others showed that the plasma clotting times with these agents are absolute and depend only on the prothrombin concentration when it becomes limiting.

Summary. Statistical evidence is presented for a relationship between the coagulation time of recalcified plasma and the coagulation time accelerated by the addition of a thromboplastic lipid. This relationship is thought to imply that the thromboplastic substance in plasma responsible for the clotting of recalcified plasma has the same physiological characteristics as the lipid thromboplastic agent and that both require for activity some limiting factor present in plasma.

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Effect on Prothrombin of Acute Massive Plasmapheresis with Simultaneous Chloroform Intoxication. (17330)

JOHN R. CARTER, GEORGE H. CHAMBERS, AND E. D. WARNER.
From the Department of Pathology, College of Medicine, The State University of Iowa, Iowa City, Iowa.

From the earliest work on blood clotting, plasma prothrombin has been recognized as an integral component in the mechanism of thrombin formation. The effects on prothrombin of diverse pathologic and physiologic states have been studied extensively in the clinic and laboratory. Because prothrombin is so intimately related to liver function, an appreciable understanding of the origin, mode of action, and utilization of prothrombin may be acquired from subjecting the liver to various abnormal chemical and physical conditions. Experimental evidence and clinical experience with certain forms of liver and biliary tract disease indicate that plasma prothrombin is produced through the vital activity of the liver.[1-7] Thus experimentally induced hepatic necrosis,[1,2] and partial[3] or complete hepatectomy[4,5] result in hypoprothrombinemia.

Many factors, in addition to the fundamental integrity of the liver, are known to operate in the maintenance of plasma prothrombin. An adequate intake of Vitamin K is essential. Certain drugs, notably dicumarol, cause hypoprothrombinemia without any morphologic evidence of damage to liver tissue. Yet very little is known about the mechanism by which prothrombin is produced and utilized in the body.

There is considerable evidence to suggest that plasma prothrombin will gradually disappear over a period of 1-3 days if the production of new prothrombin is impaired. Thus the minimum prothrombin concentration is reached in 36-48 hours following severe chloroform poisoning,[1,2] or partial hepatectomy.[3] If animals are rendered hypoprothrombinemic as a result of Vitamin K deficiency,[8] or dicumarol poisoning,[9] transfusions with normal blood result in a temporary elevation of prothrombin concentration. The effect of transfusions lasts only 1-2 days. Purified bovine prothrombin administered intravenously lasts 2-3 days in a dog made hypoprothrombinemic with dicumarol.[10] On the other hand, when prothrombin production is impaired, the tendency of plasma prothrombin concentration

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to stabilize at a subnormal level, rather than to continue to decrease, suggests a decrease in the rate of prothrombin utilization. Thus, with some types of liver disease, with continuous dicumarol administration, or with a continuous but inadequate Vitamin K intake, the prothrombin concentration tends to reach an equilibrium. Were the utilization rate constant, it would seem that the decline resulting from inadequate production should continue. Also, the rapid fall of prothrombin concentration following total hepatectomy[4,5] suggests that prothrombin "turn over" may be more rapid than indicated by experiments which impair production.

The purpose of the experiments reported in this paper was to determine the effect of massive plasmapheresis on the disappearance rate of prothrombin from the blood stream after severe chloroform intoxication, and to correlate the changes in prothrombin concentration with those of the total protein content of the plasma in dogs.

The data resulting from the present experiments entirely confirm previous findings[1-7] relative to the essential role of the liver in the manufacture of prothrombin. In addition, the experiments indicate that 1) no significant reserve stores of prothrombin exist, 2) when the prothrombin production rate is decreased, there is a concomitant decrease in the rate of its utilization, and 3) following chloroform administration, the impairment of the prothrombin producing capacity of the liver develops gradually over a period of 1-2 days.

