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Intravascular Coagulation with Generalized Shwartzman Reaction Induced by a Heparin-Like Anticoagulant (Liquoid)*

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Liquoid is a synthetic acid polymer (sodium polyanethol sulfonate) with an anti-coagulant effect similar to that of heparin both *in vitro* and *in vivo* (1, 2). In rabbits, a large intravenous dose produces intravascular deposits of a homogeneous eosino-philic material (3, 5) with the ultrastructural characteristics of fibrin (6, 7). Extensive organ damage follows, including bilateral cortical necrosis of the kidneys. This lesion can not be differentiated from the generalized Shwartzman reaction produced by two appropriately spaced intravenous injections of endotoxin (8).

The generalized Shwartzman reaction is caused by acute intravascular coagulation in a properly prepared animal, and heparin *protects* against this reaction (9). It is peculiar, therefore, that Liquoid *provokes* the reaction. The most widely accepted explanation is that of Thomas et al. (10). In rabbits prepared by endotoxin, an intravenous injection of Liquoid resulted in the abrupt disappearance of circulating fibrinogen. They suggested, therefore, that Liquoid *precipitated* fibrinogen in the vessels. This concept is supported by the fact that Liquoid precipitated fibrinogen *in vitro* (2, 11). However, it has also been suggested that Liquoid acts through intra-vascular *coagulation*, since the morphological lesions (6, 7) and the clotting defects (5, 12) are so similar to those of the generalized Shwartzman reaction.

We noted that Liquoid produced bilateral cortical necrosis of the kidneys in doses that were too small to precipitate fibrinogen, as judged from *in vitro* experiments. Therefore, we reinvestigated the effect of Liquoid *in vitro* and *in vivo*. In this article, we present evidence that Liquoid acts by triggering intravascular coagulation.

Materials

Animals. Albino rabbits of both sexes weighing 1550–2750 g were used. They were fed rabbit pellet food (Statens Institutt for Folkehelse, Oslo) and housed for 5–7 days before experiments.

Buffer. A veronal buffer of pH 7.35 and ionic strength of 0.154 was made as previously des-cribed (13).

Citrate anticoagulant was prepared by mixing 6 parts of 0.1 M sodium citrate and 4 parts of 0.1 M citric acid.

Heparin (Nyegaard & Co., Oslo) contained 5,000 I. U./ml.

Human purified fibrinogen was kindly supplied by Dr. N. O. Solum, Institute for Thrombosis Research, Oslo.

Liquoid, a synthetic acid polymer (sodium polyanethol sulfonate), was generously supplied by Hoffmann-La Roche, Basle, Switzerland. Freshly dissolved neutral solutions of Liquoid (Batch A 183627, mol wt. 21,500 ± 1,500, sulfur content 12.7%) in saline were used.

Polybrene (Abbott Lab., Chicago, Ill.), 10 mg/ml, was diluted in saline.

Sodium warfarin was kindly supplied by Nyegaard & Co., Oslo.

Thrombin. Bovine thrombin (Topostasine from Hoffmann-La Roche, Basle, Switzerland) was diluted in buffer to desired strength.

Methods

Anticoagulant Therapy with Warfarin

Animals were assigned at random to 2 groups, receiving *either* 2 ml saline (saline-pretreated) *or* 30 mg warfarin in 2 ml saline (warfarin-pretreated) intravenously for 3 consecutive days. On the 4. day, about 20 hrs after the last injection the effect of the anticoagulant therapy was checked. Anticoagulated animals with a Quick-time of more than 100 sec (normal 10–15 sec) and a decrease in hematocrit during treatment of less than 5 volume % were accepted as properly pretreated.

Autopsy

At termination of an experiment, surviving animals were killed with Nembutal (Nembutal Sodium, Abbott Lab., Chicago, Ill.). Lung, liver and kidneys were fixed in 10% formalin, and the tissue sections were stained with hematoxylin-erythrosin-safran and with Lendrum's stain for fibrin (14).

Preparation of Test Samples

Blood samples from rabbits were obtained in 3 ways.

1. From the ear veins by the method of Rapaport et al. (15).

2. From the aorta of animals anesthetized with Nembutal, using a 20 gauge needle attached to plastic tubing.

3. From the femoral artery, using an indwelling cannula (Nylon intravenous cannula, Portex FG 4, from Portland Plastics Ltd., Kent, England). During Nembutal anesthesia the artery was dissected free, and the cannula was inserted 5–7 cm, always well below the renal artery. A 3-way stopcock allowed rapid blood sampling over at least 24 hrs. The cannula was filled with saline between samples and it was rinsed with saline before and after each sample.

In all methods 9 parts of blood were allowed to flow into a plastic tube containing 1 part of citrate anticoagulant.

Plasma was removed after centrifugation at 1250 g for 10 min and stored at −28° C.

Platelet-poor plasma used for *in vitro* experiments was obtained by centrifugation at 14,400 g for 10 min. The supernatant was respun for the same period of time.

Assay Methods

Fibrinogen was measured by the method of Blombäck and Blombäck (16) as modified by Godal (17).

Factor V was measured as described by Stormorken (18). Preliminary experiments showed that the Liquoid content in a 1:10 dilution of plasma obtained from animals receiving Liquoid did not influence this assay.

Platelets were counted "blindly" in duplicate by the method of Brecher et al. (19), slightly modified.

