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From the Department of Pathology, Norwegian College of Veterinary Medicine, Oslo, Research Institute for Internal Medicine, Medical Department B and Institute for Surgical Research, National Hospital, Oslo, Norway.

LIQUOID-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION IN THE BLUE FOX

By

B. Lium, J. Dale and *A. O. Aasen*

LIUM, B., J. DALE and A. O. AASEN: *Liquoid-induced disseminated intravascular coagulation in the blue fox.* Acta vet. scand. 1982, *23*, 570—580. — The present experiments were performed to study the effects of a single high intravenous dose of Liquoid (10 mg/kg body weight) upon platelets, coagulation activities and hematocrit in blue foxes, and their correlation with the survival time. Both "short-living" (< 9 h) and "long-living" (24 h or more) blue foxes showed a marked consumption of coagulation factors, initial fall in fibrinogen, positive ethanol gel test and a gradual decrease in platelet counts. In addition "short-living" animals developed a marked rise in hematocrit, reflecting a considerable increase in vascular permeability. We conclude that activation of plasma proteases has as one of its effects increased permeability in microvasculatory vessels and that this may play a central role for the course and outcome of Liquoid-induced disseminated intravascular coagulation.

Liquoid; coagulation; platelets; hematocrit; blue fox; DIC.

Disseminated intravascular coagulation (DIC) is defined as an acute coagulation occurring in the flowing blood frequently leading to formation of microthrombi and obstruction of the microcirculation. It includes aggregation of platelets, red and white blood cells, and transformation of fibrinogen into fibrin (*Hardaway* 1978). It should be emphasized, however, that DIC is not a disease per se, but is secondary to a wide variety of clinical conditions in man (*McKay* 1965, *Sharp* 1977) and animals (*Schiefer & Searcy* 1975, *Hoffmann* 1976). The coagulation system may be triggered by different pathways (*Dixon* 1973,

Hoffmann 1976). Classically, DIC is induced in experimental animals by systemic application of bacterial endotoxins (*McKay* 1965). Liquoid® (sodium poly-anetholsulfonate) is another substance which, when injected intravenously to rabbits or rats, results in DIC (*Rodriguez-Erdmann et al.* 1960, *Evensen et al.* 1967, *Müller-Berghaus & Lasch* 1970, *Urizar et al.* 1975). Intravenous injections of Liquoid may therefore be used as a simple experimental model to investigate the mechanisms of DIC. Previous reports on Liquoid-induced DIC do not in particular contain information about changes in blood parameters in relation to the survival time. The present experiments were performed to study possible differences in the coagulation disturbances and certain other blood parameters in animals that died shortly after Liquoid injection, as compared with those that lived for 24 h or survived. Recent experiments have shown that the blue fox is suitable for studies of deposition of fibrinoid material within glomerular capillaries after systemic application of Liquoid (*Nordstoga* 1979). We therefore decided to use blue foxes as experimental animals. Patho-anatomical changes will be reported later.

MATERIALS AND METHODS

Animals

Seventeen blue foxes (Alopex lagopus) of both sexes, weighing between 3.6 and 7.2 kg and 2—3 years of age were used as experimental animals. Four animals served as controls (group C). The foxes were bred and raised at the Research Station for Fur Bearing Animals, Heggedal, Norway, and fed the ordinary feed used for the non-experimental animals at the Station. Animals that died within 9 h constituted group A, while animals who lived for 24 h or survived were called group B.

Liquoid ®

Sodium polyanetholsulfonate, (Hoffman-LaRoche, Basel, Switzerland) was freshly prepared by dissolving 10 mg of Liquoid per ml sterile, pyrogen free, isotonic saline. Ten mg of Liquoid per kg body weight were injected intravenously by vena cephalica antebrachii in the experimental animals, and pyrogen free saline was similarly injected into the controls.

Sampling procedure

Immediately before the injection of Liquoid or saline, and thereafter every 4 h, two 4.5 ml samples of blood were withdrawn from the vena cephalica antebrachii or vena saphena into plastic test tubes containing 0.5 ml of 3.2 % sodium citrate. Sampling continued until the animals died, or to 12 h after the injection of Liquoid or saline. From the control animals and 4 of the experimental animals blood samples were also obtained after 24 h.