Methods. Adult mongrel dogs, weighing from 5.3-12.9 kg, were used in all experiments. No attempt was made to control the diets rigidly, nor was any correlation between the diet and experimental observations noted. The low protein, high carbohydrate diet consisted of hospital scraps which contained bread, primarily, with very little meat. The stock diet was composed of hospital scraps supplemented with purina dog chow. The high protein diet consisted of meat and dog chow only. Donor blood was obtained from dogs maintained on a stock diet. Following removal of the plasma by centrifugation, the cells were washed twice with, and subsequently resuspended in, Locke's solution. No col-

TABLE I. Analysis on Data on Dogs Subjected to Plasmapheresis and/or Chloroform Intoxication.

Dog	45-1	45-2	45-3	45-4a	45-2	45-4b	45-8	45-9	45-10a	45-4c	45-10b	45-14	45-9	45-16
Type of experiment	P&C*	P&C	C	C	P	P&C	P&C	P&C	P&C	P	P	P	P&C	P&C
Hours of fasting before $CHCl_3$ and/or pheresis	0	30	50	50	0	50	72	50	50	50	0	50	50	50
% calculated blood volume removed by pheresis	102	65	—	—	69	73	84	95	95	87	87	78	105	69
% plasma proteins removed by pheresis	50	36	—	—	35	51	34	55	54	37	35	45	—	—
% prothrombin removed by pheresis	69	52	—	—	51	55	48	78	94	73	78	71	86	64
Condition of dog after pheresis and/or $CHCl_3$	Death 2½ hr	Shock	Good	Good	Good	Shock	Shock	Shock	Shock	Shock	Good	Good	Shock	Good
Complete recovery of prothrombin (days)	—	18	8	8	1	10	8	6	6	2	2	2	7	6

* P = Plasmapheresis. C = Chloroform.

loid substances were supplemented. A period of starvation preceded the chloroform anesthesia. (Table I.) Anesthesia was induced first with ether. The dogs subjected to chloroform and plasmapheresis were given deep chloroform anesthesia for from 75-100 minutes. Under ether anesthesia, immediately following chloroform, the femoral vessels were exposed and cannulated. The bottle containing warmed donor blood cell suspension was attached to an air pressure system so that the transfused blood entered the vein at virtually the same rate as blood was removed from the artery. Throughout the actual exchange, equal volumes of recipient and donor blood were constantly maintained. The rate of exchange was not allowed to exceed 100 cc per minute. The fraction of the calculated blood volume removed is indicated in per cent in Table I. The calculated blood volume was based on 90 cc per kg of body weight. Naturally, the blood removed during the pheresis procedure represents an admixture of the recipient's own blood and donor cells. The percentage of prothrombin and plasma proteins removed by the pheresis procedure was determined by calculation of differences of the levels before and immediately after plasmapheresis. Blood for the analyses was drawn from the jugular vein into a syringe wet with oxalate solution, and gently expressed into an 8 cc hematocrit tube containing 1.85% potassium oxalate. Prothrombin determinations were done by the two-stage method of Warner and Smith.[1,2] Crude lung extract which is rather rich in accelerator factor(s) was used as the source of thromboplastin. Protein values were determined by the semi-micro Kjeldahl method using the Parnas-Wagner distillation apparatus.

Experimental Observations. Prothrombin, total plasma protein, and hematocrit values in a typical experimental dog (45-4a) following prolonged chloroform anesthesia are recorded graphically in Fig. 1. The fall of prothrombin is extreme, the lowest level occurring at 40-48 hours. It is at this time that the icteric index is maximal, although the first appearance of icteric plasma is usually noted several hours earlier. Depending upon the depth and length of chloroform anesthesia, and the degree of relative anoxia, the prothrombin concentration at the end of 48 hours may be very low. There is a concomitant elevation of hematocrit values indicating moderate hemoconcentration. Prothrombin recovery begins promptly on the third day and is complete by the eighth day. This recovery coincides with liver repair which begins in about 48 hours and is almost complete in 5-6 days. It is noteworthy that the plasma protein values fall moderately during the period of maximal liver regeneration and prothrombin recovery. This phenomenon was repeatedly observed, and its possible significance will be discussed later.

