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Scand. J. Haemat. (1966) **3**: 59–75

Consumption of Serum Factors and Prothrombin During Intravascular Clotting in Rabbits

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We have studied the effect of experimental intravascular clotting upon Factors II, VII, IX, and X in rabbits. Animals were given sodium warfarin intravenously to block the synthesis of these factors and, 4 hours later, were infused with either dilute tissue thromboplastin or saline. The tissue thromboplastin induced intravascular clotting extensive enough to halve fibrinogen and platelet levels and to reduce markedly Factor V and Factor VIII levels. These changes resulted from a consumption during clotting of about 10 per cent of the available prothrombin.

Each of the serum factors fell further during the infusion of tissue thromboplastin than during the infusion of saline; apparently, serum factors activated during clotting do not circulate in the rabbit. Net losses due to intravascular clotting equalled about 15 to 30 per cent of the Factors VII, IX, and X initially present. These data suggest that consumption during intravascular clotting may account for the low levels of Factors VII, IX, and X described in patients with diffuse intravascular clotting complicating septicaemia.

The evidence of consumption of Factor IX raises the possibility that tissue thromboplastin may activate *intrinsic* clotting during hemostasis *in vivo*.

When blood clots in a glass tube, platelets, fibrinogen, prothrombin (Factor II), and Factors V and VIII are consumed. The level of Factor X changes very little, whereas Factor VII and IX levels appear to rise. Because they persist in serum, Factors VII, IX, and X are often called the serum factors.

Extensive clotting *in vivo* also produces thrombocytopenia and reduced

* Work done at Medical Department A, Rikshospitalet, Oslo, Norway during sabbatical leave supported by a special fellowship from the National Heart Institute, USPH.

Supported by grant HE–6128–04 from the National Heart Institute, USPH, by The Norwegian Research Council for Science and the Humanities, and by The Norwegian Council on Cardiovascular Disease.

levels of fibrinogen and Factors V and VIII; indeed, this combination of findings is considered the hallmark of diffuse intravascular clotting. What happens to the serum factors? Data are scanty and somewhat contradictory. Nilsen (1963) reported that Factor VII levels rose in two of four rabbits injected with placental thromboplastin. Goossens & Walcher (1957) stated that Factor VII fell in rabbits injected with thromboplastin, but they did not furnish data. Serum factors decreased after rabbits were injected twice with bacterial endotoxin (Kleinmaier *et al.* 1959), a procedure which also triggers intravascular clotting. Reduced levels of Factors VII, IX, and X have also been described in patients with massive intravascular clotting complicating septicaemia (Pfau *et al.* 1960, Lasch *et al.* 1961, Ratnoff & Nebehay 1962, Rapaport *et al.* 1964). However, these patients were extremely ill, and their low levels could have had multiple causes.

Because of this uncertainty, we have re-examined the effect of intravascular clotting upon Factors II, VII, IX, and X. Rabbits were given a large intravenous dose of sodium warfarin to block the synthesis of these factors. Four hours later, the animals were infused with either dilute tissue thromboplastin or saline. Clotting factors were measured before and at frequent intervals after the infusions. We hoped that the elimination of synthesis would make plasma levels more accurate and sensitive indicators of consumption, activation, and clearance of these factors during the episode of clotting.

MATERIALS

Citrate anticoagulant refers to a solution 0.06 M in sodium citrate and 0.04 M in citric acid. *Citrated diluting fluid* contains one part of this citrate anticoagulant plus five parts of isotonic saline solution. *The thromboplastin for the Factor VII assay* was made from rabbit brain by Owren's (1949) technique with care to free the tissue of blood. Other reagents are described in the references cited below with the assays.

METHODS

1. *Experimental protocol*

Female New Zealand rabbits, weighing 1.3 to 2.8 kg, were given 30 mg of sodium warfarin (Marevan, Nyegaard & Co., Oslo, Norway) intravenously. The first blood sample was taken 15 minutes later and the second sample 4 hours later. After the second sample an infusion was started containing *either* 70 ml of sterile isotonic saline (controls) *or* from 8 to 20 ml of rabbit tissue thromboplastin (Simplastin, Warner-Chilcott, Morris-Plains, New Jersey) suspended in sterile distilled water according to the manufacturer's instructions and then made up to a final volume of 70 ml in sterile isotonic saline. We gave the 70 ml into a marginal ear vein, attempting to obtain even flow over 30 minutes. The third blood sample was taken 5 minutes after the infusion. Five more blood samples were taken at hourly intervals thereafter, and the final sample was taken the next morning, about 24 hours after the injection of the warfarin.