Quick-time was determined in the following test system: 0.2 ml human thromboplastin and 0.2 ml test plasma were incubated for 3 min at 37° C and then recalcified with 0.2 ml 20 mM $CaCl_2$.

Thrombin time was determined by methods described under the appropriate experiments.

Statistical Calculations

To evaluate the significance of differences between groups, Student's t-test was used. A p-value of less than 0.05 was considered significant.

Results

A. The Anticoagulant and Precipitating Effects of Liquoid In Vitro

1. The anticoagulant effect. Liquoid prevented coagulation for hours when added to rabbit whole blood in concentrations exceeding about 0.15 mg/ml. This anti-coagulant effect, which is immediate, was best demonstrated in a thrombin time test system (Fig. 1). Polybrene, a heparin-neutralizing agent, corrected the prolonged thrombin time of citrated plasma containing Liquoid, and Fig. 2 shows that 1 mg of Polybrene neutralized 1 mg of Liquoid. In large doses, Polybrene may accelerate the thrombin time, but the neutralization points were well defined. During neutraliza-tion a white precipitate appeared which could easily be packed by centrifugation. Plasmas containing larger amounts of Liquoid were not completely corrected due to low concentrations of fibrinogen (precipitated fibrinogen was removed by centri-fugation before neutralization); compare curves 2 and 3 in Fig. 2.

These experiments demonstrate an antithrombin effect of Liquoid similar to that of heparin. The effect on the first phase of coagulation was not investigated.
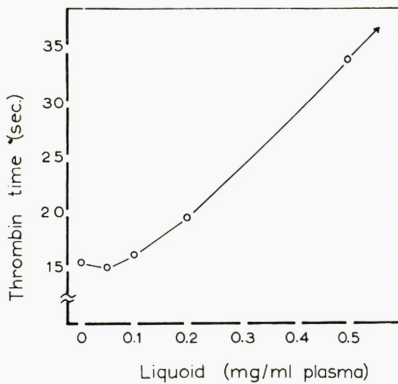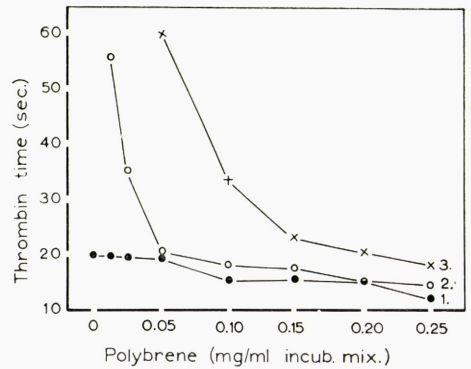

Fig. 1

Fig. 2

Fig. 1. *The effect of Liquoid on the thrombin time of citrated plasma.* One part of Liquoid was in-cubated with 9 parts of citrated platelet-poor plasma for 10 min at 20° C. After centrifugation for 10 min at 1250 g, 0.2 ml of the supernatant plasma was incubated for 3 min at 37° C before 0.1 ml of thrombin (6 NIH-u/ml) was added.

Fig. 2. *Neutralization of Liquoid with Polybrene.* One part of saline or of Liquoid in different concentrations was incubated with 9 parts of citrated platelet-poor plasma for 10 min at 20° C. After centrifugation for 10 min at 1250 g, 0.2 ml of the supernatant plasma was incubated with 0.1 ml of Polybrene of varying concentrations for 3 min at 37° C and then clotted with 0.1 ml of thrombin (7.5 NIH-u/ml). ●———● (1) Saline; ○———○ (2) Liquoid 0.05 mg/ml incu-bation mixture; ×———× (3) Liquoid 0.15 mg/ml incubation mixture.

2. Determination of fibrinogen in plasmas containing Liquoid. Our fibrinogen assay (16) is based upon clotting of fibrinogen by thrombin. Since Liquoid inhibits the effect of thrombin, we examined whether it was necessary to neutralize Liquoid before fibrinogen was assayed. Curves 1 and 2 in Fig. 3 show that Liquoid should be neutralized if its concentration exceeds about 0.4 mg/ml plasma. Curve 3 demonstrates that the neutralization procedure was effective, and curve 4 shows that Polybrene did not influence the fibrinogen assay. The same curves were obtained with double-strength

thrombin solution in the assay. Following injection of 20 mg Liquoid/1500 g body weight, the maximum plasma concentration is about 0.32 mg/ml. Therefore, only the test samples from the *in vitro* part of this study were neutralized. Since excess Polybrene did not influence the fibrinogen assay, a simple neutralization procedure was used (see legend to Fig. 4).

3. Fibrinogen-precipitating effect of Liquoid. Liquoid precipitated plasma proteins from citrated plasma (Table 1). This reaction was characterized by the immediate appearance of small floccules which coalesced to an opaque gelatinous mass at the bottom of the tube. A concentration of about 2 mg/ml plasma produced maximal precipitation. Further addition of Liquoid increased the solubility of the precipitate initially formed. Protein was precipitated to the same extent in plasma from saline- and warfarin-pretreated animals (Table 1). The washed precipitate was insoluble in saline and in strong heparin solutions (1000 I. U./ml) at 37° C; it dissolved slowly in excess Liquoid and rapidly in 30% urea and in 40% alkaline urea.

Table 1. Protein Precipitation in Citrated Plasma by Liquoid.

