CONVULSIVE SYNDROME IN RABBITS INJECTED WITH FAT EMULSION AND HEPARIN

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It is well known that when no glucose is administered to a hepatectomized animal, it soon shows signs of hypoglycemia. The most striking symptoms of this condition are physical weakness and convulsions, followed by death. An adequate amount of intravenously injected glucose easily prevents or reverses this syndrome (1, 2).

During experiments designed to follow the fate of intravenously injected fat emulsion in functionally hepatectomized rabbits we observed that such animals injected with both fat emulsion and heparin had convulsions within 2 hours, despite intermittent administration of glucose in amounts that kept other rabbits in good condition for over 6 hours. Since glucose had no effect on these convulsions, we investigated more thoroughly the circumstances leading to this syndrome and some of its possible mechanisms. It was found that massive hydrolysis of the injected fat was the probable cause of this convulsive syndrome.

Materials and Methods

Mongrel rabbits weighing 2.0 to 3.0 kg were used.

Functional hepatectomy was performed as described previously (3). The operated animals were injected intravenously with a solution containing glucose (5 per cent), insulin (0.05 IU per ml), and norepinephrine (0.01 mg per ml), every 30 minutes, starting immediately after the operation. For sham operations we used the same procedure as for the hepatectomy, but loose knots around the vessels replaced the ligatures.

The triglyceride emulsion used was a 50 per cent suspension of coconut oil in water (ediol®). Triton WR-1339 (Winthrop Labs, Inc., New York) is a para-tert-octyl phenol polyether. All injections were given intravenously in the marginal ear vein. The dose of fat emulsion was 4 ml per kg body weight; of triton 400 mg per kg body weight and of heparin 5 mg per kg body weight.

Plasma non-esterified fatty acid levels were determined following the method of Dole and Meinertz (4); plasma total esterified fatty acids according to the method of Morgan and Kingsbury (5). The blood samples for both these determinations were obtained by heart puncture and immediately cooled in ice water and centrifuged in the cold.

Plasma bicarbonate concentrations were determined titrimetrically by the method of Van Slyke, using a mixture of phenol red and methylene blue as the indicator (6). The blood samples for these determinations were obtained from the inferior vena cava *via* a polyethylene catheter inserted through the femoral vein under local anesthesia.

EXPERIMENTS

1. Induction of Convulsive Syndrome in the Rabbit.—Functional hepatectomy under ether anesthesia could be performed in 20 minutes. The rabbits recovered almost immediately from the operatory shock and assumed a normal sitting position as soon as they were released from the operation table. They were alert and showed normal reactions toward light, sound, or needle pricks. When no glucose was administered the animals became gradually weaker and shaky, collapsed slowly, and lost consciousness; convulsions and death soon followed these prodromes. The symptoms usually started within the 1st hour after the hepatectomy. Intravenous injection of 5 to 10 ml of a 5 per cent glucose solution, to which 5 IU of insulin and 1 mg of norepinephrine per 100 ml were added, stopped the convulsions within seconds and the other symptoms within 1 or 2 minutes. In all the experiments which are now to be reported the hepatectomized rabbits received intravenous injections of 5 ml of this glucose solution every 30 minutes, starting immediately after the operation. With this treatment all symptoms of hypoglycemia were prevented, and the animals could be followed for more than 6 hours if necessary. They were placed freely upon a cloth on the floor and continuously observed. Sham-operated rabbits received the same treatment.

Forty rabbits underwent functional hepatectomy and were injected intravenously with 4 ml of a 50 per cent coconut oil emulsion per kg body weight, and 5 mg of heparin per kg body weight. These injections had no visible immediate effect on the rabbits, but roughly 1½ hours later the animals seemed to become somnolent and responded less to environmental stimuli. In typical cases the rabbit slowly dropped its head in a spasmodic way between its forepaws. A few moments later the animals had convulsions of the whole body; these stopped after about 1 minute leaving the animals lying on their sides with a strong opistotonus, the legs resisting passive exercising. After these attacks respiration was very superficial and the animals no longer reacted to needle pricks, light, or sound. A second attack usually came within 15 minutes. In most cases it was milder than the first, but sometimes it was heavier. Third and fourth attacks, usually milder, occurred at irregular intervals. The animals remained comatous between the attacks. Two of the 40 rabbits that received the emulsion and heparin after hepatectomy did not show this syndrome; one died 50 minutes after the operation, the other was killed after 6 hours of observation. Nothing unexpected was found at the autopsy.

