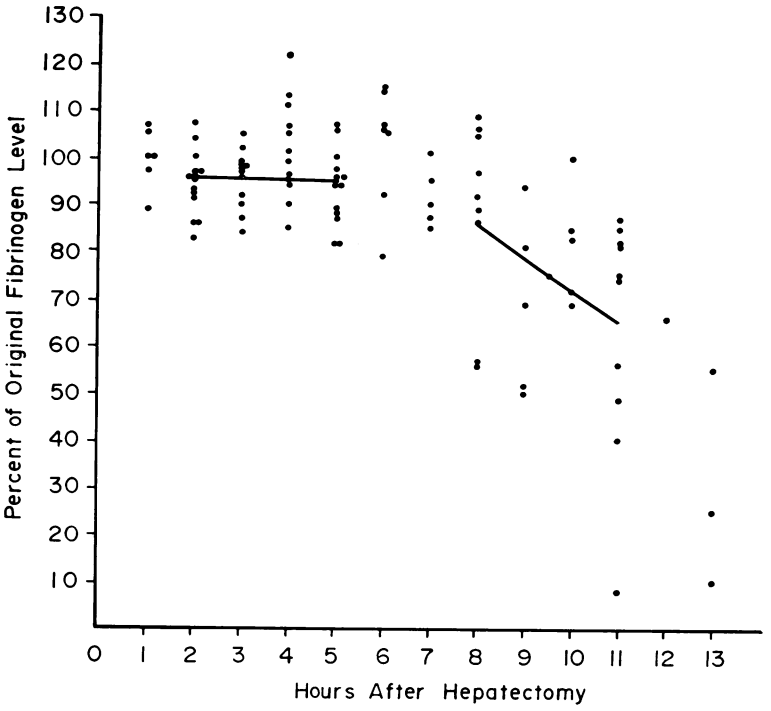

FIG. 1. Fibrinogen values from all hepatectomized dogs (Group I, 17 dogs) are plotted against time. Regression lines are drawn for an early and late 4-hour period showing a 10-fold increase in slope.

and 11th hours (average 6.5 hr.) after hepatectomy, while in six the initial gradual rate was maintained. For purposes of discussion these variations will be referred to as Groups Ia and Ib.

That this change in rate of fibrinogen disappearance is an objective phenomenon unrelated to selection of points of reference on the fibrinogen plots is demonstrated in Figure 1. All fibrinogen values from Group I are plotted against time and regression lines are drawn for an early (2–5 hr.) and a later (8–11 hr.) 4-hour period. Regression coefficients with 95 per cent confidence limits are -0.03 ± 0.28 per cent/hr. for the first period and -6.74 ± 1.1 per cent/hr. for the later period. Thus, despite the fact that in six dogs this late accelerated drop did not occur and time of onset varied over a 6-hour period in the others, this changing rate of disappearance of fibrinogen is significant even in the unselected data ($p < 0.001$).

Having established this late accelerated decrease as an objective phenomenon, we attempted to quantitate more accurately the rates represented by the two periods by calculating two regression lines, one for all values representing the initial or gradual phase and another for values from the late or rapid phase. As depicted in Figure 2, the slope for the initial phase is -0.65 ± 0.75 per cent/hr. and for the late phase, -7.28 ± 2.2 per cent/hr., about a tenfold difference in slope. Slopes from "unadjusted" values for the same data would be -1.4 ± 1.1. per cent/hr. and 8.8 ± 2.9 per cent/hr., respectively. (The range for each slope is shown for one standard deviation.) Thus data unadjusted for hemodilution also demonstrates the same phenomenon, differing moderately in degree but showing a greater scatter.

None of seven hepatectomy dogs receiving heparin (Group II) had late accelerated decrease in fibrinogen level and the mean slope is -0.36 ± 0.82 per cent/hr. (Fig. 3).