Assays

The activity of the extrinsic coagulation system was studied with the Thrombotest (TT) (Nyegaard & Co. A/S, Oslo, Norway) (*Owren* 1959), and of the intrinsic by Cephotest (Nyegaard & Co. A/S, Oslo, Norway) (*Janson & Grimmer* 1976). Fibrinogen was quantitated with the electroimmunoassay technique of Laurell (*Laurell* 1972). The ethanol gelatin test for soluble fibrin monomers was carried out as described by *Godal & Abilgaard* (1966). Platelets were counted in a hemocytometer using a modification of Nyegaards method (*Hellem* 1960).

Statistical methods

To measure centrality and distribution of the data, median values and 25 and 75 percentiles were determined. The Wilcoxon rank sum test and test for paired differences were used to test statistical significance. P-values ≤ 0.05 were considered significant.

RESULTS

Survival time

Six of the animals died within 9 h after the injection of Liquoid. Eight of the remaining 11 animals died or were killed about 24 h after the injection and 3 survived. On this background the experimental animals were divided into 2 groups for statistical evaluation of the blood parameters.

Coagulation activities

Thrombotest. During the first 8 h both groups injected with Liquoid showed significantly increasing thrombotest times (TT) as compared with the controls and with the preinjection

values (Fig. 1). In group B the TT coagulation times shortened from 12 to 24 h and were not significantly longer than pre-injection values at 24 h. In group A, the TT values were not statistically different from those observed in group B at 4 h. At 8 h, however, a considerably reduced activity of the coagulation factors in the extrinsic system was observed.

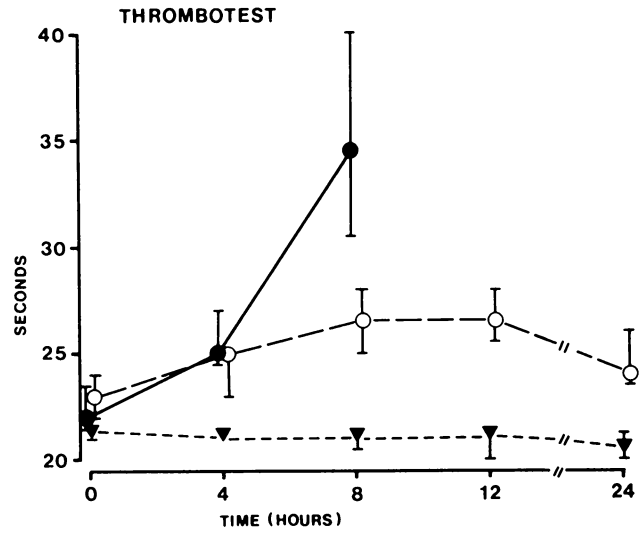

Figure 1. Effect of intravenous injection of a single high dose of Liquoid on the thrombotest times in blue foxes. Group A experiments: (●——●). Controls: (○——○). Group B experiments (▼——▼). (Median, 25 and 75 percentiles are indicated).

Cephotest. After Liquoid injection a significant prolongation of the Cephotest times was observed during the first 8 h in groups A and B compared with controls and with the pre-injection values (Fig. 2). No significant difference in the Cephotest values of groups A and B was observed during the first 8 h, but the mean clotting time was on average 90 s in group A and only 60 s in group B at 8 h. Although the Cephotest gradually became shorter in group B from 8 to 24 h, the values were significantly prolonged throughout the experiments.

Fibrinogen. A significant decrease in the plasma fibrinogen concentration was noted at 4 h after Liquoid. Thereafter the fibrinogen levels in the experimental groups increased progressively from 4 h to circulatory collapse, or to the end of the experiments

(Fig. 3). After 24 h fibrinogen levels were significantly increased when compared with the controls and pre-injection values.

