THE LIVER AS THE SOURCE OF FIBRINOGEN

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The present work embodies studies on the problem of fibrin regeneration in rabbits deprived of the entire liver (1).

In the past the formation of fibrinogen has been ascribed to various organs. Claude Bernard in 1848 (2), Lehmann (3), and Brown-Séquard (4) believed they had found the blood of the mesenteric veins rich in the substance and the content of the hepatic and renal veins relatively poor. Years later these findings seemed to be confirmed by the work of Dastre (5–8). As result the liver was credited with the function of fibrinogen destruction while certain other organs, particularly the intestine, were looked upon as its chief site of origin. By others, in the meantime, the lungs and skin (6), the bone marrow (9, 10), and leucocytes (11) have been thought to form it. The more important of these views will be considered further on in connection with our findings.

In 1905 Doyon (12, 13) and his associates found that extensive degenerative changes in the liver were accompanied by a fall in the blood fibrinogen content of dogs poisoned by chloroform and phosphorus. They suggested the liver as a probable source of fibrinogen. In the last quarter of a century this view has steadily gained acceptance. Nearly all workers on the question of the origin of fibrinogen are now agreed that the liver is its chief source. Whipple and Hurwitz (14) have determined a striking correspondence between the extent of liver damage and the decrease of blood fibrinogen in dogs poisoned by chloroform and phosphorus, and they have suggested an hepatic origin of this blood protein. Goodpasture (15), studying fibrinogen regeneration in dog's blood, agreed with this view in part but concluded that the intestines act as a controlling if unessential factor in fibrinogen formation. More will be said of this view further on.

It remained for Meek (16) to demonstrate clearly a rôle for the liver in fibrinogen formation. This author proved that the substance is regenerated in the Eck fistula dog. But if ligation of the hepatic artery was practiced in addition and the occlusion of the portal vein made close to the liver after formation of the Eck fistula, no regeneration whatever occurred. Indeed there followed a depletion of the amount already present in the blood.

In a series of studies on fibrin metabolism in the dog, under normal and pathological conditions, Foster and Whipple (17–20) have recently brought further

evidence to show that the liver is the chief if not the sole source of fibrinogen formation, a view strengthened by the work of Schultz, Nicholes and Schaefer (21).

From the foregoing it is clear that the studies of recent years have been converging upon the liver as the source of fibrinogen. But certain cogent objections have prevented a definite conclusion in the matter. Much of the evidence for a liver origin of fibrinogen has been obtained by injuring the organ with chloroform, phosphorus or carbon tetra chloride. To accept this evidence one must assume that the drugs act only upon the liver, not elsewhere in the body. Williamson and Mann's (22) observations upon hepatectomized animals have clearly shown that this is not the case.

It has seemed wise to us to study again the problem of fibrin regeneration in liverless animals not subjected to the effects of poisons and not suffering from circulatory obstruction to organs other than the liver (1).

Fibrinogen Regeneration in the Normal Rabbit

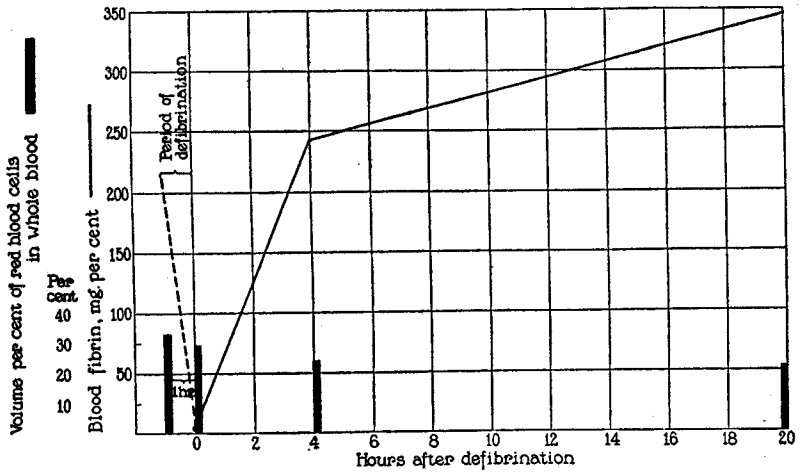
The span of life of the hepatectomized rabbit is short, on the average 24 to 30 hours (1, 23). Before proceeding to studies on the liverless animal it became necessary to estimate the rate and extent of fibrinogen regeneration during such a period in the normal animal.