Similarly, none of four eviscerated dogs in Group III showed a terminal decrease

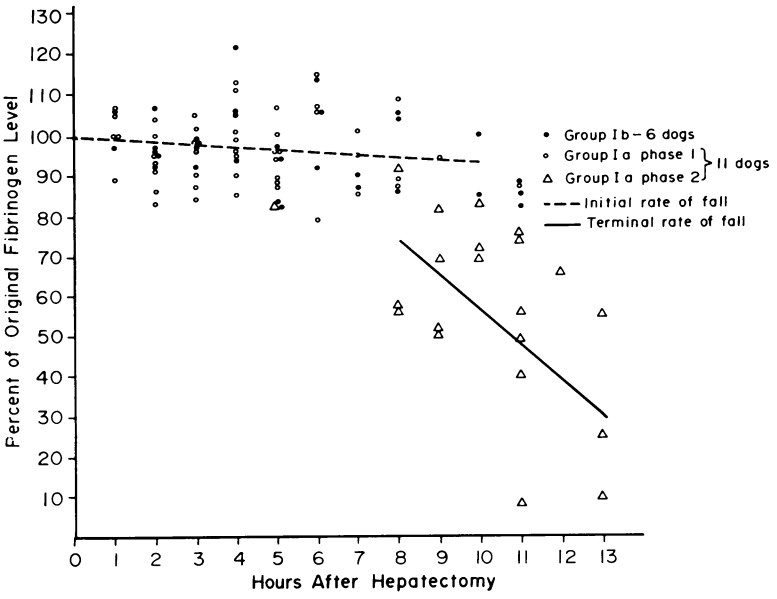

FIG. 2. The same fibrinogen values shown in Fig. 1 have been subgrouped according to whether they represent the initial, gradual or late, rapid phase of fibrinogen fall. Regression lines were then drawn for the two phases.

in fibrinogen. The mean slope is -0.31 ± 0.84 per cent/hr. (fig. 4).

There is no significant difference between the slope for the gradual phase in untreated hepatectomy dogs (-0.65%/hr.), the slope for heparinized hepatectomy dogs (-0.36%/hr.) and the slope for eviscerated dogs (-0.31%/hr.).

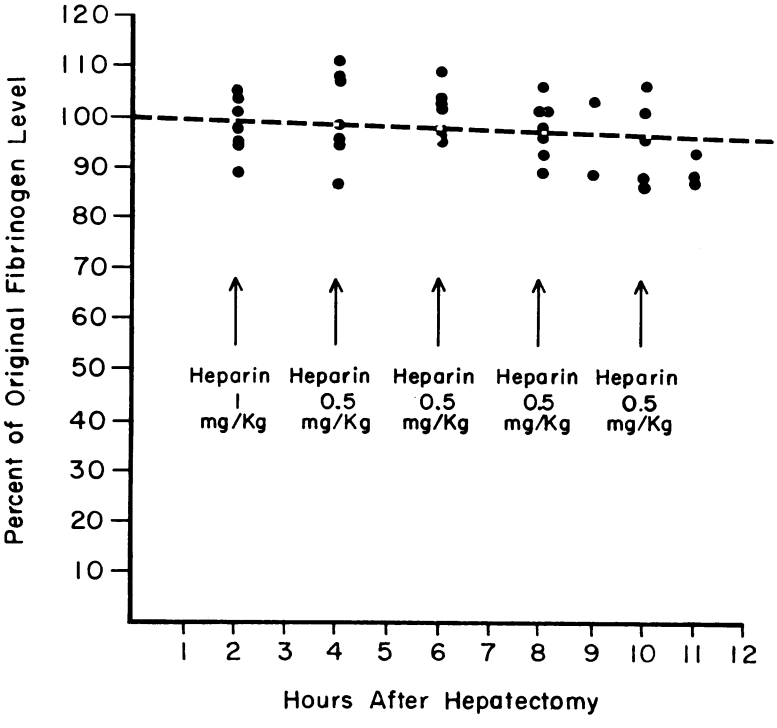

FIG. 3. Fibrinogen levels in Group II (7 dogs) given heparin after hepatectomy are plotted against time.