Liquoid mg/ml plasma	Plasma from	
	saline-pretreated animals	warfarin-pretreated animals
20	−	−
10	−	−
8	±	−
5	+ +	+
2	+ + +(+)	+ + +
1	+ + +	+ +
0.8	+	+
0.6	+	+
0.5	+	+
0.2	±	−
0.1	−	−
0	−	−

One part of Liquoid was added to 9 parts of citrated platelet-poor plasma. Precipitation was read visually after incubation for 1 hr at 37° C, pH 7.4. The rabbit donors had been pretreated with 3 daily injections of saline or warfarin, see Methods.

Highly purified human fibrinogen (90–95% clottable) was precipitated by Liquoid in the same way as citrated rabbit plasma.

Fig. 3 shows that fibrinogen was one of the plasma proteins precipitated by Liquoid. This precipitation was investigated further to determine whether it required an intact coagulation system. The answer to this problem was essential for the interpretation of the *in vivo* findings described below. Therefore, the precipitation of fibrinogen was examined in whole blood from normal and warfarin-anticoagulated animals. Fig. 4 shows that equal amounts of fibrinogen were precipitated in citrated whole blood from animals pretreated with saline or with warfarin (curves 1 and 3) and also in noncitrated whole blood from warfarin-pretreated animals where Liquoid itself was the only anticoagulant (curve 4). When Liquoid was the only anticoagulant for whole blood from saline-pretreated animals (curve 2), the blood clotted within 10 min at Liquoid concentrations below 0.10 mg/ml. In the interval 0.10–0.20 mg/ml, however, a thread-like precipitate formed within 30 min at 20° C. This precipitate was easily observed since all tubes were centrifuged 10 min after sampling. The
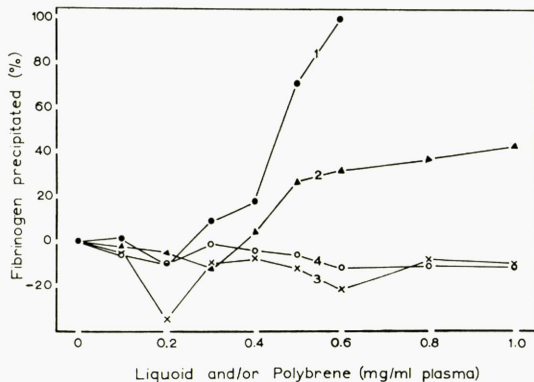
Fig. 3. *The importance of neutralizing large concentrations of Liquoid with Polybrene before fibrinogen is assayed.* Nine parts of citrated platelet-poor plasma were incubated with 1 part of Liquoid in varying concentrations or with saline for 10 min at 20° C and then centrifuged for 10 min at 1250 g. Polybrene in the same concentrations as Liquoid, or saline was then incubated with the supernatant plasma for 10 min at 20° C. After centrifugation for 10 min at 1250 g, the fibrinogen was measured. ●————● (1) Liquoid added alone; △————△ (2) Liquoid added alone. Supernatant neutralized by Polybrene; ×————× (3) Liquoid and Polybrene added together before first centrifugation; ○————○ (4) No Liquoid, Polybrene added alone.

washed precipitate was insoluble in excess Liquoid and also in 30% urea, but it dissolved easily in 40% alkaline urea. In samples with these threads 70% or more of the fibrinogen had disappeared. Higher concentrations of Liquoid, enough to block coagulation completely, precipitated fibrinogen to the same extent as shown in
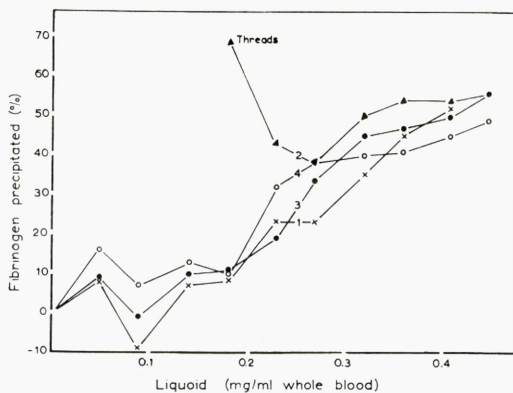

Fig. 4. *Precipitation of fibrinogen by Liquoid in whole blood from saline- and warfarin-pretreated rabbits.* From the aorta 0.9 ml blood was allowed to flow into plastic tubes containing 0.1 ml saline or Liquoid in varying concentrations, plus 0.1 ml saline or citrate anticoagulant. After incubation for 10 min at 20° C the tubes were centrifuged at 1250 g for 10 min. Forty min after sampling, 0.1 ml citrate anticoagulant and 0.1 ml Polybrene (10 mg/ml) were added to all the separated supernatants. After a second centrifugation for 10 min, fibrinogen was assayed. ×————× (1) Saline-pretreated; incubation with citrate; ▲————▲ (2) Saline pretreated; incubation without citrate; ●————● (3) Warfarin-pretreated; incubation with citrate; ○————○ (4) Warfarin-pretreated; incubation without citrate.

curves 1, 3 and 4. This is clearly seen in Fig.4 where curve 2 joins the other curves at higher concentrations of Liquoid.

Two important conclusions follow from this experiment. First, concentrations of Liquoid below 0.20 mg/ml could not prevent slow fibrin formation in whole blood from the saline-pretreated animals. Secondly, Liquoid precipitation of fibrinogen did not depend on the coagulation mechanism.