Method.—Subtotal defibrination was carried out in the classical manner in four normal rabbits of about 2 kilograms each. Under ether anesthesia, cannulae were placed in the left carotid artery and jugular vein, and a sample of blood withdrawn for fibrin determination. A supply of blood roughly equivalent to the animal's estimated blood volume, already obtained from donor rabbits, was defibrinated after cross agglutination tests had ensured its compatibility with that of the experimental animal. The defibrinated blood was slowly injected through the cannula inserted in the jugular vein while at the same time an equal volume of arterial blood was removed from the carotid. This, defibrinated in turn, was reinjected into the animal during the removal of another equivalent volume of blood. Five or six repetitions of the procedure reduced the circulating fibrinogen to less than 5 per cent of its original amount. Blood specimens taken at intervals thereafter and examined for fibrinogen content disclosed the rate of its regeneration.

Using duplicate specimens, fibrinogen was estimated in the form of fibrin by the method of Foster and Whipple (17), as modified by Schultz, Nicholes and Schaefer (21). To ensure the complete precipitation of fibrin by the presence of ample thrombin, controls were run, as routine, to which fresh serum had been added. These controls showed no increased amounts of fibrin.

In normal rabbits fibrinogen regeneration was exceedingly rapid. Within five or six hours after a 90 per cent reduction of the substance

in the circulating blood a complete return to the previous amount was observed. Text-figs. 1 and 2 depict the findings in two of the four experiments. In the first one the blood fibrinogen had returned to the normal amount in less than 4 hours after total defibrination and 20 hours later had exceeded this by approximately 50 per cent. In the second instance a slightly less pronounced rise in blood fibrinogen during the first 5 hours was followed by a progressive increase to more than the normal amount in 21 hours and to double the original amount



TEXT-FIG. 1

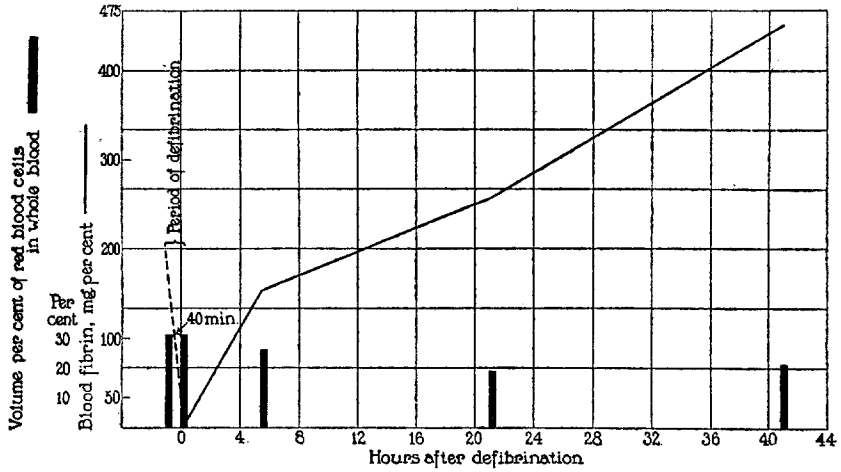
by the second day. The findings in the remaining two experiments were comparable to these in every way.

Influence of the Absence of the Liver on Fibrinogen Regeneration

The findings were wholly different in the liverless rabbit. Instead of a rapid fibrinogen regeneration, after partial defibrination, a speedy decrease in the small amount still remaining was the invariable rule.

Under ether anesthesia 10 rabbits of about 2 kilos body weight were subjected to a modified Markowitz operation (24) for inducing a collateral circulation about the liver, as already described by one of us (1). For two months thereafter they were kept on a full mixed diet on which they thrived, as evidenced by a gain in body weight. Then, after a fast of 48–72 hours, hepatectomy (1) was performed under ether and cannulae were placed in the left carotid artery and jugular vein.