TABLE I

Summary of experimental conditions

Except for the number of animals, the figures are means with ranges given in parenthesis

	Saline group	Thromboplastin group	
		Group 1	Group 2
Number	7	10	8
Weight (kg)	2.4 (2.2–2.7)	2.0 (1.3–2.6)	2.5 (2.3–2.8)
Inj. time (min.)	30 (20–55)	36 (20–73)	29 (21–34)
Thromboplastin			
ml	0	12.8 (8–20)	16
ml/min.*	0	0.43 (0.19–0.80)	0.57 (0.52–0.76)
ml/min. per kg*	0	0.21 (0.09–0.42)	0.23 (0.20–0.28)
Factor measured			
Prothrombin	+	+	+
Factor X	+	+	+
Factor VII	+	+	–
Factor IX	+	–	+

* Calculated as ml of undiluted Simplastin prepared according to the manufacturer's instructions. For the lot used in most of the animals, a 1:7 dilution (equivalent to 10 ml made up to a final volume of 70 ml) clotted rabbit plasma in 12 to 13 sec.

Seven animals were given saline and 18 animals were given thromboplastin. Ten animals (group 1) received various amounts of thromboplastin: two animals, 8 ml; four animals, 10 ml; two animals, 16 ml; and two animals, 20 ml. In a second group, each of eight animals received 16 ml of thromboplastin. Prothrombin and Factor X were measured in both groups; Factor VII was measured only in the first group and Factor IX only in the second group. Levels of fibrinogen and Factors V and VIII were also determined in the first group. Platelets were counted in two animals in the first group, in all animals in the second group, and in four additional animals given 16 ml of thromboplastin for another purpose. Experimental conditions are summarized in Table I.

2. *Preparation of test samples*

All blood was taken from ear veins. After shaving the ear, we rubbed it gently with gauze moistened with xylene to dilate the vessels. The ear was then dried with gauze,

and a thin layer of vasoline was applied to make a non-wettable surface. A number 21 gauge needle was quickly inserted into the vein to the end of the bevel and then withdrawn. The first drop of blood was discarded.

"Micro samples" of diluted whole blood were used for the test samples in all assays except fibrinogen. This was necessary to allow serial sampling with minimal blood loss. Two to four "micro samples" were taken at each bleeding. With a capillary pipette 0.05 ml of freely flowing blood was mixed with 0.45 ml of citrated diluting fluid in a 1 ml plastic vial. The samples were then frozen at − 20° C. All samples, except those taken at 24 hours, stood overnight before being thawed for the assay of Factors II, VII, IX, and X; they were stored for approximately 48 hours before being thawed for the assay of Factors V and VIII. The freezing and thawing lysed the red cells and gave an evenly hemolyzed sample that was used without further dilution in assays for Factors II, VII, VIII, and IX, and in most Factor X assays. It was diluted 1:2 in citrated diluting fluid in some Factor X assays and 1:5 in all Factor V assays. The dilution of plasma in the test sample was calculated from the microhaematocrit, which was determined each time samples were taken.

Larger amounts of blood for fibrinogen determinations were collected at the following times: 15 minutes, 4 hours, 5 minutes after the infusion, 6 hours and 24 hours. Rapidly flowing drops of blood were collected in a plastic tube marked at 1 ml and containing 0.1 ml of citrate anticoagulant. The plasma was removed after centrifugation and stored at − 20° C.

3. *Assay methods*

Prothrombin was measured by a modification of the method of Hjort *et al.* (1955). "Proconvertin reagent" (Hjort 1957) supplied Factor X; the reagents were incubated together for exactly 3 minutes at 37° C after the addition of the viper venom. *Factor VII* was assayed by the technique of Aas (1952) modified by the addition of adsorbed ox plasma as an added source of Factor V. To avoid activation of Factor VII (Rapaport *et al.* 1955), the diluted test sample was not exposed to a glass surface until its transfer to the clotting mixture. *Factor X* was determined by a modification of the method of Hougie (1962) in which cephalin (Rapaport *et al.* 1954) was used as the lipid; the incubation time from the addition of the viper venom was exactly 3 minutes. *Factor IX* was measured in a partial thromboplastin time assay in which 0.1 ml of Factor IX deficiency plasma, of cephalin 1:100 in 10 mg per ml kaolin suspension, and of the "micro sample" were incubated together for 8 minutes at 37° C and then recalcified with 0.1 ml calcium chloride solution. Rabbit plasma contains many times the Factor V and VIII activity of human plasma; these factors fell during intravascular clotting. For the experimental series, therefore, the assay was fortified with 0.1 ml adsorbed ox plasma as an added source of Factors V and VIII.

The accuracy of using diluted ear blood as the test sample in the above assays was checked in two ways. Three animals were given sodium warfarin for 3 days; disappearance curves for Factors II, VII, IX, and X obtained from assays of "micro samples" were very similar to disappearance curves obtained from assays of plasma prepared as described above for fibrinogen determinations. In three other rabbits, we compared the levels of Factors II, VII, and IX obtained in diluted ear blood, in plasma from ear blood, and in plasma from heart blood. The values did not differ significantly in fresh samples; after storage for 24 hours the values for Factor VII activity were slightly lower in the diluted ear blood samples.