B. Evidence of Intravascular Coagulation Induced by Liquoid in Vivo

1. Protection by warfarin. The *in vitro* experiments showed that warfarin treatment did not prevent precipitation. If Liquoid produces renal cortical necrosis by precipitation, therefore, warfarin should not prevent this lesion. This was tested in the following experiment.

Two groups of animals were pretreated with saline or with warfarin (see Methods). After an initial sample from the ear vein for fibrinogen assay and platelet count had been taken, 20 mg of Liquoid per 1500 g body weight was injected intravenously in all animals. Further samples were taken from the ear 20 min and 2 hrs later. Surviving animals were killed 20 hrs after the injection.

A dose of 20 mg Liquoid per 1500 g body weight was chosen, because it decreased fibrinogen and produced renal cortical necrosis in normal animals (Fig.5). In this dose Liquoid produced marked clinical symptoms in saline-pretreated animals. Within a few hours the animals were severely ill; their ears were cold and the respiration shallow. Autopsy performed on 5 animals that died within 20 hrs suggested that pulmonary edema and hemorrhage were the main causes of early death. In contrast, the warfarin-pretreated animals quickly recovered from mild respiratory symptoms and then remained clinically well.
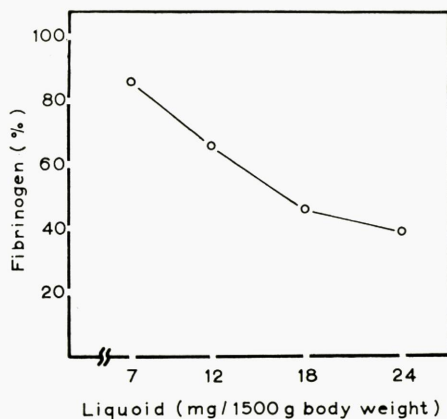

Fig. 5. *The effect of intravenously injected Liquoid on the fibrinogen level of plasma.* Eighteen rabbits were injected with various doses of Liquoid. The points represent the mean fibrinogen level 1 hr after injection in % of the preinjection level.

After the injection, the mean platelet count dropped in both groups, but more in the saline-pretreated animals (Fig.6). The counts varied markedly, especially in the anticoagulated animals. The difference between the 2 groups was not significant 20 min after the injection ($0.10 > p > 0.05$), but it was significant after 2 hrs

(0.02 > p > 0.01). Fig. 7 illustrates the significant difference between the slight drop in fibrinogen in the warfarin-pretreated animals and the marked drop in the saline-pretreated animals 2 hrs after the injection (p < 0.01).
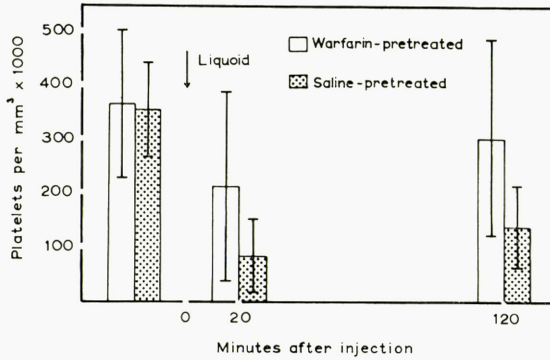

Fig. 6. *The effect of an intravenous injection of 20 mg Liquoid/1500 g body weight on the platelet level in saline- and in warfarin-pretreated rabbits.* The mean and standard deviations are indicated; 6 animals in each group.
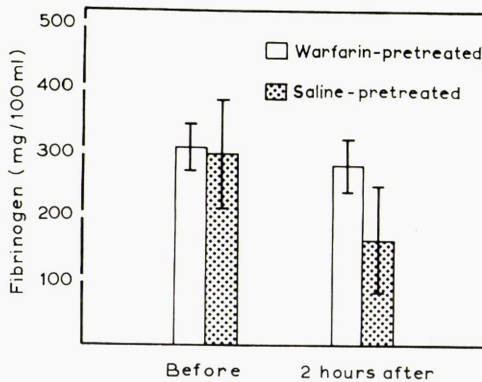

Fig. 7. *The effect of an intravenous injection of 20 mg Liquoid/1500 g body weight on the fibrinogen level in saline- and in warfarin-pretreated rabbits.* The mean and standard deviations are indicated; 8 animals in the saline-pretreated and 7 animals in the warfarin-pretreated group.

This difference in fibrinogen corresponded well with morphological findings in the 2 groups. The saline-pretreated animals developed severe renal cortical necrosis, as in the generalized Shwartzman reaction. The glomeruli were swollen, and the capillaries were filled with masses of a homogeneous material staining like fibrin (Fig. 8). The lungs showed edema and large areas of hemorrhage, and thrombi or emboli were often seen. In the liver, areas of necrosis and hemorrhage were found.

Table 2 and Fig. 8 show that anticoagulant therapy with warfarin completely prevented the lesions induced by Liquoid in normal animals. The organs appeared normal except for occasional petechiae.