In all the experiments blood specimens were drawn from the cannula placed in the carotid artery 10–15 minutes after hepatectomy. Partial defibrination of the circulating blood was next accomplished by the method described in the experiments on normal animals, save that it was not carried quite do far. The procedure accomplished a reduction of approximately 50–70 per cent of the circulating fibrinogen. Ten to fifteen minutes after completion of the partial defibrination another blood specimen was invariably taken. Further specimens were obtained at various time intervals in the different instances, as the charts



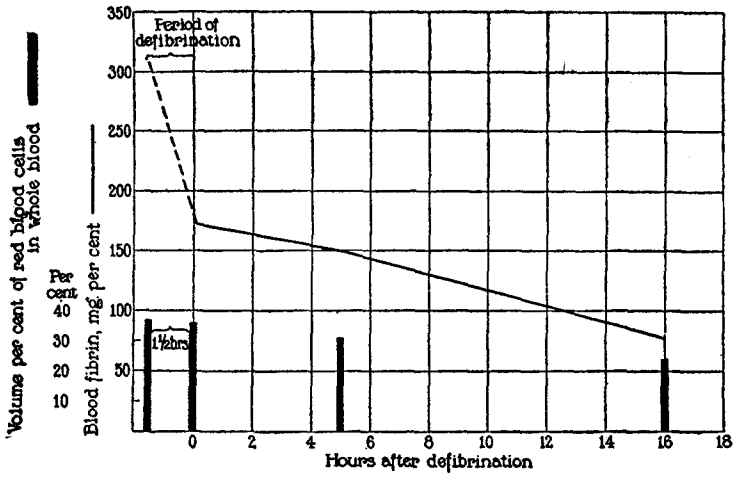
TEXT-FIG. 2

The Rapid Rate of Fibrinogen Regeneration in the Normal Rabbit

Text-figures 1 and 2 depict the rate of fibrinogen regeneration in the normal rabbit after almost complete defibrination of the circulating blood, as described in the text. It was exceedingly rapid. The line represents the concentration of fibrinogen in the blood after defibrination; the dark columns indicate the volume per cent of red blood cells in whole blood.

show, and the fibrin determinations carried out as in the preceding experiments. In the specimens taken after defibrination, controls were again run to which serum was added to make up for a possible thrombin lack. This addition did not appreciably increase the amount of fibrin clot obtained, showing that the fall in blood fibrinogen encountered was due to a lack of this protein and not to a fibrin ferment (thrombin) deficiency.

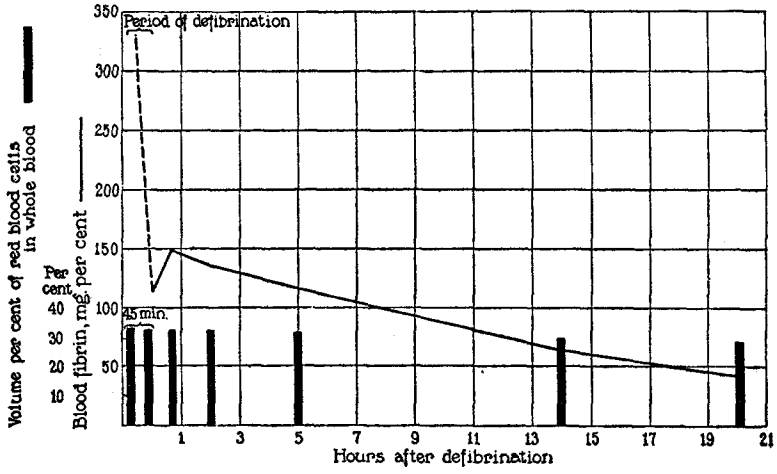
No evidences of fibrinogen regeneration appeared after hepatectomy in any of the experiments. Three typical examples, charted in