Factor VIII was measured by a kaolin-activated partial thromboplastin time method (Rapaport *et al.* 1965).

Factor V was measured in the assay described by Borchgrevink *et al.* (1960). *Fibrinogen* was determined by the technique of Jacobsson (1955) as modified by Blombäck & Blombäck (1956) and by Godal (1961). Platelets were counted in duplicate by a slight modification of the technique of Brecher *et al.* (1953).

Clotting times were converted to per cent activity from mean dilution curves prepared from 5 to 10 dilution curves from normal rabbits.

4. *Statistical calculations*

Because the dilution curves were logarithmic plots, log per cent activity was used to calculate the mean disappearance curves of Figures 4–7 and for the means listed in Table III.

The mean loss during the infusion for each factor (see Table V) was calculated from the formula:

$$L = (\bar{x}_2 - \bar{x}_3)/\bar{x}_2 \times 100$$

where $\bar{x}_2$ is the mean pre-infusion level, $\bar{x}_3$ is the mean level 5 minutes after the infusion, and L is the loss in per cent of the pre-infusion value.

To evaluate the significance of differences between the mean loss for the saline group and for the thromboplastin group, we determined "t" from the equation:

$$t = \frac{\bar{L}_t - \bar{L}_s}{\sqrt{\frac{(S_t)^2}{N_t} + \frac{(S_s)^2}{N_s}}}$$

where $\bar{L}_t$ is the mean loss for the thromboplastin group, $\bar{L}_s$ is the mean loss for the saline group, S_t and S_s are the corresponding standard deviations, and N_t and N_s are the corresponding numbers of animals. Six degrees of freedom were used to obtain a p-value, because the control group contained seven animals. We considered a p-value of less than 0.05 as "significant".

RESULTS

Most of the animals tolerated the thromboplastin well, but two animals died. A 2.4 kg rabbit which was given 16 ml of Simplastin over 29 minutes (0.23 ml/min per kg) died just after the infusion; gross thrombi were found in the right heart and pulmonary vessels. A 1.9 kg rabbit which received 20 ml of Simplastin over 25 minutes (0.42 ml/min per kg) seemed to tolerate the infusion, but, shortly afterwards, its ears became cold and bloodless and its breathing became rapid and labored. It died 1 hour after the infusion; we found no thrombi or other reason for death on gross examination. Judged by the fall in fibrinogen (from 224 to 48 mg per cent) and in platelets (from 525,500 to 20,500 per μl), this rabbit had more extensive intravascular clotting than any other animal.

1. *The falls in fibrinogen, platelets, and Factors V and VIII induced by thromboplastin*

These factors were measured to assure ourselves that infusing diluted thromboplastin over 30 minutes would produce extensive intravascular coagulation. Figure 1 illustrates the clear contrast between the slight drop in fibrinogen in the animals infused with saline and the marked drop in the animals infused with thromboplastin. In the latter, about one-half of the circulating fibrinogen was consumed (mean plasma level before infusion, 235 mg per cent; mean plasma level after infusion, 120 mg per cent). Fibrinogen levels had already begun to rise in the samples taken 3 hours after the infusion. By the next morning, about 20 hours after the infusion, the mean level exceeded the mean initial level.

Figure 2 and Table II show that the mean platelet level fell to about one-third of the pre-infusion value during the infusion of thromboplastin. The counts rose slightly but significantly ($p < 0.05$) over the next 2 hours but then levelled off. The next morning, 20 hours after the infusion, the counts were not significantly higher than the 2-hour counts. We conclude, therefore, that the intravascular clotting was extensive enough to destroy at least one-half of the circulating platelets.

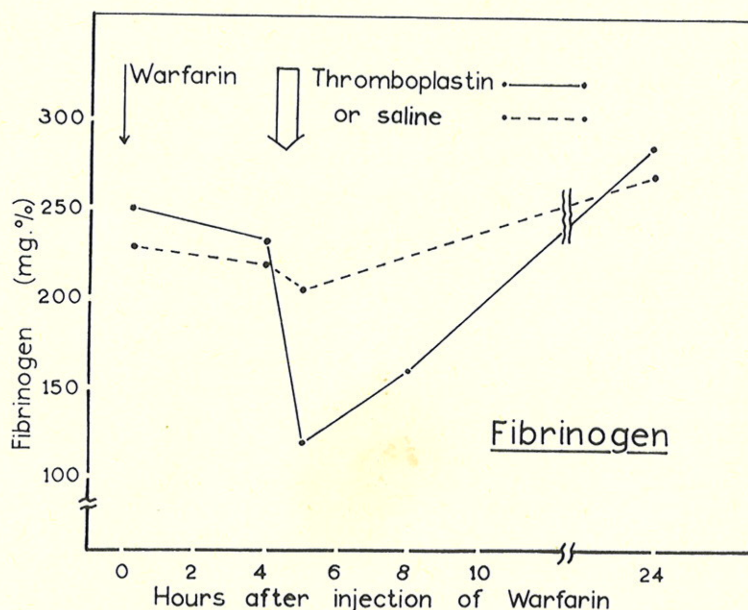

Figure 1. *The effect of the infusions upon fibrinogen.* The points on the control line represent means of 6 or 7 animals. The points on the experimental line represent means of 7 to 10 animals (only 5 animals for the 8 hour point).