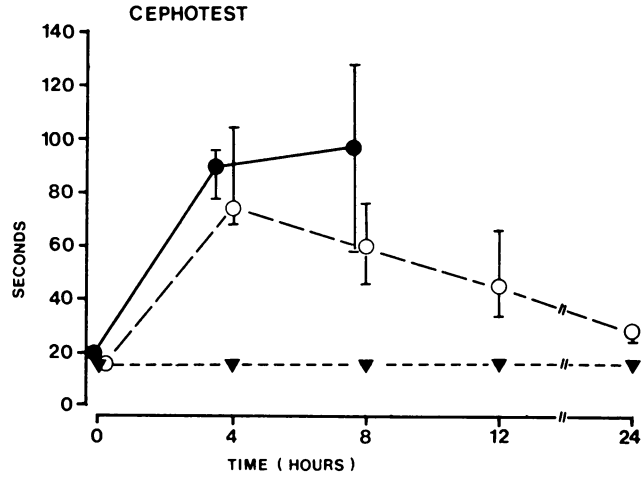

Figure 2. Effect of intravenous injection of a single high dose of Liquoid on the cephotest times in blue foxes. Symbols as in Fig. 1.

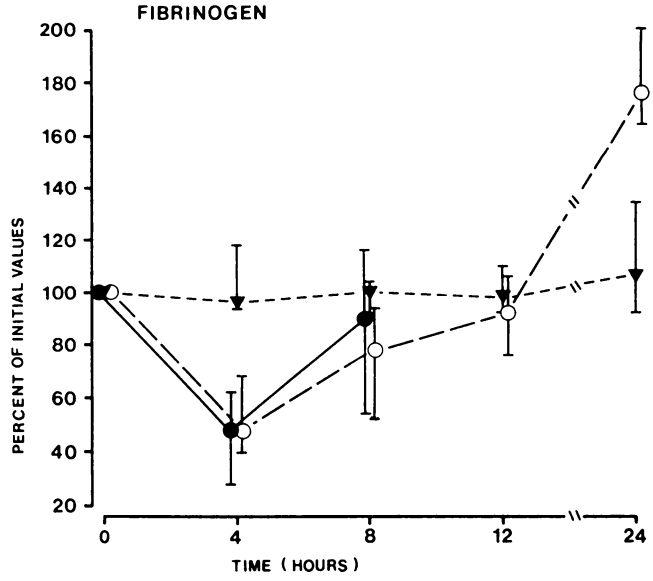

Figure 3. Changes in fibrinogen levels after injection of a single high dose of Liquoid into blue foxes. Symbols as in Fig. 1.

Fibrin monomers. The ethanol gelation test was positive in all samples from group A and in 5 of 7 samples examined in

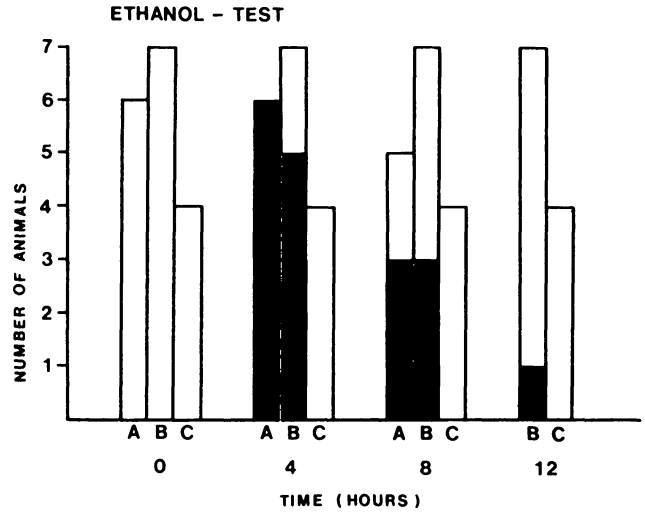

Figure 4. Effect of intravenous injection of a single high dose of Liquoid on the ethanol gelation test. Negative ethanol test: □ Positive ethanol test: ■ Group A experiments: A. Group B experiments: B. Controls: C.

group B after 4 h (Fig. 4). After 8 h, 3 of 5 samples in group A, and 3 of 7 in group B were positive. Only 1 of 7 samples from the latter group was positive after 12 h. In the control animals the ethanol gelation test remained negative throughout the experiments.