TEXT-FIG. 3

Failure of Fibrinogen Regeneration in the Hepatectomized Rabbit

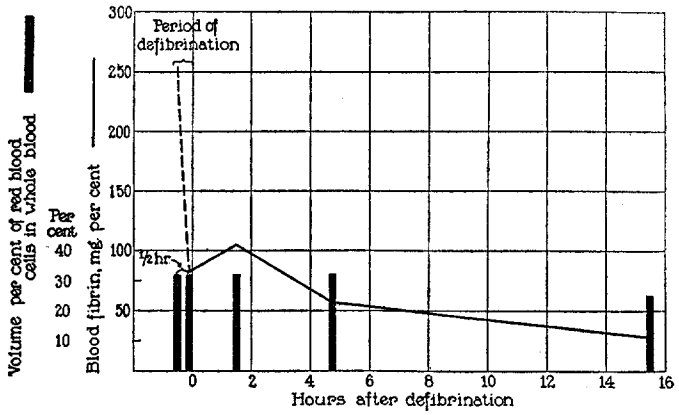
In Text-figures 3, 4 and 5 are charted the changes in blood fibrinogen of hepatectomized rabbits after partial defibrination of the circulating blood. The curves, which are fully discussed in the text, demonstrate the failure of fibrinogen regeneration in the liverless rabbit.



TEXT-FIG. 4

Text-figs. 3 to 5, show this fact well. Not only was there no rapid new formation of fibrinogen, such as occurs in the normal rabbit, but a swift fall in the amount of the substance in the blood signified a rapid utilization of the fibrinogen remaining in the organism.

The curves plotted in the text-figures differ in some details but the general findings are the same, and the variations can be safely attributed to the different time intervals at which blood specimens were taken.



TEXT-FIG. 5

Blood Fibrinogen after Partial Defibrination in the Liverless Rabbit

In the experiment plotted in Text-fig. 3, the fibrinogen content of the blood apparently decreased progressively, for a third specimen taken 5 hours after defibrination showed slightly less fibrin than the preceding one, and a fourth removed 16 hours later contained but half as much. In later experiments blood specimens taken at shorter intervals after defibrination showed a transient rise in the quantity in the blood, a phenomenon indicative of a fibrin reserve within the body as already noted by Foster and Whipple (20). The rapidity with which this occurred (Text-fig. 4) suggested an inflow of the substance with the lymph. In this experiment the third blood sample was obtained 35 minutes after the second, and a fourth an hour later. The fourth contained less fibrinogen than the third and each subsequent

blood specimen contained progressively less. Three similar experiments not plotted in the text-figures yielded like findings, no increase in fibrinogen being observed in the blood of hepatectomized rabbits later than 45 minutes after defibrination, that is to say after a transient rise. It is to be noted in Text-fig. 4 that the concentration of blood fibrinogen 5 hours after defibrination was still slightly above the figure obtained immediately following this procedure, that is to say in the second blood specimen. In Text-fig. 5 the plotted findings of

TABLE I

Blood Fibrinogen Content and Percentage of Red Blood Cells in Rabbit's Blood before and after Hepatectomy

No.	Preoperative		Postoperative		Hours after operation
	Fibrinogen, mgs. per cent	*Volume per cent of red blood cells in whole blood*	*Fibrinogen, mgs. per cent*	*Volume per cent of red blood cells in whole blood*	
1	380	33	130	31	30
2	408	32	156	34	27
3	447	36	222	33	27
4	345	34	201	33	24
5	262	30	115	32	16
6	426	30	282	28	15
7	310	35	171	34	15
Control Experiment. Ablation of 70 Per Cent of the Liver					
1	246	34	239	35	24

another experiment show the amount of fibrinogen in the fourth blood specimen, taken 5 hours after defibrination, to be distinctly less than in the second specimen which was removed a few minutes after defibrination. Such an experiment, without the determination shortly after hepatectomy, would have yielded a curve like that given in Text-fig. 3.

The investigation of the source of the fibrinogen reserve has not been attempted. Unfortunately fibrin determinations require so much plasma that frequent sampling is precluded and one is unable to follow at short intervals the changes in concentration of this substance in the blood of any one animal.