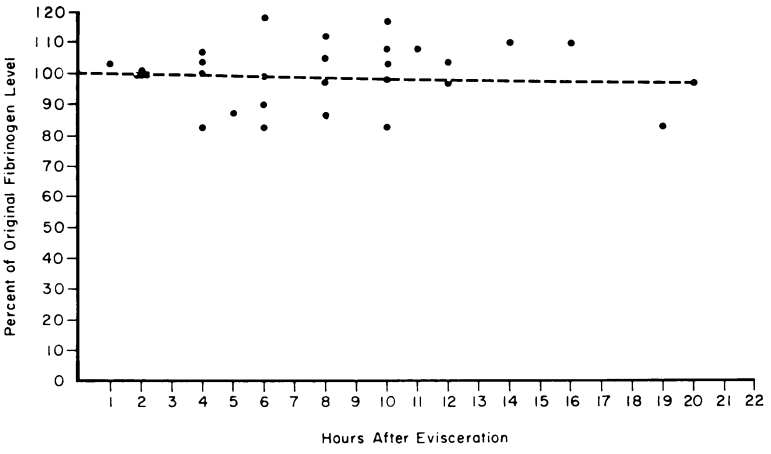

FIG. 4. Fibrinogen values after evisceration in Group III (4 dogs).

Because of the minimal slope and scatter points there is also no significant difference between these three slopes and the horizontal, unlike the late phase which is significantly different ($p < 0.001$, $t_{31} = 6.17$).

A comparison of survival times between the different groups could explain some differences observed. In Group I, six animals with only gradual decrease in fibrinogen level (Group Ib) survived an average of 9 hours compared to 15 hours for those with a terminal drop (Group Ia). Only one of the Group Ia dogs died earlier than the latest death in Group Ib. Most early deaths were abrupt and unexpected and appeared to be respiratory arrest, whereas late deaths followed progressive shock. These six dogs may not have had a terminal drop because of early death.

Animals in Group II (hepatectomy and heparin) survived an average of 9.6 hours, an early demise which may be related to increased intraperitoneal bleeding observed at autopsy. Group III (eviscerated) dogs lived an average of 15.5 hours, slightly longer than the average of 13 hours for Group I (hepatectomized) dogs.

Correlations between rate of fibrinogen decrease and other clotting factors measured are imperfect because of insufficient data for the same detailed analysis as with fibrinogen.

Shock and Acidosis. In four of five dogs in Group I in which arterial pressure was monitored, the abrupt decrease in fibrinogen level began within an hour of the onset of shock and oliguria. Arterial and venous pH, pCO_2, and pO_2 were measured in 15 dogs. Five dogs monitored in Group Ia showed marked progressive metabolic acidosis with widening of A-V O_2 differences, while none of three dogs of Group Ib studied were acidotic at the time of death. Two of three heparinized dogs were markedly acidotic, and one mildly so. All four eviscerated dogs showed progressive acidosis. There was not the strong correlation between time of onset of acidosis and terminal rapid decrease in fibrinogen level as with the onset of shock.

Other Clotting Parameters. Platelet counts were done on three dogs of Group Ia. Two showed an abrupt decrease in number of platelets accompanying the decrease in fibrinogen level. In the third, the number of platelets dropped gradually from 338,000/mm.^{3} to 110,000/mm.^{3} during 10 hours while fibrinogen level dropped abruptly only in the last 2 hours. Prothrombin times and glass and silicone clotting times were measured in eight dogs in Group I. There was moderate initial prolongation of silicone clotting times without much change in the glass clotting times,