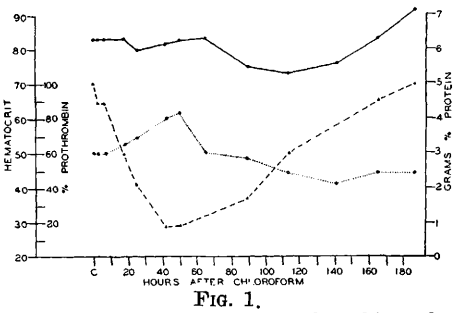

FIG. 1.

Effect of chloroform on prothrombin, plasma protein, and hematocrit values.

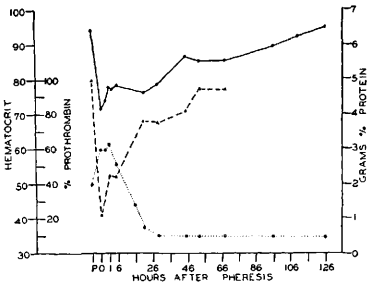

FIG. 2.

Effect of plasmapheresis on prothrombin, plasma protein, and hematocrit values.

Fig. 2 shows the prothrombin, total plasma protein, and hematocrit values of a typical experimental dog (45-10b) following acute massive plasmapheresis. The precipitous fall of prothrombin and plasma proteins is roughly proportional to the amount of blood removed. At such low prothrombin levels, bleeding from venapuncture wounds frequently occurred; no spontaneous bleeding was observed, however. The recovery curve of prothrombin follows a rather characteristic

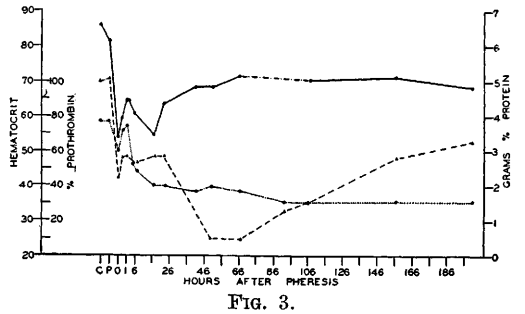

FIG. 3.

Effect of chloroform and plasmapheresis on prothrombin, plasma protein, and hematocrit values.

pattern, with a return to about 75% of normal in from 16 to 20 hours following plasmapheresis. By the end of 50 hours, prothrombin values have returned to normal. The recovery rate of the total proteins is more prolonged. It will be noted that after an abrupt rise of approximately 20% above the minimum value, the plasma proteins remain relatively constant for a period of about 26 hours, while during the same period, the prothrombin recovery rate is maximal. From 26-46 hours, the recovery rates of both prothrombin and plasma proteins are virtually the same, although for the latter, this period marks the maximal recovery rate. At the end of 116 hours, the plasma protein level has returned to normal. The sharp rise followed by the abrupt fall of the hematocrit is adequately explained by hemoconcentration followed by relative anemia.

When dogs are subjected to both acute massive plasmapheresis and chloroform intoxication, the graphical result is similar in several respects to a superimposition of the chloroform curve upon the pheresis curve. There are certain significant differences, however. Fig. 3 shows the results of a typical experiment of this type (dog 45-4b). The fall of prothrombin concentration is abrupt following plasmapheresis. This is followed by an exceedingly rapid increase in prothrombin values, probably due to hemoconcentration initially. The prothrombin concentration is maintained at this elevated level for a period of approximately 20 hours. This is in sharp contrast to the very transitory elevation of plasma protein and hematocrit values. Numerous attempts were made to alter the character of this segment of the prothrombin curve, but regardless of diet, the quantity of exchanged blood, or the degree of chloroform intoxication, a postpheresis plateau invariably was obtained. At about 26 hours, prothrombin values again begin to fall and continue to do so for an additional 20 hours. If one graphically compares the decrease in prothrombin concentration after 26 hours with that of the comparable period in dogs subjected to chloroform intoxication only, it is observed that the curves are similar. This indicates that the termination of the postpheresis plateau and the subsequent decline in prothrombin concentration is the result of liver damage initiated some 30 hours previously by chloroform. As in the case of chloroform alone, the minimum prothrombin concentration is reached by the end of 48 hours. This is followed by a slow, but progressive recovery period.