Blood Fibrinogen after Hepatectomy without Defibrination

In seven hepatectomized rabbits, which were employed for other experiments which involved no other complicating factors, the blood fibrinogen was estimated prior to removal of the liver and again 15–30 hours later. The results summarized in Table I are consistent. In the absence of the liver the fibrinogen concentration of the circulating blood rapidly decreased in all these animals while the hematocrit readings showed no significant changes. The findings are included here as further evidence not only of the lack of fibrinogen regeneration in the hepatectomized organism but of its destruction.

In a single experiment, also summarized in Table I, blood fibrinogen estimations were made before removal of 70 per cent of the liver of a rabbit and again 24 hours afterwards. As is well known, this procedure fails to induce clinical signs of liver insufficiency in the rabbit (25, 26). The blood fibrinogen showed no decrease.

DISCUSSION

It is certain from our results that the liver is absolutely essential to the maintenance of the normal quantity of fibrinogen in the blood. But is the liver the only source of fibrinogen? This question has not been answered by these experiments, for they do not preclude the possibility of an inconsiderable extra hepatic formation of fibrinogen, one wholly insufficient to make up for its destruction in the course of normal events. An enormous over production of fibrinogen took place in the normal animals stimulated to regeneration of the substance by defibrination of the blood. Surely a similar activity should have been observed in liverless rabbits after defibrination had there been any other important source of fibrinogen besides the liver. And any considerable compensation should have prevented the speedy decrease of the substance in the blood.

Theories of Extra-Hepatic Fibrin Formation

In the past many theories of extra hepatic fibrin formation have been offered. A little will be said of these.

The work of Mathews (11) and others (15) has tended to show an important activity of the intestinal tract in the origin of fibrinogen. At the present time it is evident that the findings of these authors were due to functional derangement

of the liver and not to injury or removal of the intestine. The experiments (11) had involved surgical interference with the blood supply of the intestinal tract, by ligations of the coeliac axis and of the superior and inferior mesenteric arteries. In addition ablation of segments of the intestine, or total extirpation of the organ, was practiced. For example, in one experiment (11) removal of the intestine from pylorus to rectum led to a pronounced fall in blood fibrinogen. In another experiment involving partial removal of the intestine in a cat, the stomach, pancreas, spleen and a segment of small intestine were allowed to remain. The animal, surviving 5 hours, regenerated fibrin but only half as rapidly as the normal cat. Procedures such as these reduced the circulation through the liver considerably.

The finding of fibrinogen in the blood of the mesenteric veins in greater concentration than in the general circulation has repeatedly been stressed in the literature as evidence for an intestinal origin of the protein (2, 3, 4, 11). The figures presented by the advocates of this view show changes sufficiently small to be accounted for by alterations in the concentration of the blood. The findings lie well within the errors of the methods used.

Müller (9) working with infections in guinea pigs found an increase in the fibrinogen content of the marrow in these animals and concluded that the marrow furnished this protein. In the light of more recent pathological knowledge these conclusions seem unwarranted. It is now well known from the work of Foster and Whipple and others (20, 11) that almost any infection or inflammation in the body leads to fibrinogen increase in the blood and accumulations of fibrin in various locations. Morawitz and Rehn (10) after withdrawing blood, defibrinating and reinjecting it into animals found myelocytic proliferation in the marrow and spleen and believed that this activity showed that fibrin deficiency was being made up in these regions. It is now recognized that red blood cells become fragmented and partially hemolyzed by the process of removal and defibrination. The myelocytic proliferation may have been a secondary consequence of this destruction.

One further point deserves mention. The experiments here described serve well to demonstrate the rapid rate of fibrinogen utilization, a phenomenon already emphasized by Foster and Whipple (19). It indicates an important function of the protein as yet unknown but worthy of investigation. As our experiments have indicated, absence of the liver brings about a true fibrinogen lack but no deficiency in thrombin (fibrin ferment), for an excess of the latter when added to the blood gave rise to no further coagulum of fibrin.

SUMMARY

In hepatectomized rabbits a progressive decrease in blood fibrinogen occurs. Partial defibrination in the liverless rabbit invariably

results in a progressive decrease in blood fibrinogen preceded by a temporary and slight rise. No evidence has been secured of fibrinogen regeneration in the absence of the liver. From this it follows that the liver is the essential source of fibrinogen and in all probability the sole one.

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