Table 2 also includes a control experiment which indicates that this prevention by warfarin was not due to a direct effect of warfarin in the blood stream: 6 animals

received 30 mg of warfarin intravenously 1 min before the Liquoid injection; all of them developed renal cortical necrosis.
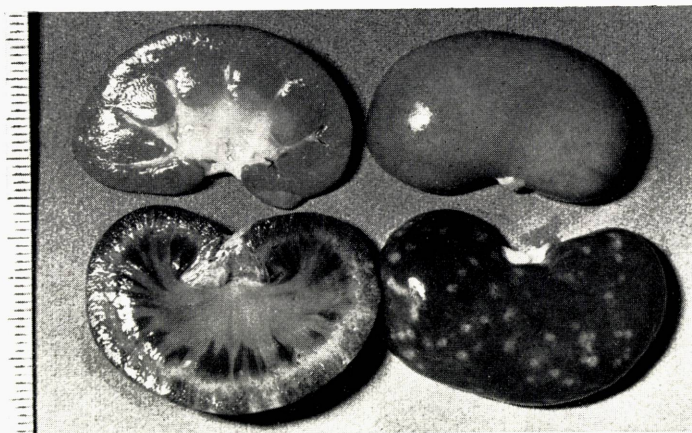
Fig. 8 A. The saline-pretreated animal had severe bilateral cortical necrosis (below); the warfarin-pretreated animal had normal kidneys (top). The scale is in mm.

Fig. 8 B. A swollen glomerulus with characteristic intracapillary deposits of fibrin-like material from a saline-pretreated animal (right), and a normal glomerulus from a warfarin-pretreated animal (left). Lendrum's stain for fibrin; ×900.

Fig. 8. *The effect of an intravenous injection of 20 mg Liquoid/1500 g body weight on the kidneys in saline- and in warfarin-pretreated rabbits.*

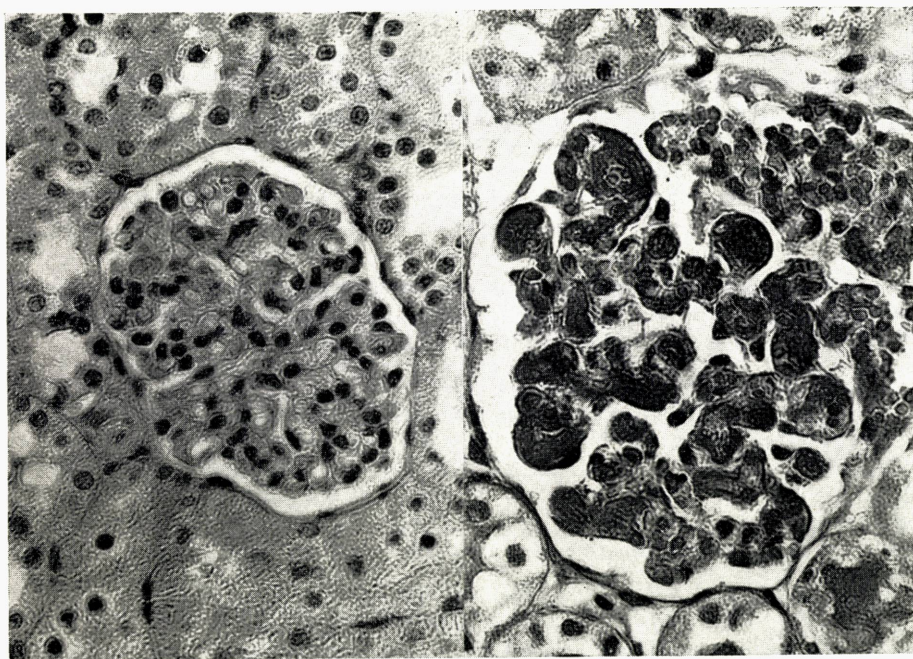Table 2. Protection by Warfarin against Death and Bilateral Cortical Necrosis of the Kidneys Produced by an Intravenous Injection of 20 mg Liquoid/1500 g Body Weight.

	Saline-pretreated animals	Warfarin-pretreated animals	Warfarin given with Liquoid
Drug injected	Liquoid	Liquoid	Liquoid
Number with renal cortical necrosis/total number injected	10/11	0/10	6/6
Number dead within 20 hrs after injection	5	0	3
Drop in hematocrit (vol. %) during saline- or warfarin-pretreatment	1.4 (42.8)[1]	1.4 (41.3[1])	—
Quick time (sec) before Liquoid injection	12.2 (10.0–14.2)	>180	—

1) Mean hematocrit before injection of Liquoid.

The first and second columns include a preliminary experiment with 6 animals from which no blood samples were taken. The third column presents a control group that received a single injection of 30 mg warfarin 1 min before the injection of Liquoid.

2. Observations on the duration and mechanism of the in vivo effect of Liquoid. The previous section gives strong evidence that Liquoid acts by triggering intravascular coagulation. This raises two obvious questions. First, why does not Liquoid itself protect against intravascular coagulation? One explanation could be that its anti-coagulant effect disappears too quickly. Secondly, is it possible to obtain further evidence that Liquoid triggers coagulation? In this section we present some observations relevant to these questions.

Seventeen rabbits were randomly divided into 3 groups. All of them had a cannula in the femoral artery for repeated and reliable blood sampling. Autopsy was done at termination of the experiment.

Group A (5 animals) were injected with 2 ml saline only.

Group B (8 animals) were pretreated with saline for 3 days and then received an intravenous injection of 20 mg Liquoid/1500 g body weight.

Table 3. Fate of Animals in the Second in Vivo Experiment.

