Effects of Fat on Blood Viscosity in Dogs

By Roy L. Swank, M.D.

Intravenous fat injections cause significant increases in blood viscosity. Nonemulsified or poorly emulsified oil produces pulmonary and other embolization. Plasma is lost rapidly and in alarming degree. The hematocrit increases rapidly and is accompanied by a nearly parallel increase in blood viscosity. Oil in stable emulsion in blood plasma or lymph can be injected in greater doses. Slow, but significant increases in the blood viscosity develop to maximum in 10 to 24 hours. Varying degrees of paralysis follow. The loss of plasma is not so marked, and increase in hematocrit is not sufficient to account for increase in viscosity.

In a previous paper it was shown that the feeding of large fat meals to dogs or to human beings caused significant in vitro changes in the suspension stability of the blood consisting of adhesiveness and aggregation and changes in the rate of sedimentation of the red blood cells. Changes were also observed in the plasma protein patterns in paper chromatography. The changes in the red blood cells and plasma became maximal 6 to 8 hours after fat feedings and several hours after the peak of the lipemia. Subsequent studies in the cheek pouch of the golden hamster demonstrated the adhesiveness and aggregation of the red blood cells and slowing of the circulation in vivo after fat feedings. Other studies demonstrated that these visible in vivo changes in the golden hamster were accompanied by significant increases in the viscosity of the blood. To measure these changes in viscosity in small amounts of blood containing no anticoagulant a method was developed employing an old principle employed before by other investigators.

The present studies were undertaken to determine whether the ingestion or intravenous injection of fat in dogs would produce changes in the viscosity of the blood similar to the changes observed in the hamster, and to observe the effects of changes in viscosity of the blood on the behavior of the dog.

Material and Methods

Dogs usually weighing between 10 and 15 Kg., were used. Relative blood viscosities were measured on venous blood containing no anticoagulant by the method of Swank and Rogh. The blood viscosity is expressed in terms relative to the viscosity of water. One small modification was necessitated by the fact that the calibrated viscosity needles sometimes suffered a reduction in their caliber after fat injections. This was due to deposition of a layer of amorphous gray material in the lumen of the viscosity needle and pipette. This material could not be removed by ether or alcohol, but was readily removed by washing with papain in a slightly alkaline or in aqueous solution. We now calibrate the viscosity needles before and after each viscosity determination and wash the needles with a solution of papain whenever their calibration is altered. All needles used for duplicate determinations have an even closer calibration constant.

Blood volumes were measured by a radioactive chromium method of Hutchens, sedimentation rates were determined in Wintrobe tubes and read at the end of 1 hour, and hematocrits were determined in Wintrobe tubes read first after being centrifuged 30 min., and then again at 40 min. at 3000 rpm in an International centrifuge.

Results

Feeding Fat to Dogs. Cream fat meals varying in size from 2 Gm. to 8 Gm./Kg. body

This method involves a closer evaluation of the radioactive material injected. This is accomplished by counting the syringe assemblies before and after injection. With this information corrections can be made in the amount of delivered radioactive chromium. At the same time a standard is prepared and aliquots of the diluted standard are counted at the same time that the blood samples are counted. We are indebted to Dr. Tyra Hutchens for assistance in our blood volume studies.

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weight were fed in more than 100 experiments. There were 24 successive experiments in which all measurements were in duplicate and hematocrits were determined on the same blood. In control studies in human beings and more recently in dogs, it was observed that the viscosity (and hematocrit) normally decreased about 10 per cent from 8:00 A.M. to midday. The viscosity returned to its fasting levels in the late evening. After the feeding of fat to dogs, the morning drop in viscosity failed to occur and in many instances an increase in viscosity of from 10 per cent to 15 per cent was observed in blood samples drawn 2 to 4 hours after fat feeding. The blood viscosity then dropped below the control level.

Repeated fat meals, daily, twice weekly and weekly revealed a surprising adaptation by the dog for periods as long as 6 to 12 months. The resulting chylomicronemia and slight viscosity change became less marked despite increases in the size of the test meals up to as much as 8 Gm./Kg. body weight, and the dogs remained normally active and vigorous.