The first segment of the plasma protein curve (Fig. 3) follows the pattern of the prothrombin curve rather closely. The rapid fall of plasma proteins parallels the fall of prothrombin. In every instance, the edema level was reached or exceeded, but, probably because this was only transitory, no clinical edema was noted. The postpheresis recovery curves of plasma protein values were quite variable, but generally speaking, there was a transitory rise with a secondary fall comparable to that of the hematocrit values. In the experiment shown in Fig. 3, concomitant with the fall of prothrombin, incident to the hepatotoxic effect of chloroform, plasma protein values gradually rose from 3.5 g% to 5.2 g%. This level was maintained for approximately 80 hours, during which time prothrombin recovery was most rapid. The general pattern of the plasma protein curve showed considerable variation in different animals, however. In some animals, there was a definite but minimal fall of plasma proteins during the period of maximal liver injury. Often, an additional drop in plasma proteins was observed during maximal prothrombin recovery. In other animals, plasma protein recovery paralleled prothrombin recovery. The

hematocrit values frequently showed a slight decrease immediately following plasmapheresis. A transitory rise, presumably due to hemoconcentration, then occurred in all of the dogs. This rise lasted only one to 2 hours in contrast to the postpheresis rise in prothrombin values, however. Considerable hemolysis was unavoidable in the massive pheresis procedure so that even though the hematocrit values of donor cell suspension and recipient blood were virtually the same, many of the washed red blood cells were hemolyzed. The result was a moderate anemia. The hematocrit values leveled off at from 30 to 40% and gradually returned to normal after 2-3 weeks.

Discussion. The profound fall in plasma prothrombin concentration in dogs having been subjected to chloroform intoxication confirms the previous work of Warner, Smith, and Brinkhous.[1,2] The evidence indicates that the liver is the source of prothrombin production, since the fall of prothrombin concentration rather closely parallels the degree of liver injury, and the prothrombin returns to normal as the liver regenerates.

Kerr, Hurwitz, and Whipple,[11] showed that severe liver injury in dogs by means of phosphorous or chloroform intoxication will cause, or at least be associated with, a moderate fall in blood plasma proteins. With 1½ hours of chloroform anesthesia, the fall began on the second day and progressed for the next 3 or 4 days during which period liver repair was most active. With comparable liver injury, our data (Fig. 1) revealed a similar plasma protein response, the minimal level occurring at about 4½ days. For almost 3 days following the administration of chloroform, during which period the prothrombin concentration had reached its lowest level and had begun to return toward normal values, the plasma proteins had remained relatively stable at a normal level. This lag period, occurring concomitantly with that of the greatest liver injury, could be explained by the presence of reserve stores of plasma proteins, if one assumes that the liver is the source of albumin and globulin and that the rate of utilization remains constant at a normal level. If this assumption is valid, there must be some decrease, if not a total arrest, in the rate of plasma protein production resulting from liver injury. The lag period might also be taken as evidence, although inconclusive, that tissues other than liver fabricate albumin and globulin. Nevertheless, the extensive work of Whipple, Madden *et al.* strongly favors the liver as the source of the plasma proteins.[12] There are at least two other possible explanations for the decline of plasma proteins during the period of liver repair and maximal prothrombin regeneration. A smooth line curve of the plotted data accentuates the fact that the decline of plasma proteins coincides with an increase in prothrombin concentration, thereby suggesting a relationship between the two. Although prothrombin is only a very small fraction of the total proteins, it is conceivable that in order for prothrombin to be fabricated rapidly following liver injury, relatively large amounts of plasma proteins are diverted from the circulation to provide a pool from which the prothrombin molecule is constructed. Or, it is possible that the circulating proteins are called upon to supply the amino acid moiety necessary to fabricate liver protein itself, since the fall in plasma proteins occurs during the period of regeneration of liver tissue.