	Group A	Group B	Group C
Pretreatment	None	Saline	Warfarin
Drug injected	Saline	Liquoid	Liquoid
Number with renal cortical necrosis/total number injected	0/5	7/8	0/4
Number dead within 20 hrs after injection	0	5	2
Quick time (sec) before Liquoid or saline injection	—	—	>180
Hematocrit drop (vol. %) during the first 4 hrs after injection	6.8	7.0	6.5

After appropriate pretreatment, animals were injected intravenously with saline or with Liquoid (20 mg/1500 g body weight). See text for details.

Group C (4 animals) were pretreated with warfarin for 3 days and then received the same dose of Liquoid.

Table 3 summarizes the experiment. In group B one animal died 20 min after the injection of Liquoid, totally depleted of fibrinogen. This animal died before necrosis had time to develop, but all the other animals in this group had severe bilateral cortical necrosis of the kidneys with fibrin-like deposits in the glomerular capillaries. The animals in group A and C remained unaffected for an observation period of 4 hrs. However, 2 of the warfarin-pretreated animals (group C) bled to death during the following night. At autopsy, all animals in group A and C had normal kidneys.
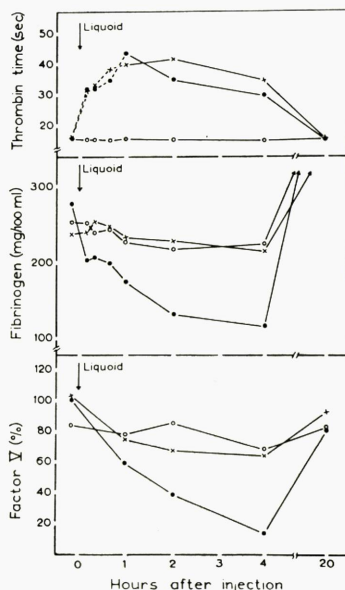

Fig. 9. *The duration of anticoagulant effect and the progressive fall in fibrinogen and factor V after an intravenous injection of Liquoid (20 mg/1500 g body weight)*. The following thrombin time test system was used: 0.1 ml citrated test plasma was mixed with 0.1 ml citrated platelet-poor normal rabbit plasma and incubated at 37° C for 3 min before 0.1 ml thrombin (7.5 NIH-u/ml) was added. The curves represent the mean for each group. During the first hour after Liquoid injection solid clots did not form in the thrombin test system, and formation of threads was taken as the end point. Later, solid clots were the end point. For this reason, the initial parts of the curves in the upper chart are broken. At 20 hrs, mean values are uncertain due to the small number of animals surviving for this period of time. O———O Group A. Saline only; ●———● Group B. Saline-pretreated, then Liquoid; ×———× Group C. Warfarin-pretreated, then Liquoid.

The duration of anticoagulant effect after injection of Liquoid. The thrombin time test system (see legend to Fig. 9) contained normal rabbit plasma as a reagent. It was therefore fairly insensitive to changes in the fibrinogen concentration of the test samples and reflected mainly the heparin-like effect of Liquoid. Fig. 9 shows no difference between the animals in groups B and C. Therefore, pretreatment with warfarin did not influence the potency or the clearance rate of Liquoid. Clearly, Liquoid exerted an immediate anticoagulant effect which decreased little during an observation period of 4 hrs.

We conclude that a considerable heparin-like effect was present for several hours after the injection, i. e., during the time period when fibrinogen was decreasing and renal cortical necrosis was developing.

The progressive fall in fibrinogen and factor V after Liquoid injection. In saline-pretreated animals Liquoid produced a progressive fall in fibrinogen and factor V (Fig. 9). Four hrs after the injection more than half of the fibrinogen and about 80% of factor V had disappeared. The warfarin-pretreated animals showed a small and insignificant drop, as did also the animals injected with saline only. Twenty hrs after the injection, the mean factor V level had returned to normal in the saline-pretreated animals, while the mean fibrinogen concentration in all 3 groups exceeded the mean initial level by nearly 100%. This increase in fibrinogen was probably due to the stress of the experiment. The mean drop in hematocrit during the first 4 hrs was about the same in all 3 groups (Table 3).

It is difficult to measure clotting factors in the presence of Liquoid. However, the fall in fibrinogen and factor V cannot be explained simply by the presence of Liquoid in the assay system, because it was progressive and because it was not found in the warfarin-pretreated animals. For the same reasons, the fall in fibrinogen cannot be due to a chemical precipitation by Liquoid.

Cold-precipitation of fibrin-like material in plasma after Liquoid injection. Citrated plasma from all animals was incubated at 4° C for 24 hrs and inspected for precipitates. In saline-pretreated animals the samples taken during the first hour after Liquoid injection became turbid within 15–20 min after chilling. During the next 2 hrs a whitish thready precipitate formed in most of these samples, especially in those taken 40–60 min after the injection. Occasionally, precipitates were also seen in slightly hemolysed samples taken later. The precipitates sometimes formed during incubation at 20° C but never at 37° C. They were soluble in 40% alkaline urea but insoluble in 30% and in excess Liquoid. The appearance of these precipitates is illustrated in Fig. 10.

In the plasma from warfarin-pretreated animals (group C) a faint precipitate might form, and during prolonged incubation at 4° C small precipitates occasionally appeared also in the plasma from animals injected with saline only.