The average time at which the first convulsions occurred was 76 minutes (standard deviation, ±25.6, with normal distribution according to the test of Dixon and Massey, reference 7).

These rabbits were extremely weak after the first attack, so that taking blood samples or too much manipulation was often lethal. For this reason we determined the survival time up to 6 hours in 12 animals that were not touched after the operation and injections, except for the injections of glucose. Two other groups were hepatectomized and injected with either heparin or fat emulsion; a fourth group received both injections but was sham-operated. In Table I it is shown that 11 of the 12 rabbits that were hepatectomized and given both

injections showed the convulsive syndrome. Four of these animals died within ½ hour after the first attack; 5 survived throughout the observation period. In 4 of these last rabbits the opistotonus diminished towards the end of the observation; they gradually regained consciousness and could sit upright again.

TABLE I

Occurence of Convulsive Syndrome after Intravenous Injection of Fat Emulsion and Heparin in Hepatectomized Rabbits, and Its Prevention by Triton

Treatment*	No. of rabbits	No. of rabbits displaying convulsive syndrome	Survival time
Hepatectomy Fat emulsion, intravenously Heparin, intravenously	12	11	4 rabbits died within 30 min after the first convulsive attack; 5 survived for more than 4 hours, or 6 hours after the operation.
Hepatectomy Fat emulsion, intravenously	10	None	All rabbits survived for more than 6 hours after the injection of fat emulsion and/or heparin.
Hepatectomy Heparin, intravenously	8	None	
Sham hepatectomy Fat emulsion, intravenously Heparin, intravenously	4	None	
Hepatectomy Fat emulsion, intravenously Heparin, intravenously Triton, intravenously	6	None	

* For detailed description see Materials and Methods.

In the other groups convulsions, opistotonus, or coma were completely absent, and all animals survived.

Since neither heparin nor fat emulsion alone caused convulsions in hepatectomized rabbits, we concluded tentatively that this syndrome could be related to the level of non-esterified fatty acids in the plasma of animals injected with both. It is well known that a lipase appears in the plasma after intravenous injection of heparin in rabbits as well as in other species including man (8). This enzyme, lipoprotein lipase or clearing factor, could have acted on the unusually high supply of substrate in rabbits that had also received an injection of fat emulsion, whereas in those that received only heparin or fat

TABLE II

Plasma Fatty Acids

	Treatment*		Pre	5 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
I	Hepatectomy‡	No.	7	8	9	6	2	1	—	5
	Fat emulsion, intravenously	Average§	0.4	2.0	8.4	7.9	11.6	8.7	—	2.0
	Heparin, intravenously	Range§	0.3–0.6	0.9–3.2	5.8–13.3	4.0–11.4	11.3–11.9	—	—	1.7–2.4
II	Hepatectomy	No.	8	8	8	6	3	3	3	3
	Fat emulsion, intravenously	Average	0.4	2.0	3.2	4.0	5.9	5.7	2.7	2.4
		Range	0.1–0.6	1.4–4.2	1.1–6.7	1.4–7.6	4.1–8.9	3.5–9.4	1.8–3.8	1.0–5.0
III	Hepatectomy	No.	5	6	6	6	4	2	2	—
	Heparin, intravenously	Average	0.4	0.6	0.5	0.5	0.6	0.7	0.6	—
		Range	0.2–0.6	0.3–0.8	0.4–0.6	0.4–0.6	0.5–0.8	0.5–0.9	0.5–0.6	—
IV	Sham hepatectomy	No.	4	4	4	4	4	4	4	3
	Fat emulsion, intravenously	Average	0.7	2.0	5.2	3.3	0.9	0.7	0.6	0.5
	Heparin, intravenously	Range	0.4–1.1	1.4–3.1	3.8–6.8	1.5–6.7	0.7–1.2	0.6–0.8	0.5–0.7	0.4–0.5
V	Sham hepatectomy	No.	5	5	5	5	5	5	—	—
	Fat emulsion, intravenously	Average	0.7	2.5	3.7	3.2	2.2	1.1	—	—
		Range	0.4–0.8	1.5–3.3	2.5–5.6	2.7–3.7	1.3–2.7	0.8–1.8	—	—
VI	Hepatectomy	No.	8	8	8	8	5	3	3	3
	Fat emulsion, intravenously	Average	0.4	1.1	0.6	0.5	0.5	0.7	0.5	0.7
	Heparin, intravenously	Range	0.2–0.6	0.8–2.1	0.3–0.8	0.3–1.1	0.4–0.8	0.4–1.1	0.4–0.8	0.4–1.1
	Triton, intravenously									
VII	Hepatectomy	No.	6	6	6	6	6	5	3	2
	Fat emulsion, intravenously	Average	0.7	1.7	0.8	0.6	0.6	0.7	0.9	0.9
	Triton, intravenously	Range	0.4–1.1	1.2–2.6	0.4–1.2	0.4–1.0	0.4–0.8	0.6–0.9	0.6–1.3	0.8–1.0

