Effect of Heparin on Fate of Intravenously Administered Fat Emulsions in Rats.* (20880)

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The injection of heparin is known to produce clearing of the turbidity of plasma associated with alimentary lipemia(1). A previous report from this laboratory(2) described experiments showing that plasma from rats which had received an intravenous injection of heparin was capable of clearing *in vitro* the turbidity caused by addition of fat emulsion to the plasma.

The present study was undertaken to determine what effect simultaneous injection of heparin and fat emulsion would have on the rate of disappearance of fat from the bloodstream and on the uptake of fat by various organs. Several related phenomena were also investigated.

Methods. The fat emulsion used in this study was prepared by and kindly supplied by Dr. Douglas Frost and his associates of Abbott Laboratories, North Chicago, Ill. It contained 10 g of sesame oil, 0.5 g of Tween 60 and 5 g of glucose per 100 ml. It was prepared by high pressure homogenization and the particle size was one μ or less. Forty-eight male Sprague-Dawley rats weighing between 200 and 250 g received a single rapid injection of 33 ml/kg of this emulsion into the tail vein. One-half of the animals received 10 mg/kg of heparin (Abbott) intravenously simultaneously with the fat emulsion. Three rats from the heparin-treated group and 3 from the group without heparin treatment were sacrificed at each of the following intervals after injection: 5, 10, 20, 40, 80, 120, 240, and 480 minutes. At the time of sacrifice blood samples were drawn from the abdominal aorta under ether anesthesia. The liver and spleen were removed for total lipid analysis by the method of Geyer *et al.* (3). The total esterified fatty acid concentration of the blood serum was determined by the method of Bauer and Hirsch(4). Six rats which had not been injected with fat emulsion were used to obtain control values. In the studies on hemolysis, plasma or serum hemoglobin was determined by the method of Flink and Watson(5). Before performing hemoglobin determinations on lipemic plasma or serum samples these were clarified by shaking with an equal volume of chloroform, centrifuging and separating the supernatant plasma.

Results. Fig. 1 presents the results of serum esterified fatty acid determinations at various intervals after injection of fat emulsion with and without heparin. Each point on the graph represents the mean value of determinations on 3 rats. The blood lipid levels were approximately the same in the 2 groups of animals during the first 10 minutes after injection but thereafter the group which received heparin showed a much more rapid return to control levels. As early as 20 minutes after injection there was a striking

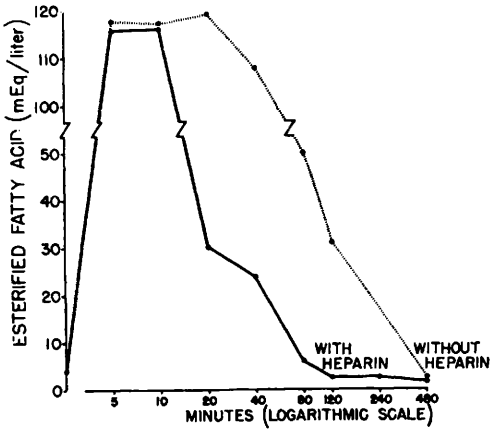

FIG. 1. Serum esterified fatty acid levels of rats at various times after intravenous injection of 33 ml/kg of fat emulsion with and without 10 mg/kg of heparin.

* The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any governmental agency.

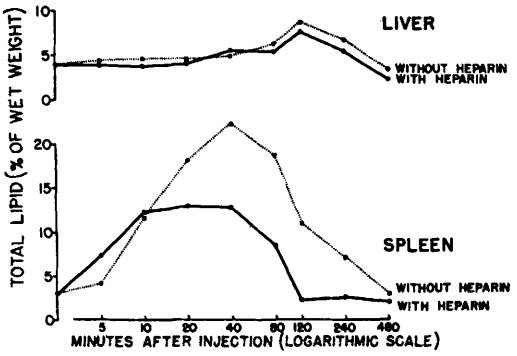

FIG. 2. Total lipid concentration of liver and spleen of rats at various intervals after intravenous injection of 33 ml/kg of fat emulsion with and without 10 mg/kg of heparin.

difference in blood fat levels. The centrifuged blood specimens at the 40-minute interval from the group which did not receive heparin showed marked turbidity whereas those from the group which received heparin showed no turbidity in spite of the fact that the serum fat level of the latter group was still substantially elevated above the control value at this time. This indicates that loss of turbidity proceeds more rapidly than removal of fat from the bloodstream but that both processes are accelerated by heparin.