TABLE II

The decrease in platelets after infusing thromboplastin

Time	No. of animals	Platelet count (x 1000 per μl)		Per cent of mean initial count
		Mean	SD	
Before tpl.				
4 hr.	14	603	113	100
5 min.	14	582	101	97
After tpl.				
5 min.	13	187	101	31
2 hr.	12	282	102	47
20 hr. (approx.)	12	312	156	52

Table III lists the mean levels for Factors V and VIII just before and just after the infusions. As expected, thromboplastin strikingly reduced Factors V and VIII. But we must point out that our technique of assaying Factors V

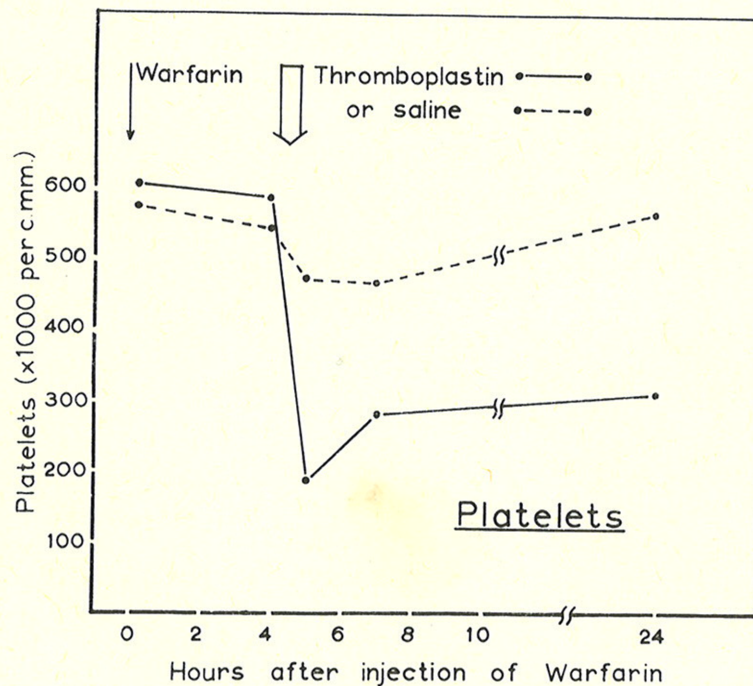

Figure 2. *The effect of the infusions upon platelets.* The points on the control line are means of 2 animals; the points on the experimental line are means of 12–14 animals.

TABLE III
Levels of Factors V and VIII before and after the infusions

Infusion	No. of animals	Time	Mean (per cent)	
			Factor V	Factor VIII
Saline	7	Before inf.	114	113
		After inf.	96	98
Thrombopl.	10	Before inf.	155	104
		After inf.	32	22

and VIII had two sources of error. First, we have recently discovered that diluted ear blood gives too high a value for Factor V (by the Russell's viper venom technique) and for Factor VIII, probably because these factors are activated by traces of thrombin formed either within the ear vessels or during sampling. Second, we now know that Factors V and VIII in diluted blood will deteriorate significantly during 2 days of storage, which was the interval most of these samples were stored before assay. Thus, in opposite directions and to unknown degrees, two errors influenced the accuracy of the values we obtained. Despite this, the differences between the control and thromboplastin group were so great that the infusions of thromboplastin must have produced a large drop in Factors V and VIII.

2. *The greater fall in haematocrit of the animals receiving thromboplastin*

As Figure 3 shows, beginning 1 hour after the infusions, we consistently found lower mean haematocrit levels in the animals given thromboplastin than in the animals given saline. Because of the haemostatic defects caused by intravascular clotting, the experimental animals lost more blood than the control animals during the collection of two – 1 ml samples for fibrinogen determinations just after the infusion. But this can not explain a mean difference of 2.9 per cent (control animals, 37.6 per cent; thromboplastin animals, 34.7 per cent) in the haematocrit of the two groups 1 hour later. A 2.3 kg rabbit with an estimated blood volume of 70 ml per kg would have to lose almost 13 ml of blood to drop its haematocrit this amount.

We can not explain the differences in the haematocrit, which, although small, were highly significant statistically. Neither microangiopathic changes in the red cells (Brain *et al.* 1962) nor gross evidence of haemolysis was noted.