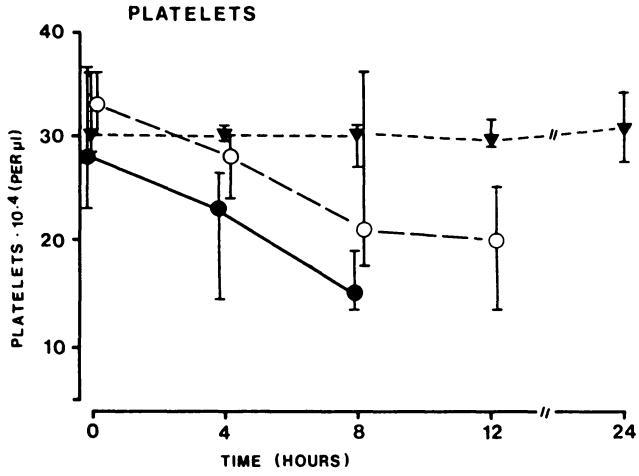

Figure 5. Changes in platelet counts after injection of a single high dose of Liquoid into blue foxes. Symbols as in Fig. 1.

Platelets. In both experimental groups the number of circulatory platelets decreased until 8 h after Liquoid administration (Fig. 5). The reduction was then near 50 % in group A. In group B the number of circulating platelets had decreased by 35 % after 12 h. Both these values were significantly lower than the preinjection values and the controls.

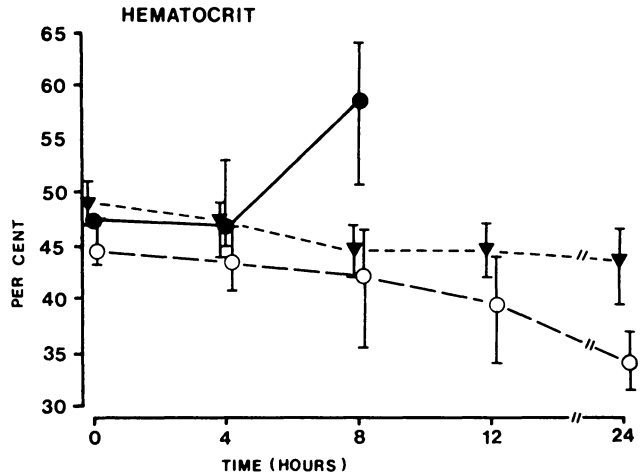

Figure 6. Changes in hematocrit values after injection of a single high dose of Liquoid into blue foxes. Symbols as in Fig. 1.

Hematocrit. The hematocrit values in blood samples from group B and controls decreased slightly for the first 12 h (Fig. 6) and more markedly thereafter in animals which had received Liquoid. By contrast, the hematocrit values increased considerably in group A, reflecting a pronounced terminal hemoconcentration.

DISCUSSION

The present study clearly demonstrates that a single intravenous injection of Liquoid induces activation of the intrinsic and extrinsic pathways of coagulation in blue foxes. The TT-values at 8 h indicate a more pronounced consumption of extrinsic coagulation factors in the foxes that died early than in the others. This might reflect a more severe tissue damage in the former group of animals. A characteristic difference between animals that died within 9 h of experimentation and animals that lived longer was the very pronounced terminal hemoconcen-

tration observed in the former group. Such a rise in hematocrit leads to increased blood viscosity and reduced microvasculatory blood flow (*Guyton* 1976). Slowly moving blood is acidotic and hypercoagulable and predispose to thrombosis (*Hardaway* 1979). Endothelial injury is thought to play an important role in the pathogenesis of Liquoid-induced intravascular coagulation (*Evensen & Shepro* 1973). Endothelial damage and higher vascular permeability increase the amount of plasma that comes in contact with collagen and the basement membrane. This is thought to result in activation of the Hageman factor (*Griffin & Cochrane* 1979) and thereby of the intrinsic coagulation mechanism, but also of the plasma kallikrein-kinin systems, the fibrinolytic and the complement systems (*Murano* 1978).