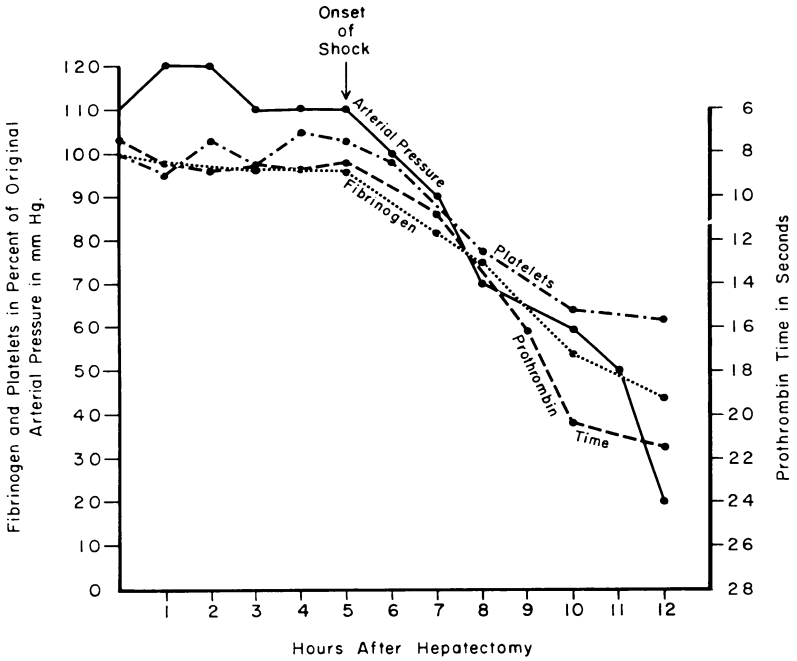

FIG. 5. Mean arterial pressure is plotted along with fibrinogen, platelets and prothrombin time in a hepatectomized dog to show the rough correlation between the onset of shock and an increase in the rates of decline of these clotting parameters.

but after onset of the terminal phase decrease in fibrinogen level all but one blood specimen failed to clot by either method. There was a gradual prolongation of prothrombin time up to 1 to 2 seconds initially, with marked increase within 1 hour of the beginning of the rapid decrease in fibrinogen level in six dogs, and within 2 hours in the other two.

The approximate correlation between onset of shock and change in the rate of decrease of fibrinogen level, number of platelets and prothrombin times is seen in data from two dogs (Fig. 5, 6).

Discussion

The two obvious methods for estimating "natural" decay rate of fibrinogen are 1) measuring the level in the blood after hepatectomy and 2) radioisotope tagging. The isotope method has several difficulties: 1) uneven tagging of fibrinogen, 2) possible modification of the half-life of fibrinogen by the tagging process, 3) a phase of rapid disappearance after infusion due to either escape into the extravascular fibrinogen pool (20–50% of the total) or initiation of intravascular coagulation by the infusion, 4) shift of the tag to other proteins of different biologic half-life, 5) impure fibrinogen. Many of these sources of error have been eliminated by technical refinements reported by MacFarlane.[13] By considering the second or equilibration phase to represent the "natural" decay rate, reasonable agreement in estimates for the biologic half-life of fibrinogen has been obtained in the range of 2½ days [1, 12] for dogs and 4 days for humans.[3, 6, 7]

Previous hepatectomy experiments[4, 5, 11, 14, 16] included fibrinogen levels on only a few dogs; there were only 17 unmodified hepatectomized dogs on which serial fibrinogen levels were recorded in five reports. These data suggest that the half-life of fibrinogen is about a half day [5, 11, 14] and Munro [16] reports more rapid disappearance. In general they show an early, fairly rapid decrease, although Jones and Smith [11] observed a more gradual initial rate of fall. However their data are not compatible with estimates from tagged-fibrinogen de-

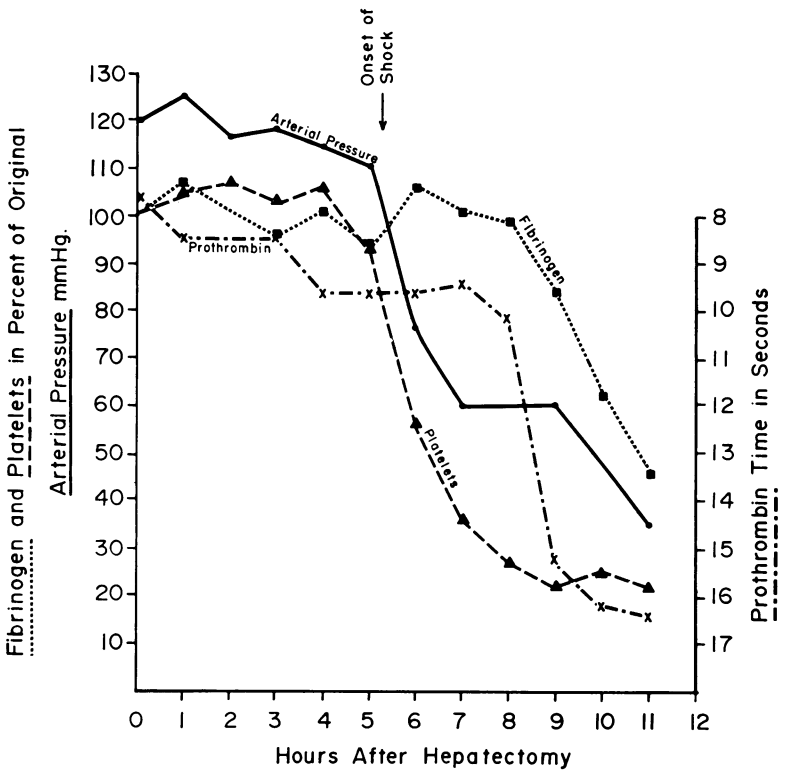

FIG. 6. Mean arterial pressure is plotted along with fibrinogen, platelets and prothrombin time in hepatectomized dog to show the approximate correlation between the onset of shock and an increase in rates of decline of these clotting parameters.