3. *The falls in prothrombin and Factors VII, IX, and X induced by thromboplastin*

Mean disappearance curves for these factors in the control and experimental animals are given in Figures 4–7. In addition, Table IV lists the mean losses during the infusion for the two groups of animals.

a. *Prothrombin.* Because the mean amount of thromboplastin given per kg/min was almost the same in the two groups receiving thromboplastin (see Table I), we combined the data from all 18 experimental animals. As Table IV shows, and as expected, the animals given thromboplastin lost more prothrombin during the infusion than the control animals. However, the difference between the mean loss of activity for the two groups was not statistically significant. In one control animal we obtained a value of 41 per cent activity lost during the infusion. This figure, obviously in error, had to be included in calculating the mean and standard deviation for the control group. Had it been omitted, the mean for the controls would have dropped to 7.8 per cent, with a standard deviation of 4.5 per cent, and the difference

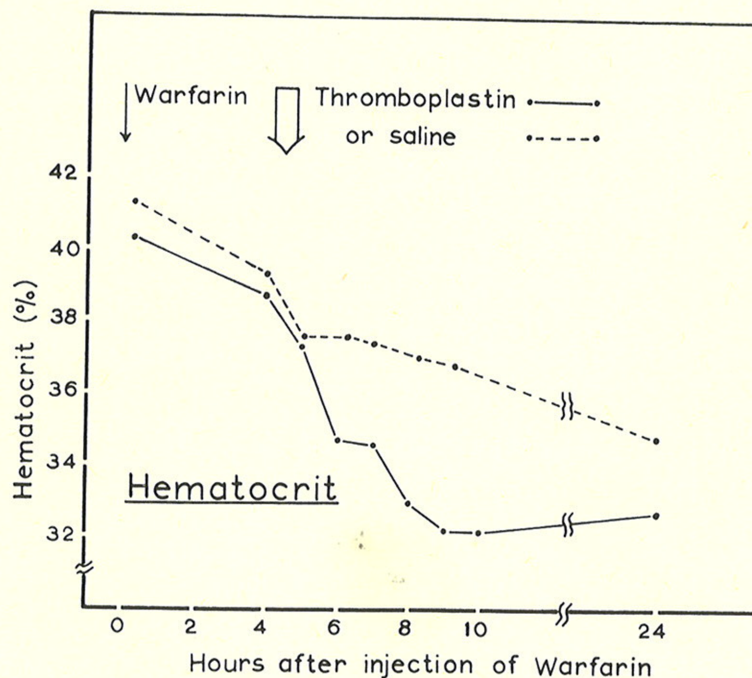

Figure 3. *The fall in the microhaematocrit during the experiment.* The points on the control line represent the mean values from 7 animals (except for the last point, 6 animals). The points on the experimental line represent mean values from a minimum of 16 animals.

TABLE IV

The falls in prothrombin and the serum factors during the infusions
Per cent loss of activity during the infusion was calculated from the formula
(sample 2 — sample 3) / sample 2 x 100
Means and one SD are given

Factor	No. of animals		Mean per cent loss of activity during inf.		Significance*	
	Sal.	Tpl.	Sal.	Tpl.	"t"	p
Proth.	7	18	12.5 ± 13.2	20.3 ± 8.9	1.44	0.20
Fact. X	7	18	10.6 ± 9.8	32.6 ± 10.5	4.95	< 0.005
Fact. VII	7	10	22.1 ± 17.9	49.5 ± 16.6	3.20	< 0.025
Fact. IX	7	8	14.6 ± 17.9	33.6 ± 28.1	1.58	< 0.20

* n = 6 degrees of freedom.

between the means for the animals receiving saline and receiving thromboplastin would have been highly significant ($p < 0.001$).

The disappearance curves shown in Figure 4 support the conclusion that significantly more prothrombin was consumed during the infusion in the experimental animals than in the control animals. Each mean for the experimental group after the infusion was lower than the corresponding mean for

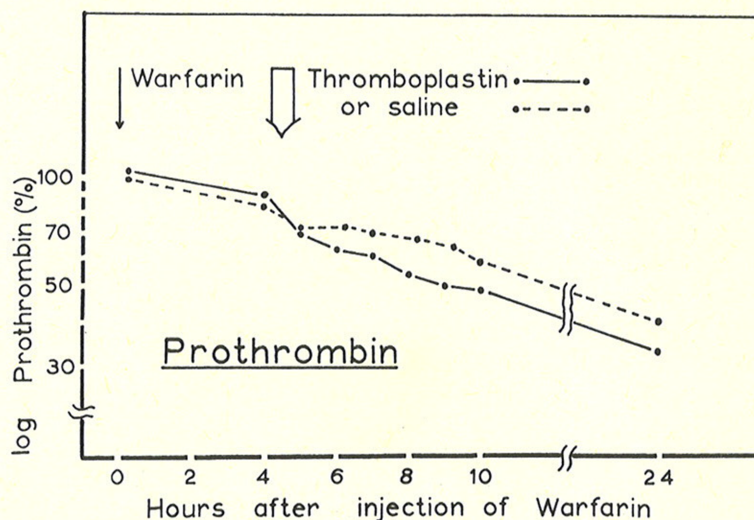

Figure 4. *Mean disappearance curves for prothrombin.* The points on the control curve represent means of 7 animals (except the last point, 6 animals). The points on the experimental curve represent means of 15 to 18 animals.