Thromboplastin is found in high concentrations in the plasma membranes of all vascular endothelia (*Zeldis et al.* 1972) and Liquoid-induced trauma to the vascular endothelium might therefore initiate coagulation via the extrinsic system. Furthermore, the prostacyclin synthesis is disturbed in damaged endothelial cells, predisposing to platelet aggregation and thrombosis (*Moncada & Vane* 1979). Electron microscopic studies have demonstrated that even minimal injury to the vascular endothelium results in platelet adhesion and fibrin formation (*Ashford & Freiman* 1968).

In our experiments platelet counts decreased gradually. Others have reported that the platelet counts drop immediately and reach a minimum value within 10 min after the intravenous injection of Liquoid (*Evensen & Jeremic* 1968, *Müller-Berghaus & Lasch* 1970). The observed fall in platelet counts may be caused by an interplay of many factors. Liquoid appears to aggregate platelets directly with release of platelet factors (*Evensen & Jeremic* 1968, *Iatridis et al.* 1980). Thrombin formed by the Liquoid-activated coagulation systems stimulate thromboxane synthesis which induces the platelet release reaction and platelet aggregation (*Gorman* 1979). Platelets do not seem to be essential for initiation of the coagulation activation caused by Liquoid injections (*Evensen & Jeremic* 1968). A marked hemolysis was observed in the present experiments (unpublished data). This indicates erythrocyte damage and thereby release of ADP and thromboplastic material (*Quick et al.* 1954, *Bishop et al.* 1959, *Gaarder et al.* 1961, *Hardaway* 1979), which stimulate platelet aggregation and intravascular coagulation, and thereby contribute to maintain DIC in this model.

The initial fall in fibrinogen values in the experimental groups is thought to be due to consumption of fibrinogen in intravascular coagulation, which leads to a transient elevation of fibrin monomers reflected by the positive ethanol gelation test. This seems to initiate an acute phase response leading to significantly increased fibrinogen levels later after the Liquoid injection. High fibrinogen levels is a common response to DIC (*Hardaway* 1978).

The blue foxes reacted somewhat differently to Liquoid administration, and the time period from 4 to 8 h after the injection appeared to be critical. The homeostatic mechanism of some animals collapsed at this time. They underwent an anaphylaxis-like reaction, probably associated with increased vascular permeability with hemoconcentration, widespread and intense intravascular clotting, resulting in irreversible shock and death. In other foxes a more moderate DIC was initiated, and the animals' defence mechanism better managed to correct the disturbances. These animals did not develop hemoconcentration. The cause of death among these animals most likely was multiple organ failure secondary to focal necrosis caused by microinfarcts in vital organs (*Rodriguez-Erdmann et al.* 1960, *Nordstoga* 1977). In conclusion activation of plasma proteases, and perhaps direct endothelial damage with increased permeability in microvasculatory vessels and marked hemoconcentration seems to be essential pathogenetic factors for the development of DIC and irreversible shock in this experimental model.

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SAMMENDRAG

Liquoid-indusert disseminert intravaskulær koagulasjon hos blårev.

I denne artikkelen beskrives forandringene i blodplatetall, koagulasjonsaktiviteter og hematokrit etter at en enkelt, høy dose, Liquoid (10 mg/kg) ble gitt intravenøst til blårev. Resultatene diskuteres i relasjon til dyrenes overlevelsestid etter injeksjonen. Både dyr som døde kort tid etter injeksjonen (< 9 t) og dyr som levde 24 t eller mer viste markert forbruk av koagulasjonsfaktorer, signifikant nedgang i fibrinogen nivå, positiv ethanol-gel test og gradvis fall i blodplatetall. Rever som døde innen 9 t etter injeksjonen utviklet dessuten markert stigning i hematokrit umiddelbart før de døde, trolig som følge av økt karpermeabilitet. Vi konkluderer med at aktivering av plasma proteaser og økt karpermeabilitet er sentrale mekanismer ved utvikling av Liquoid indusert sjokk.

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Reprints may be requested from: Bjørn Lium, the Department of Pathology, Norwegian College of Veterinary Medicine, P.O. Box 8146, Dep., Oslo 1, Norway.