cay studies. On the other hand, the present report demonstrates an initial period after hepatectomy wherein the rate of fibrinogen disappearance approximates 1 per cent/hr. The rate of decrease during this period probably reflects the natural decay rate and the range is comparable with that in the equilibration phase of tagged-fibrinogen studies. This initial period was variable and may be related to the technic of hepatectomy. Rough handling of the liver, excessive hemorrhage, prolonged or repeated portal occlusions or failure to prevent pooling or release of substances from ischemic viscera into the systemic circulation may greatly foreshorten the initial phase, so that all that may be detected is the rapid secondary phase of the decrease in fibrinogen level. This rapid phase, which probably would occur in dogs surviving long enough, seems to accompany the onset of shock and acidosis, a feature noted by Markowitz[15] and Starzl[19] to be a late sequel in hepatectomized dogs.

That the rapid phase was associated with the time of onset of shock, was accompanied by a decrease in clotting and did not occur in hepatectomized dogs treated with heparin suggest that this secondary acceleration of fibrinogen disappearance is due to superimposition of increased intravascular coagulation on the usual rate of fibrinogen consumption.

Though this study did not show that increased fibrinolytic activity, known to occur after hepatectomy,[2, 5] does not contribute significantly to the rapid disappearance of fibrinogen during this secondary phase, other indirect evidence suggests this to be unlikely. Gans[5] demonstrated an increase in fibrinolytic activity after hepatectomy and yet found that epsilon amino caproic acid (EACA) did not significantly modify the observed rate of fall of fibrino-

gen levels. Bono *et al.*[2] showed that a prompt marked increase in fibrinolytic activity, probably due to excluding fibrinolysin inhibitors of hepatic origin, occurred within 10 minutes of bypassing the liver. One would thus expect this factor to be operative immediately after hepatectomy and long before the accelerated phase of fibrinogen decline began. One problem in interpreting studies of fibrinolytic activity is that there is no direct relationship to fibrinogen levels. Hunter[10] proposed a test in which duplicate fibrinogen assays are incubated for 1 hour at 37° C. and EACA is added to one of the specimens. Studies using this method are in progress.

Of interest is failure of eviscerated dogs to demonstrate late accelerated decrease in fibrinogen level even though the dogs survived longer than those which were hepatectomized and also developed shock and acidosis. This suggests that substances released into portal blood may contribute to increased coagulation seen in hemorrhagic shock in dogs, as suggested by Gans.

Finally, we do not imply that estimates of the initial rate of decrease after hepatectomy are *precisely* the natural decay rate of fibinogen, but rather an approximation; the true rate of decay is in this general range, and comparable with estimates of biologic half-life by fibrinogen-tagging experiments. Nor can we prove that the transition to the accelerated phase is abrupt, for more frequent determinations might show a curve in the slope at this point. In addition, we do not hold that increased fibrinolytic activity does not contribute to this terminal acceleration, but rather that its contribution to the decrease in fibrinogen level is a minor one as is that from lack of new fibrinogen formation.

Summary

An initial gradual decrease in fibrinogen level was observed after one-stage total hepatectomy in 17 dogs, which, unlike previous observations by this method, suggests a gradual "natural" decay rate for fibrinogen, agreeing with estimates derived from the equilibration phase of isotope studies.

A secondary, approximately tenfold acceleration in rate of fibrinogen disappearance began after a variable period in most untreated dogs, particularly in longer survivors.

This rapid terminal decrease in fibrinogen level was related to time of onset of shock and was associated with similar decreases in other measurements of clotting factors.

No accelerated phase was observed in seven dogs given intravenous heparin after hepatectomy, nor in four dogs which were totally eviscerated.

The implications of these findings are discussed.

Acknowledgment

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