The rapid removal of as much as 50% of the circulating prothrombin results in the expected abrupt reduction of the level in the blood. On the basis of evidence cited above, that prothrombin in the blood lasts 1-3 days,[1-5,8-10] the removal of such amounts of prothrombin in animals subjected to chloroform intoxication would be expected to result in a conspicuously lower prothrombin level throughout the remainder of the experimental period. On the contrary, plasmapheresis does not appear to increase the severity of the hypoprothrombinemia, nor does it appear to alter the rate of prothrombin regeneration during the recovery period. The postpheresis plateau in chloroformed dogs could be explained on

[11] Kerr, W. J., Hurwitz, S. H., and Whipple, G. H., *Am. J. Physiol.*, 1918, **47**, 379.

[12] Madden, S. C., and Whipple, G. H., *Physiol. Rev.*, 1940, **20**, 194.

the basis of available prothrombin from reserve stores. Were this the case, however, once the reserves were exhausted, the prothrombin concentration would be expected to fall rapidly to a point below that of the chloroformed animals not subjected to plasmapheresis. Also, the rate of disappearance of prothrombin following total hepatectomy suggests that no significant stores exist. Further, with massive plasmapheresis in the normal animal, the fall in prothrombin concentration approximates the expected decrease from the amount of blood exchanged, and the rate of recovery is comparable to that seen in chronic Vitamin K deficiency following administration of specific therapy. Were appreciable quantities of prothrombin present in stores, it would be expected that the fall would be less and the recovery rate more rapid in the acute pheresis animal.

The relative ineffectiveness of massive plasmapheresis to materially alter the prothrombin level beyond 24-36 hours, despite greatly impaired liver function, could be explained on the basis of a very rapid turnover of the plasma prothrombin. Thus, if under normal conditions, the prothrombin of the blood was replaced several times per hour, the amount removed by plasmapheresis would become relatively insignificant. However, such rapidity of turnover does not seem compatible with the rate of fall of prothrombin following total hepatectomy.[4,5]

Evidence now available suggests that for any given level of hepatic function relative to prothrombin production, a balance is established between the rate of utilization and rate of formation. As a result, the plasma prothrombin becomes stabilized at a subnormal level, the height of which is determined more by productive capacity, than by any semi-constant rate of utilization. This is in accord with the tendency for the plasma prothrombin to become stabilized at a subnormal level when animals are maintained on a low Vitamin K or continuous dicumarol intake.

Our observations would strongly indicate, therefore, that no significant reserve stores of prothrombin exist, but rather that a nice balance between prothrombin production and utilization best explains the experimental findings. Indeed, even the postpheresis plateau is probably an artefact, since if we compare the fluctuation of plasma protein and prothrombin values with those of the hematocrit, it will be seen that fluctuations of plasma volume might well result in an apparent increase per unit volume of plasma proteins and prothrombin.

Calculations involving total grams of plasma proteins and total units of prothrombin, respectively, 1) before plasmapheresis, 2) actually removed, and 3) remaining in the circulation, 1-10 minutes after pheresis, were made. The respective values were corrected for oxalate and hematocrit. The results were in accord with the fact that reserve stores of plasma protein exist, but indicated that no significant, if any, reserve stores of prothrombin are present. Since no total plasma volume determinations were performed, however, the calculations are inconclusive. Wide fluctuations in total plasma volume could readily distort the total values of prothrombin and plasma proteins.

From the experimental data, it can also be concluded that hepatotoxic damage with the concomitant decrease in prothrombin concentration incident to chloroform intoxication, is a progressive phenomenon which develops gradually over a period of 1-2 days. If the liver were completely functionless after the first hour of chloroform, the postpheresis plateau would then be impossible. Rather the prothrombin would continue to fall at a rate determined by utilization alone.

Summary. Dogs subjected to both chloroform intoxication and acute massive plasmapheresis reveal changes in prothrombin concentration which rather conclusively indicate that no significant reserve stores of prothrombin exist. The stabilization of prothrombin at subnormal levels, as well as the rate of recovery of prothrombin, represents, instead, a balanced equilibrium between production and utilization. The data also substantiate the impression that hepatotoxic injury, with a concomitant decrease in prothrombin concentration, is a gradual progressive phenomenon.

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