* For detailed description see Materials and Methods.

‡ All rabbits of this group showed the convulsive syndrome.

§ Given in mEq/liter.

emulsion, the production of fatty acids would have been limited by the smaller amount of either substrate or enzyme.

This working hypothesis could be tested by giving an intravenous injection of the non-ionic detergent triton to 6 hepatectomized rabbits before the fat emulsion and heparin were administered. Triton is a strong inhibitor of lipoprotein lipase (9). In these rabbits it gave full protection against the convulsive attacks, and increased the survival time as compared to the group that received the same treatment but no detergent (Table I).

2. Plasma Fatty Acids in Hepatectomized Rabbits Injected with Fat Emulsion and Heparin, and in Control Groups.—Since the previous result was in agreement with our hypothesis that the convulsions could be related to the intravascular hydrolysis of the injected triglycerides, we studied the evolution of plasma fatty acids in hepatectomized rabbits injected with fat emulsion and heparin, and in different control groups. Seven groups of rabbits were used in this experiment.

Five groups were hepatectomized and received one of the following treatments: intravenous injection of fat emulsion and heparin; fat emulsion alone; heparin alone; fat emulsion, heparin, and triton; fat emulsion and triton. Two groups were subjected to sham hepatectomy and injected with fat emulsion and heparin, and fat emulsion alone respectively. Plasma fatty acids were determined before treatment, 5 minutes after the last injection, and from then on every hour up to 6 hours. It was impossible to make a complete series of determinations for each individual rabbit. This would have required 8 blood samples of 5 ml per rabbit within a period of 6 hours, which would have influenced the results. We already mentioned that rabbits with convulsive syndrome were to weak to survive this trauma. Therefore, we took not more than 4 blood samples from the same rabbit. The preparation and extraction of the plasma samples are described in the section on Materials and Methods. Total esterified fatty acids were also determined.

The results of this experiment are summarized in Table II, which shows the average plasma fatty acid values, their range and the number of determinations on which these figures are based. The plasma fatty acids of all groups injected with fat emulsion were raised significantly after 5 minutes, which may be ascribed to the presence of free fatty acids in the fat emulsion. All animals of the first group showed the convulsive syndrome. One hour after the injections of fat emulsion and heparin their average plasma fatty acid level was 8.4 mEq per liter. Comparable high values were found until 4 hours after the injections.

In hepatectomized rabbits injected with fat emulsion alone (group 2) the average plasma fatty acid level showed a smaller rise than in the first group. This increase can be ascribed to the hydrolysis of the injected triglycerides by a lipase appearing in the plasma after intravenous injection of fat emulsion. It has indeed been described that when plasma of rabbits injected with fat emulsion was incubated at 37°C the *in vitro* production of fatty acids was much higher than in normal rabbit plasma to which the fat emulsion was added