Figure 2 depicts the results of the total lipid determinations on the spleens and livers. In the spleen the lipid concentration rose progressively during the first 40 minutes and then gradually returned to control values. In the group of rats which received heparin the rise in lipid concentration in the spleen was smaller and the return to the control level was more rapid. The magnitude of the uptake of the injected fat by the spleen is striking. In a separate study the mean weight of the spleen was found to increase from a control value of 2.2 g/kg body weight to 3.7 g/kg one hour after intravenous injection of 33 ml/kg of fat emulsion. The mean per cent of injected fat recovered from the spleen at this time was 30%. In the liver (Fig. 2) the increase in lipid concentration takes place much later after fat injection than in the case of the spleen and is much smaller in magnitude. However, since the weight of the liver is roughly 10 times that of the spleen a given percentage rise in lipid concentration in the liver represents 10 times as much fat on an absolute basis. The increase in liver lipid concentration takes place at a time when the spleen lipid concentration is rapidly decreasing. Although the lipid concentration in the liver is lower in the animals which received heparin, the difference is small.

In the course of the studies on the rate of disappearance of fat from the blood after intravenous injection it was noted that the lipemic samples always showed considerable hemolysis. A study was made of the effect of heparin injection on the degree of hemolysis in lipemic sera. A study also was made to determine whether alimentary lipemia would produce hemolysis. The results of this study appear in Table I and show that both oral administration and intravenous injection of fat lead to hemolysis. The lesser degree of hemolysis seen in the plasma of the rats given the fat emulsion orally as compared with those given the emulsion intravenously is probably due to the lower fat concentration in the serum of the former group. The results of the experiments in which heparin was given together with fat emulsion intravenously appear in Fig. 3. The values in the graph are the means of determinations on sera from 3 animals in each group. It will be noted that marked and equal elevation of plasma hemo-

TABLE I. Serum Hemoglobin and Lipid of Rat Blood after Oral or Intravenous Administration of Fat Emulsion.

No. of rats	Treatment	Esterified fatty acids (mEq/l)	Serum hemoglobin (mg %)
10	Untreated controls	4.64 ± .78	7.17 ± 1.64
12	10 ml/kg fat emulsion by stomach tube 4 hr before drawing blood	14.61 ± 1.29	86.5 ± 9.58
10	10 ml/kg fat emulsion intrav. 5 min. before drawing blood	118.12 ± 14.17	427.7 ± 4.42

Figures following mean values are stand. errors.

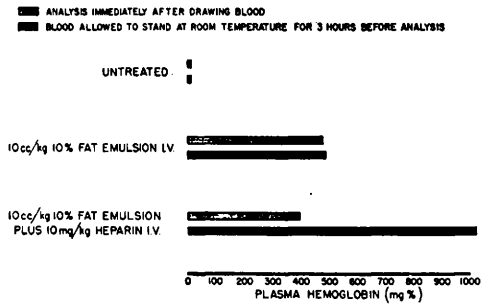

FIG. 3. Plasma hemoglobin of rats 5 min. after intravenous injection of fat emulsion with or without heparin.

globin occurred in both the group which received fat emulsion with heparin and the group which received fat emulsion alone. However, when the blood samples were permitted to stand at room temperature for 3 hours a marked further increase in plasma hemoglobin occurred in those from the group which had received fat emulsion plus heparin, whereas no change was seen in the samples from the group which had received only fat emulsion.

Discussion. The results of this study indicate that heparin is capable of producing clearing of lipemia induced by injection of fat emulsions in the same manner that it has previously been shown to produce clearing of alimentary lipemia. Under the influence of heparin not only is the physical state of the fat in the blood altered so that turbidity is decreased but also the fat leaves the blood more rapidly. This extremely rapid disappearance of fat from the blood cannot be accounted for by increased rate of uptake of the fat by the liver or spleen, as the concentration of lipid in these organs is actually decreased. Since accumulation of fat in the spleen occurs after intravenous but not after oral administration of fat, by reducing splenic uptake of fat heparin causes intravenously injected fat to behave more nearly like orally administered fat.