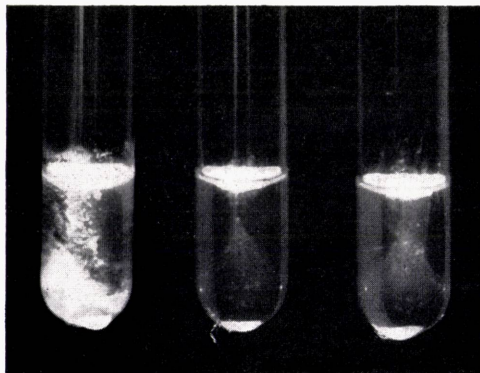Fig. 10. *Cold-precipitable material in citrated rabbit plasma obtained 1 hr after injection of Liquoid or saline.* The tubes contained plasma from a saline-pretreated animal injected with Liquoid (left), from a warfarin-pretreated animal injected with Liquoid (middle), and from a normal animal injected with saline (right). The dose of Liquoid was 20 mg/1500 g body weight. The tubes were incubated at 4° C for 20 hrs.

Thus, cold-precipitating material formed during the period of rapid fibrinogen depletion (Fig. 9), suggesting that this material was closely related to the fibrin-like material deposited in the glomerular capillaries in the saline-pretreated animals after Liquoid injection.

Discussion

A single large dose of Liquoid produces renal cortical necrosis with remarkable consistency. It is caused by intravascular deposits of an eosinophilic, homogeneous, fibrin-like material and is preceded by a rapid drop in platelets, by a more progressive drop in fibrinogen and factor V, and by the appearance of cold-precipitable material in the plasma.

Intravascular coagulation or precipitation? Our findings indicate that this sequence of events is due to intravascular coagulation and not to precipitation of fibrinogen. There are 5 arguments in favor of intravascular coagulation.

1. Pretreatment with warfarin protects against the renal cortical necrosis (Tables 2 and 3) and prevents the fall in fibrinogen and factor V (Figs. 7 and 9) which follows Liquoid injection, although warfarin does not influence precipitation *in vitro* (Table 1 and Fig. 4).

2. The fall in fibrinogen and factor V after Liquoid injection is progressive over several hours (Fig. 9) while precipitation *in vitro* is immediate.

3. Precipitation *in vitro* requires a concentration of Liquoid of at least 0.20 mg/ml whole blood (Fig. 4), while 20 mg injected intravenously produces severe effects in a rabbit weighing 1500 g. Assuming a blood volume of about 7%, this dose gives an initial blood level of at most 0.20 mg/ml whole blood.

4. The precipitates formed *in vitro* dissolved in 30% urea and in excess Liquoid, while the precipitates formed in plasma from rabbits injected with Liquoid dissolved in 40% alkaline urea only, like fibrin.

5. The low levels of platelets, fibrinogen and factor V after Liquoid injection are typical of the clotting defect resulting from intravascular coagulation. This argument, however, is not decisive; a similar defect could probably result from precipitation.

It should be noted that precipitation may contribute to the total *in vivo* effect of Liquoid, but the importance of this contribution depends on the dose. According to Fig. 4, 0.20 mg Liquoid/ml whole blood precipitated about 15% of the fibrinogen *in vitro*. This is the maximum concentration right after an injection of 20 mg Liquoid, provided the material stays in the circulation (see above). The fact that fibrinogen did not decrease in the warfarin-pretreated animals suggests that the actual maximum concentration was well below this level. However, with sufficiently large doses, precipitation will rapidly kill the animal, and intravascular coagulation will play little or no role. These predictions are based on our *in vitro* study with fresh whole blood added to increasing amounts of Liquoid (Fig. 4). In lower doses, Liquoid did not prevent coagulation; in larger doses, it precipitated fibrinogen.

How does Liquoid trigger coagulation? At present, there is no evidence for a direct activating effect of Liquoid on clotting factors, nor for an effect on tissues which could result in release of thromboplastic material. However, there is convincing evidence for an effect on platelets. Liquoid aggregates platelets *in vitro* (20) and *in vivo* (7), resulting in "viscous metamorphosis" with release of platelet factors (20). Rodriguez-Erdmann and Lasch (12) have given evidence that a similar release probably takes place *in vivo*.

Under our experimental conditions platelets decreased more rapidly than the clotting factors (Figs. 6 and 9), and pretreatment with warfarin did not prevent the abrupt drop in platelets. Thus, Liquoid appears to aggregate platelets directly, independently of the coagulation mechanisms.

These observations all fit the theory that Liquoid aggregates platelets and releases platelet factors which then trigger a progressive intravascular coagulation. However, the clot-promoting potential of platelets *in vivo* is still debated. Epstein and Quick (21) could not activate the clotting mechanism with injections of platelet extracts, and irradiated dogs tolerated large amounts of disintegrated platelets without evidence of coagulation (22). Large doses of bovine platelet factor 3, on the other hand, triggered the Shwartzman reaction in Thorotrast-prepared rabbits (23).

Although further work is necessary, we conclude that Liquoid probably triggers coagulation by aggregating and destroying the platelets.

Why does Liquoid not prevent coagulation? The fall in clotting factors after Liquoid indicates that coagulation goes on, not only in the platelet aggregates, but also in the circulating blood. This coagulation proceeds in spite of a considerable heparin-like effect (Fig. 9). Thus, 4 hrs after the injection the thrombin time was still more than doubled. We can only conclude that this anticoagulant effect (as opposed to the effect of warfarin) obviously was too small to prevent coagulation.