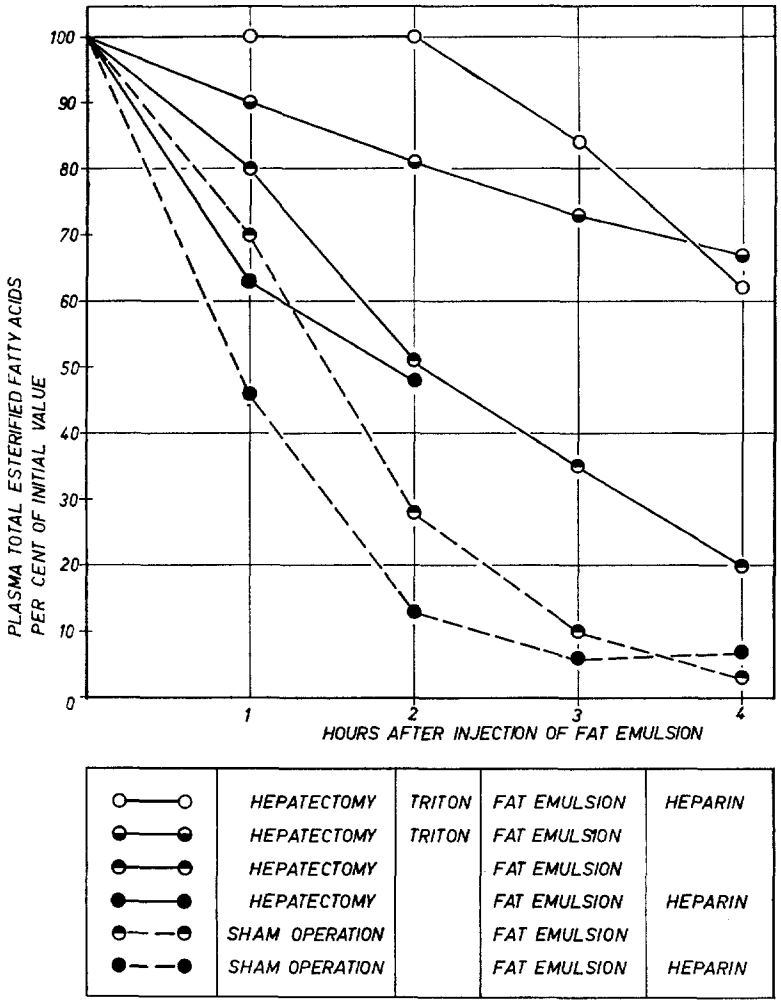

FIG. 1. Influence of heparin and triton on the plasma total esterified fatty acid levels after injection of fat emulsion in hepatectomized and sham-operated rabbits.

in vitro (10). This lipase activity however remained far below that obtained after intravenous injection of heparin (11).

In hepatectomized rabbits injected with heparin alone (group 3) the plasma fatty acid levels remained low probably because the hydrolysis of the normal low triglyceride levels of these animals cannot yield a marked increment of fatty acids.

The influence of hepatectomy on the fatty acid levels can be assessed by comparing the values obtained in the sham-operated rabbits that received

fat emulsion and heparin (group 4), with those of the first group. One and 2 hours after the injections the fatty acid levels reached a lower average in the sham-operated rabbits, and later on almost normal levels were found. Sham-operated rabbits injected with fat emulsion alone (group 5) had fatty acid levels that after 1 and 2 hours were similar to those of the hepatectomized group that had received the same injection (group 2), but from then on the levels started to decrease whereas in the hepatectomized group they still increased.

A possible explanation for the generally higher fatty acid levels found in hepatectomized rabbits may be that the sequestration of the liver directly affected the removal of fatty acids from the plasma (12, 13). A second probable factor may be sought in the concomitant higher values of plasma total fatty acid esters (Fig. 1). The uptake of fat emulsion by the liver is an important mechanism for the disappearance of injected fat (14, 3) and our hepatectomized rabbits had indeed higher plasma fatty acid ester concentrations than the sham-operated groups. It may be concluded that more substrate for the postheparin lipoprotein lipase or the lipase appearing after injection of fat emulsion was available in hepatectomized rabbits than in the sham-operated ones.

The influence of triton on the fatty acid levels was very clear; animals that had received triton showed only a small and transient rise of fatty acids immediately after injection of the fat emulsion. Whether heparin was also injected with the detergent and the fat emulsion (group 6) or not (group 7) made no difference, which demonstrates that triton is an effective inhibitor of the lipoprotein lipase as well as of the lipase mobilized by the fat emulsion itself. Fatty acid ester levels also remained very high in these groups, confirming our previous findings (3).

The results of Table II do not establish a causal relationship between plasma fatty acid levels and the convulsive syndrome. Although the average fatty acid levels were much higher in the group that showed convulsive syndrome than in any other group, the individual values obtained in several of the control groups overlapped those of the first. This overlapping was especially notable at 1 or 2 hours after the injections, which was the period in which the convulsions started for the vast majority of animals in the first group. The results thus are at variance with the hypothesis that there exists a critical plasma fatty acid level at which convulsions appear. A causal relationship between fatty acids and convulsions is possibly not to be sought in the plasma fatty acid level itself, but rather in the total amount of fatty acids absorbed by the extra hepatic tissues in the hepatectomized animals injected with fat emulsion and heparin.