Creditor(6) has shown that intravenous injection of fat emulsion produces a marked increase in mechanical fragility of red blood cells. Weld and Spitzer(7) found that the injection of heparin into dogs with alimentary lipemia produced a decrease in the antihemolytic activity of the plasma although lipemia alone caused no significant change. The question arises as to whether the elevation of serum hemoglobin seen in lipemic blood is due to *in vivo* hemolysis or whether it is due to hemolysis occurring during and after blood drawing as a result of increased red blood cell fragility. Although the studies of Johnson *et al.*(8-10) suggest that lipemia produces *in vivo* hemolysis, studies failed to show an elevation of biliary bilirubin excretion rate after intravenous injection of fat emulsion with or without heparin(11). We interpret our results to indicate that the elevated serum hemoglobin values occurring with lipemia following ingestion or injection of fat are due to *in vitro* hemolysis resulting from the increased fragility of red blood cells in the presence of fat. The intravenous injection of heparin apparently produces a delayed increase in the magnitude of this *in vitro* hemolysis. We and others(12) have found that free fatty acid is released when post-heparin plasma is incubated with a neutral lipid substrate. This release of free fatty acid might account for the increased hemolytic activity of post-heparin lipemic blood on standing at room temperature.

Conclusions. 1. Addition of heparin to intravenously administered fat emulsion in rats resulted in a marked increase in the rate of disappearance of the fat from the blood and a decrease in the accumulation of fat in the spleen and liver. Heparin apparently caused a change in the physical state of the fat in the blood resulting in decreased turbidity and this altered fat rapidly left the blood. 2. Lipemic blood from rats either fed fat or injected with fat emulsion showed elevated serum hemoglobin concentrations. Injection of heparin with fat emulsion resulted in higher serum hemoglobin levels than injection of fat emulsion alone but this difference was seen only when the blood was allowed to stand for a period after it had been drawn.

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Ultrafiltration and Electron Microscopy of Three Types of Poliomyelitis Virus Propagated in Tissue Culture.* (20881)

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Previous work on filtration of poliomyelitis virus through gradocol membranes(1-3) was performed with Type 2 strains (MV, Lansing) propagated in the nervous system of monkeys or mice, and the extracts or stock filtrates actually used in the critical filtration experiments contained as little as one and not more than 100 infective doses. Using Elford's formula and different methods of estimating the average pore diameter (APD) which held back the virus, the size was variously reported as 8-12 mμ(2), 10-15 mμ (1,4), and 15-23 mμ(3). Since filtration endpoints may be affected both by the concentration of virus and the presence of extraneous tissue particles, it appeared possible that the size of the poliomyelitis viruses might actually prove to be smaller in tests with water-clear tissue culture fluids containing 10^6 to 10^8 infective doses per ml. Poliomyelitis virus of all 3 immunologic types, propagated in cynomolgus kidney tissue cultures, was studied by filtration through gradocol membranes and by electron microscopy.

Materials and methods. With one exception, the virus used in these tests was grown in 250 ml centrifuge bottles in a roller drum. The washed, minced cynomolgus kidney tissue, suspended in chick embryo extract, was embedded over the entire inner surface of the bottles previously coated with chicken plasma. The medium consisted of 0.5% lactalbumin hydrolysate (enzymatic—Nutritional Biochemicals Corp., Cleveland), Simms ox serum ultrafiltrate, Earle's balanced salt solution, 0.05 mg of crystalline soybean trypsin inhibitor, 100 units of penicillin and 0.1 mg of streptomycin per ml; 40 ml of this medium were used per bottle. After 7 days in the roller drum at 35°C, the bottles were inoculated with 0.1 ml of undiluted, virus-containing tissue culture fluid. Virus was harvested twice at 2- or 3-day intervals. In some tests only the first harvest was used, in others a pool of the two. The virus was stored in the frozen state in a chest containing solid CO_2. Virus titrations were performed in roller tubes of cynomolgus kidney tissue cultures. The gradocol membranes were purchased from St. Mary's Hospital, London, England. Prior to filtration of the tissue culture fluid, brain and heart infusion, proteose-peptone broth (Difco) was passed through the membranes—10 ml for membranes with an APD of more than 100 mμ and 5 ml for those less than 100 mμ. Filtration was performed under positive pressure of nitrogen—10 lb per square inch for membranes over 100 mμ and 15 lb for

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