Effect of other synthetic heparinoids. All the synthetic heparinoids have similar chemical and toxic effects. Thus, dextran sulfate and other heparinoids produce platelet aggregates and widespread intravascular deposits of metachromatic material in rabbits (24, 25). Because these heparinoids precipitate fibrinogen *in vitro*, the metachromatic material is thought to be complexes between heparinoid and fibrinogen (25). Our findings suggest that intravascular coagulation may play a role in the formation of these deposits.

In animals prepared with endotoxin, small doses of Liquoid produce intravascular deposits of an eosinophilic material, presumably due to precipitation of fibrinogen (10). Again, our findings may suggest that intravascular coagulation contributes to these deposits.

Summary

Liquoid (sodium polyanethol sulfonate), a synthetic heparin-like anticoagulant, produces renal cortical necrosis in rabbits. This lesion is indistinguishable from the generalized Shwartzman reaction which is caused by intravascular coagulation in a prepared animal. We have investigated this apparently paradoxic effect of Liquoid. Our main findings are:

1. Liquoid is a potent heparin-like anticoagulant. One mg of Liquoid is neutralized by 1 mg of Polybrene. After intravenous injection the anticoagulant effect is immediate and persists for several hours.

2. In larger concentrations, Liquoid precipitates fibrinogen. The precipitation does not depend on an intact coagulation system; the precipitates are dissolved in 30% urea.

3. An intravenous injection of 20 mg Liquoid/1500 g body-weight produces an early thrombocytopenia, a progressive depletion of fibrinogen and factor V, the appearance of cold-precipitating material in plasma, and severe renal cortical necrosis.

4. Pretreatment with warfarin completely prevents all of these effects, except a moderate fall in platelets.

We conclude that Liquoid produces these effects, not by precipitation of fibrinogen, but by intravascular coagulation, probably released through aggregation and damage of the platelets. Thus, intravascular coagulation is again confirmed as the final event in the generalized Shwartzman reaction.

Résumé

Le Liquoïd (polyanétholsulfonate de sodium), un anticoagulant synthétique ressemblant à l'héparine, produit chez le lapin une néerose du cortex rénal. Cette lésion ne peut être distinguée de la réaction de Shwartzman généralisée qui est causée par une coagulation intravasculaire chez l'animal préparé. Nous avons étudié cet effet apparemment paradoxal du Liquoïd. Nos principaux résultats sont les suivants:

1. Le Liquoïd est un puissant anticoagulant ressemblant à l'héparine. Un mg de Liquoïd est neutralisé par 1 mg de Polybrène. Après une injection intraveineuse l'effet anticoagulant est immédiat et persiste plusieurs heures.

2. Aux concentrations plus élevées le Liquoïd précipite le fibrinogène. La précipitation ne dépend pas d'un système de coagulation intact; les précipités sont solubles dans l'urée à 30%.

3. Une injection de 20 mgr de Liquoïd pour 1,5 kg de poids produit rapidement une thrombocytopénie, une disparition progressive du fibrinogène et du facteur V, l'apparition dans le plasma d'un matériel précipitable au froid et une nécrose grave du cortex rénal.

4. Un prétraitement à la warfarin élimine toutes ces réactions, exceptée une chute modérée des thrombocytes.

Nous concluons que le Liquoïd produit ces effets non par précipitation du fibrinogène mais par coagulation intravasculaire, probablement mise en train par l'agrégation et l'altération des plaquettes. Ainsi, il se confirme que la coagulation intravasculaire est la dernière étape de la réaction de Shwartzman généralisée.

Zusammenfassung

Liquoid (Natrium polyanethol Sulfonat), ein synthetisches, heparinähnliches Antikoagulans, verursacht bei Kaninchen Nierenrindennekrosen. Diese Schädigung kann nicht von einer generalisierten Shwartzman-Reaktion, welche durch intravasale Gerinnung bei einem entsprechend vorbehandelten Tier auftritt, unterschieden werden. Wir haben diesen anscheinend paradoxen Effekt von Liquoid näher untersucht. Unsere hauptsächlichen Befunde sind:

1. Liquoid ist ein wirksames, heparinähnliches Antikoagulans. 1 mg Liquoid wird von 1 mg Polybren neutralisiert. Nach intravenöser Injektion setzt die antikoagulierende Wirkung sofort ein und besteht durch mehrere Stunden.

2. In größeren Konzentrationen fällt Liquoid Fibrinogen. Der Fällungsvorgang setzt nicht ein intaktes Gerinnungssystem voraus. Die Präzipitate werden in 30%-igem Harnstoff gelöst.

3. Eine intravenöse Injektion von 20 mg Liquoid bei einem Kaninchen mit 1500 g Körpergewicht verursacht frühzeitig eine Thrombozytopenie, eine fortschreitende Verminderung von Fibrinogen und Faktor V, das Auftreten von kältefällbarem Material im Plasma und eine schwere Nierenrindennekrose.

4. Vorbehandlung mit Warfarin verhindert diese Wirkungen vollkommen, abgesehen von einem geringgradigen Absinken der Thrombozytenzahl.

Wir kommen zu dem Schluß, daß Liquoid diese Wirkungen nicht durch Fällung von Fibrinogen, sondern durch eine intravasale Gerinnung auslöst, die wahrscheinlich durch Aggregation und Zerstörung von Plättchen hervorgerufen wird. So wird die intravasale Gerinnung neuerdings als wesentliche auslösende Ursache der generalisierten Shwartzman-Reaktion bestätigt.

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