the control group. Yet, clearly, only a small amount of the available prothrombin was consumed in intravascular clotting. The conversion of only about 10 per cent of the circulating prothrombin to thrombin produced the extensive falls in fibrinogen, platelets, and Factors V and VIII described above.

b. *Factor X*. Common means were also calculated for the Factor X activity of the 18 animals given thromboplastin. These animals lost about three times as much Factor X activity during the infusion than the control animals, a highly significant difference (see Table IV). The disappearance curves of Figure 5 confirm this, for the curve for the animals given thromboplastin breaks sharply during the infusion and then resumes a slope paralleling the slope for the control group. By subtracting the mean loss during the infusion of the control animals from the mean loss of the experimental animals, we calculate that 22 per cent of the circulating Factor X, was consumed as a result of the 30 minute episode of intravascular clotting.

c. *Factor VII*. In contrast to clotting *in vitro*, intravascular clotting resulted in a decrease in Factor VII activity (see Figure 6 and Table IV). Animals receiving thromboplastin lost about twice as much Factor VII activity during the infusion as animals receiving saline, a difference significant at below the 2.5 per cent level. But, as Figure 6 shows, the Factor VII level fell more between the first and the second samples (preceding the infusion) in the con-

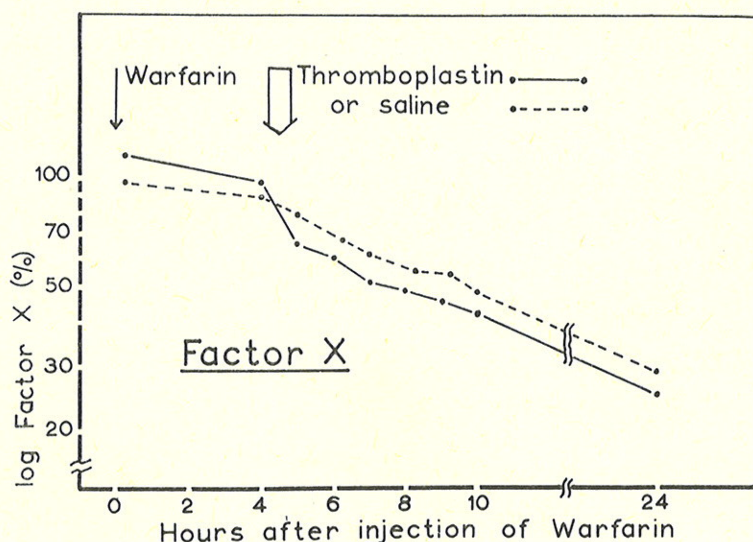

Figure 5. *Mean disappearance curves for Factor X*. The points on the control curve represent means of 7 animals (except the last point, 6 animals). The points on the experimental curve represent means of 15 to 18 animals.

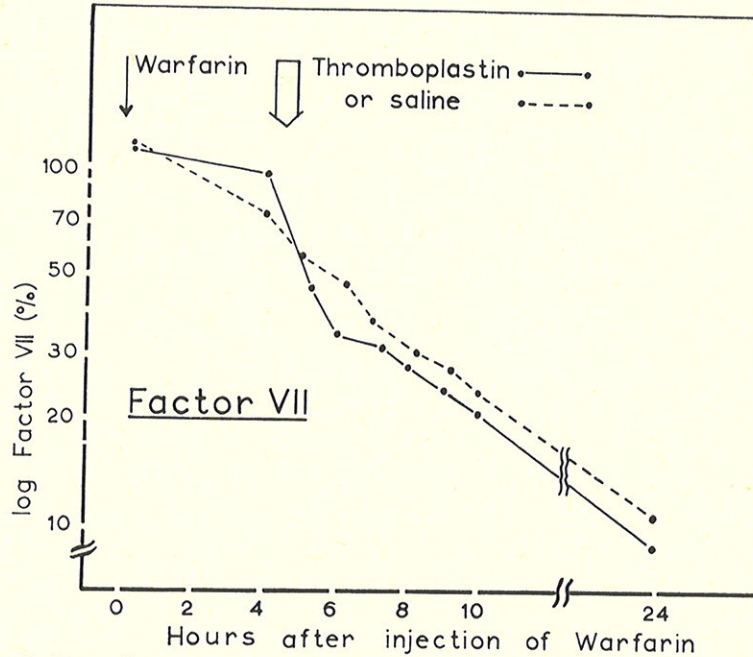

Figure 6. *Mean disappearance curves for Factor VII.* The points on the control curve represent means of 7 animals (except the last point, 6 animals). The points on the experimental curve represent means of 8 to 10 animals.