3. Reversal of Convulsive Syndrome.—We explored the possibility of reversing an established convulsive syndrome. Our attempts were based on the hypothesis that changes secondary to lipolysis might have caused the syndrome.

(*a*) A possible direct consequence of the liberation of unusually high amounts

of fatty acid in the plasma could be a disturbance of the acid-base equilibrium of the plasma. Normal rabbits were found to have a plasma bicarbonate reserve of about 24 mEq per liter (see Fig. 2). Roughly calculated, this means a total of 2.5 mEq of circulating bicarbonate in a rabbit of 2.5 kg body weight. Complete hydrolysis of the standard dose of injected fat emulsion for a rabbit of that size would yield an estimated 20 mEq of fatty acids, enough to exhaust the bicarbonate reserve many times over.

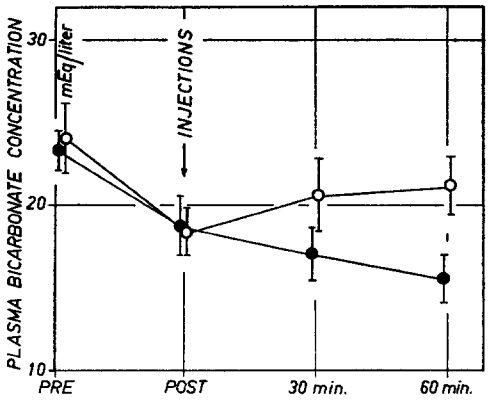

FIG. 2. Influence of triton on the plasma bicarbonate concentration in hepatectomized rabbits, injected with fat emulsion and heparin.

●, hepatectomy + fat emulsion + heparin;

○, hepatectomy + triton + fat emulsion + heparin;

I, standard deviation ($N - 1 = 5$);

PRE, before operation;

POST, after operation.

We followed the bicarbonate reserve of hepatectomized rabbits injected with fat emulsion and heparin, and compared it with that of rabbits that received the same treatment but also an intravenous injection of triton. Each group consisted of 6 animals; blood samples were obtained from the inferior vena cava before and after the hepatectomy, and 30 minutes and 1 hour after the injections. The plasma bicarbonate concentration was determined in each sample.

The initial bicarbonate concentration was between 23 and 24 mEq per liter in both groups (Fig. 2). Hepatectomy caused this value to drop to less than 19 mEq per liter. In the group injected with triton the bicarbonate concentration increased during the next hour, while in the other it decreased to less than 16 mEq per liter.

The interpretation of these results is complicated by the fact that the bicarbonate concentration was depressed more by hepatectomy under ether anesthesia than by the subsequent injections with fat emulsion and heparin. Even in the group that received triton, with minimal production of fatty acid

(see Table II), the bicarbonate did not return to its pretreatment level after 1 hour. It is clear that although the fall of the bicarbonate reserve with lipolysis was in favor of our hypothesis, the acid-base equilibrium was also disturbed by other factors.

(*b*) Since calcium and fatty acids form an insoluble complex, it seemed possible that the concentration of circulating calcium ions would have been decreased in the presence of such large amounts of fatty acid as were found in

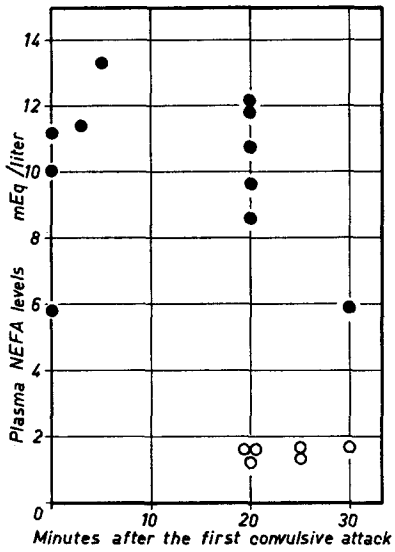

FIG. 3. Plasma non-esterified fatty acid levels at different times after the first convulsive attack in hepatectomized rabbits injected with fat emulsion and heparin.

○, animals which received triton immediately after the first convulsive attack;

●, no triton injections.

convulsive rabbits, and that the syndrome might have been caused by hypocalcemia.

We tried to prevent or reverse the convulsions by giving repeated intravenous injections of calcium chloride to 4 hepatectomized rabbits that had received fat emulsion and heparin. Although total doses of 400 to 600 mg of calcium chloride were administered, none of these animals was protected against convulsion, and the clinical picture of the syndrome was not altered. Convulsions, already established, were unaffected by treatment with calcium. The hypothesis thus could not be confirmed.

(*c*) Since trtion prevented convulsions, we investigated whether this detergent might stop convulsions that were already established.

Eight rabbits were hepatectomized and given fat emulsion and heparin. Triton was administered immediately after the first convulsive attack. Two rabbits died after about 1 minute. The 6 remaining animals showed definite signs of improvement after about 15 minutes: their opistotonus diminished and they gradually regained consciousness. Within ½ hour they all spontaneously sat upright, at which time a blood sample was taken for fatty acid determination.

In Fig. 3 the result of these determinations is given and compared to values obtained from controls that did not receive triton. At the first convulsive attack the plasma fatty acid levels ranged from 5.9 to 13.2 mEq per liter, and comparable values were found 20 to 30 minutes later in rabbits that did not receive triton. Contrastingly, the 6 animals that were given triton after their first convulsive attack all had fatty acid levels of less than 2 mEq per liter at the moment of their recovery.

This result is another indication that the production of fatty acid is closely related to the convulsive syndrome. Fatty acids have the most rapid turnover of all plasma lipids (15), and there exists evidence that triton does not decrease it (16). Our results are in agreement with the hypothesis that the lipolysis stopped with the administration of the detergent, and that a quick elimination of circulating fatty acids brought the levels down to near physiological values in less than ½ hour.

DISCUSSION

The induction of a convulsive syndrome by intravenous injection of triglycerides and heparin in hepatectomized rabbits has not been previously described. Our experiments do not explain the mechanism of this syndrome but suggest that the large amounts of fatty acid liberated from triglycerides under the influence of postheparin lipoprotein lipase are an essential link in the chain of reactions that leads to the convulsions. Although we did not find a critical concentration of plasma fatty acid at which the rabbits became convulsive, the observation that the syndrome could be prevented or reversed by intravenous injection of triton, which inhibited lipolysis, strongly suggested this relationship.

The plasma fatty acid levels found in many of our experimental rabbits were 10 to 20 times higher than in normals. In view of the rapid turnover of plasma fatty acid, this accumulation illustrates the activity of postheparin lipoprotein lipase. The intravenous administration of fat emulsion to rabbits results already in a marked rise of plasma fatty acid (Table II), probably due to lipase activity induced by fat emulsion (10, 11). It is conceivable that the appearance of this lipase is secondary to a release of endogenous heparin, and identical with lipoprotein lipase. The amount of enzyme released after injection of fat would then be limited by the available amount of endogenous heparin, since the simultaneous injection of heparin causes a still higher lipase activity.

In hepatectomized rabbits the injection of heparin with fat emulsion leads to an extremely high plasma fatty acid level, since the liver no longer participates in the removal of the fatty acids. That the peripheral tissues actively remove plasma fatty acids is illustrated by the experiment in which fatty acids promptly dropped to almost normal levels after the injection of triton into hepatectomized rabbits with convulsive syndrome (Fig. 3).

The possibility remains that the anticonvulsive effect of triton may be independent of its antilipolytic action. Triton alters the plasma lipoproteins (17), has antiatherogenic properties (18), prevents the uptake of fat emulsions by the liver (3), lowers the surface tension of the plasma (19), and is an *in vivo* tuberculostatic for many species (20, 21). Considering these divergent effects, one must recognize the possibility that the anticonvulsive action may be another as yet undefined pharmacological property of triton.

SUMMARY

Functionally hepatectomized rabbits showed convulsions and coma within 2 hours after the intravenous injection of fat emulsion and heparin.

Intravenous injection of the detergent triton prevented this syndrome, if given before the fat emulsion and heparin, and reversed it if given subsequently.

Extremely high plasma non-esterified fatty acid levels were noted in rabbits showing the convulsive syndrome, but not in animals that received the detergent.

The mechanism of the convulsions probably includes the toxicity of high amounts of non-esterified fatty acids.

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