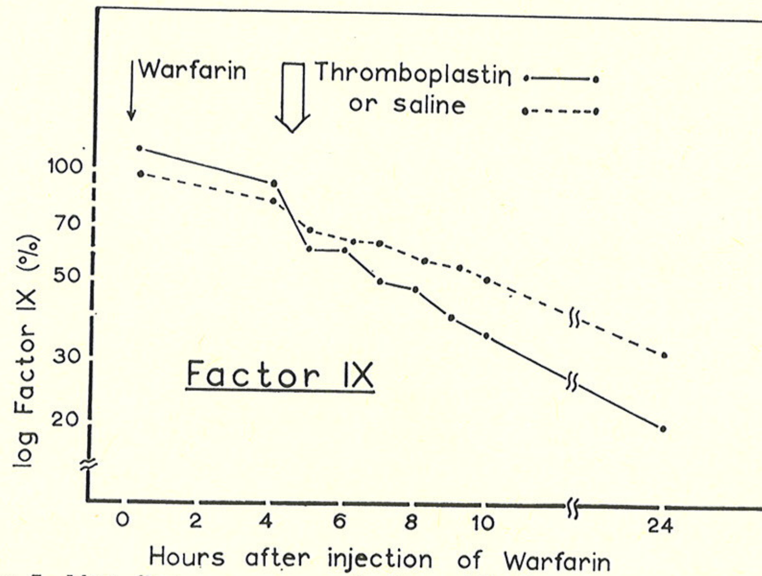

Figure 7. *Mean disappearance curves for Factor IX.* The points on the control curve represent means of 7 animals (except the last point, 6 animals). The points on the experimental curve represent means of 6 to 8 animals.

trols than in the experimental animals. We do not know why, but had this not happened, the difference in the amount lost during the infusion between the two groups would most likely have been smaller. Nevertheless, the disappearance curves of Figure 6 also confirm that more Factor VII was lost in the animals given thromboplastin, for all subsequent means in the experimental group are lower than the corresponding means in the control group. Subtracting the mean loss during infusion for the control group from the mean loss for the experimental group, we obtain a value of about 27 per cent for the amount of circulating Factor VII consumed due to the 30 minute episode of clotting.

d. *Factor IX*. As the data of Figure 7 and Table IV indicate, Factor IX activity also appeared to fall as a result of infusing tissue thromboplastin. The animals receiving thromboplastin lost about twice as much Factor IX activity during the infusion as the animals receiving saline; however, the difference between the mean losses was not statistically significant because of the large standard deviations. The disappearance curves of Figure 7 support the conclusion that some Factor IX is consumed during intravascular clotting triggered by tissue thromboplastin, for, again, all means after the infusion for the experimental group were lower than the corresponding means for the control group.

DISCUSSION

We consistently found lower values for the serum factors in the sample taken immediately after the infusion in the animals receiving thromboplastin. At that time (note the third point on the curves of Figure 3), the haematocrit was nearly the same in the two groups of animals. Therefore, greater haemodilution with fluid deficient in clotting factors can not account for the lower serum factor levels in the thromboplastin group.

Rather, our data strongly suggest that intravascular clotting triggered by tissue thromboplastin consumes Factors VII, IX, and X. Thus, after its synthesis was blocked by sodium warfarin, each of these serum factors fell further during the infusion and continued to exhibit lower means on serial samples over the next 20 hours in the animals given thromboplastin than in the control animals. Intravascular clotting extensive enough to halve fibrinogen and platelet levels was calculated to consume about 15 to 30 per cent of circulating Factors VII, IX, and X.

Factor VII is activated when blood clots *in vitro*, with or without tissue thromboplastin (Johnston & Hjort 1961), and serum may appear to possess three to four times the Factor VII activity of the original plasma. Therefore, we assume that some Factor VII was also activated during intravascular clotting in our animals. If this activated Factor VII had remained in the cir-

culation we would have found higher, not lower, Factor VII levels in the animals receiving thromboplastin. Since activated Factor VII is not inactivated *in vitro*, the mechanism for removal of activated Factor VII *in vivo* probably does not involve inactivation within the circulating blood. Spaet and co-workers, who observed the rapid removal of Product I in rabbits (Spaet & Cintron 1960) and injected "blood thromboplastin" in rats (Spaet *et al.* 1961), have suggested that activated clotting factors are cleared in the liver. Extravascular clearance of activated Factor VII would also account for the observation of Loeliger *et al.* (1960) that the T½ of transfused serum-Factor VII in man is shorter than the T½ of plasma-Factor VII.

Clearance of an activated fraction can also explain the consumption of Factor X during intravascular clotting. This explanation receives support from comparing the consumption of Factor X and prothrombin during intrinsic clotting *in vitro* and during our thromboplastin infusions. When blood clots in a glass tube, only a little of its Factor X is activated; yet, most of its prothrombin is converted to thrombin. When we infused thromboplastin, an estimated 20 per cent of the Factor X was consumed and presumably, therefore, was first activated; however, only 10 per cent of the prothrombin was converted to thrombin. We infer from this that an extravascular mechanism rapidly cleared activated Factor X from the circulating blood, and that this mechanism, rather than decay within the blood itself, is the primary physiological mechanism for inactivating Factor X.

Our data on Factor IX deserve special comment. Because it corrects the abnormality of Factor IX deficient blood, tissue thromboplastin is thought to bypass Factor IX in clotting. Yet, activation during clotting triggered by the infused tissue thromboplastin, with subsequent clearance of the activated fraction, again seems to us the best explanation for the difference between the disappearance curves for Factor IX in our control and experimental animals.

The activation of Factor IX during intrinsic clotting *in vitro* requires the presence of Factors XII and XI. Clinical observations suggest that a mechanism independent of Factors XII and XI may activate Factor IX during *in vivo* hemostasis, for patients with hemophilia B (Factor IX deficiency) bleed much more than patients deficient in Factor XI or XII.

Can tissue thromboplastin be the alternative *in vivo* activator of Factor IX? From differences in the effect of weak tissue thromboplastin upon thrombin generation in plasmas deficient in Factors XII, XI, and VIII, Biggs & Nossel (1961) concluded that tissue thromboplastin can bypass the contact activation reactions. Our data would fit the hypothesis that tissue thromboplastin, either directly or through an intermediate step of extrinsic clotting, activates Factor IX. Clearly, this hypothesis requires more rigorous testing. We used a commercial thromboplastin. To study whether or not the tissue

thromboplastin particle itself can trigger intrinsic clotting, we shall have to prepare thromboplastin from various tissues free of any possible contamination with blood by washing in a decalcifying medium followed by sedimention in an ultracentrifuge (Hjort 1957).

These data help us to understand the clotting defects which may arise from diffuse intravascular coagulation in patients. In the rabbits, fibrinogen levels returned to normal within 20 hours of the episode of clotting. Dogs also have a rapid fibrinogen replacement rate (Hardaway *et al.* 1964). These observations suggest that if a patient with suspected intravascular clotting has a very low fibrinogen level, clotting has taken place within a few hours, at the longest, of the time the sample was drawn. Conversely, if the level is normal or high, it does not rule out extensive intravascular clotting, but only extensive intravascular clotting within the preceeding 24 hours.

Thrombocytopenia may result from reversible or irreversible platelet damage. Recovery from the former is rapid, e.g., Waalkes *et al.* (1957) have reported that platelet levels drop to below 10,000 per μl during anaphylaxis in rabbits but return to normal within 2 hours. In our rabbits the platelets fell to about one-third of their initial level during the episode of clotting. Two hours later they had risen to about 50 per cent of initial levels. This early rise represents the return of reversibly damaged platelets to the circulating blood, the mobilization of a small reserve of platelets in the bone marrow (Finch 1961), or both processes. But then the rise stopped, and the levels the next day were not significantly higher than the two hour values. When intravascular clotting irreversibly damages large numbers of platelets in the rabbit, the return of the count to normal must await new platelet production, a process which may take several days. If this also holds for man, we should encounter patients a day or two after an episode of intravascular coagulation with normal clotting factors and only a moderate thrombocytopenia as residual evidence of the clotting.

Our data confirm that the levels of platelets, fibrinogen, Factor V and Factor VIII are the most sensitive indicators of diffuse intravascular clotting, for these factors dropped further than prothrombin and the serum factors. Nevertheless, the serum factors are also consumed. We would no longer be surprised to find reduced levels of Factors VII, IX, and X in patients with extensive intravascular clotting.

The amount of prothrombin consumed during intravascular clotting in our animals was small despite the impressive fall in fibrinogen and platelet levels. We have seen a patient with diffuse intravascular clotting and a prothrombin level of 22 per cent (Rapaport *et al.* 1964). Clearly, this low a level of prothrombin, measured in a specific assay, indicates massive or prolonged intravascular clotting.

Theoretically, an elevated level of a clotting factor in a patient, e.g., high Factor VII in a pregnant woman, could mean either an increased amount of the native factor or conversion of a fraction of the native factor to a more active form. Our data indicate that activated Factors VII, IX, and X do not circulate in the rabbit. We doubt, therefore, that they circulate in the human and believe that, for the serum factors at least, an elevated level always means an increased amount of the native factor.

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Received July 10, 1965.

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