**Intravenous Injection of Vegetable Oil (Production of Fat Emboli).** Seventeen dogs received cottonseed oil intravenously at a very slow rate (total injection took 1 to 2 hours). Varying degrees of gross fat embolization occurred. An example of acute, fulminating fat embolisms with death after injection of 1.3 Gm. of oil/Kg. body weight is shown in figure 1. There was a rapid and extreme increase in hematocrit readings accompanied by an almost parallel increase in blood viscosity. The plasma volume decreased with equal rapidity. Marked pulmonary hyperventilation occurred in this dog.

Less severe embolism occurred in the other dogs receiving the same or slightly less oil. The findings were essentially the same. In some cases the early increase in viscosity was followed by a delayed increase in viscosity which could not be accounted for by an increase in hematocrit readings.

In 4 experiments vegetable oil held in emulsion by a monoglyceride was injected intravenously.* The concentration of the oil varied from 3 to 6 per cent, and the emulsified particles were mostly under 4 to 5 micra in diameter. The amount of oil per Kilogram body weight which could be injected intravenously in this emulsified form about equalled the amount of nonemulsified oil that could be injected and the physiologic effects were the same. In both instances the oil had to be injected very slowly and the limits of tolerance were approximately 1 Gm. oil/Kg. body weight.

**Intravenous Injection of Lipemic Chyle into Dogs.** Because of the rapid adaptation of dogs to ingested fat, methods were sought by which larger amounts of fat could be introduced intravenously at a rapid rate. Lipemic chyle obtained from donor dogs several hours after ingestion of high fat meals was injected in 25 experiments in 8 dogs under pentobarbital or urethane-chloralose anesthesia. From 300 to 700 ml. of lipemic chyle was obtained from each donor dog. From 50 to 120 ml. of this

*Fig. 1. Percentage changes in blood viscosity, hematocrit reading and blood volume after intravenous injection of 1.3 Gm. oil/Kg. body weight.

*This emulsion was prepared by Dr. Jack Fellman, Division of Neurology, University of Oregon Medical School.
was injected in a period of 5 to 15 min. into the recipient dogs. In 7 experiments an increase of 10 to 15 per cent in the viscosity without corresponding increase in the hematocrit changes resulted during the first 2 hours after injection. One of these dogs was weak in all 4 extremities the next day, but recovered completely the following day. The remaining dogs were normally active the day after injection. In 2 dogs receiving 70 ml. and 65 ml. of chyle, respectively, the viscosity rose to much higher levels. In the first (dog A, fig. 2) the viscosity rose approximately 73 per cent in the first 40 min. accompanied by a 2 per cent increase in the hematocrit. The following day this dog was quadriplegic but conscious. He recovered completely in 4 days. In the second dog (dog B, fig. 2) the viscosity rose 60 per cent in cent in 4 hours. The hematocrit readings did not increase. The following day the viscosity was still elevated. This dog was quadriplegic for 3 days after the chyle injection. He recovered slowly and was still slightly ataxic at the end of 14 days. In neither of these dogs were there signs of pulmonary or other embolisms such as hyperventilation or peripheral vascular failure. In dog A the plasma turbidity from lipemia was higher initially than it was in dog B. No changes in viscosity were noted in the remaining 16 experiments.

Intravenous Injection of Commercial Fat Emulsions. Because of the difficulties of obtaining chyle and because the fat content of the chyle varied, 2 commercial fat emulsions and their vehicles without fat* were studied. All injections were made under light surgical pentobarbital anesthesia.

One fat emulsion containing 15 per cent vegetable oil was injected into 10 dogs in the amount of 1 Gm./Kg. body weight. The percentage changes in blood viscosity, and red cell and plasma volume were recorded and averaged. There was a prompt increase in the viscosity and hematocrit readings and concurrent decrease in plasma volume. The viscosity was still increasing 24 hours after injection, even though the hematocrit changes and plasma volume appeared to be returning to normal. A slight lipemia (optical density 0.15) was present in these dogs at the end of one hour; this disappeared in 4 hours. Twenty-four hours after injection 3 dogs were normally

*One by Upjohn Pharmaceutical and the second by the Baxter Laboratories.
active, 4 had weakness or stiffness of hind legs and 3 were quadriplegic and blind. All dogs recovered completely in 2 to 4 days.

For control of the fat emulsion an equivalent amount of vehicle (emulsifying agent; 6.67 ml./Kg. body weight) was injected into 4 dogs. Changes occurred promptly in the viscosity, plasma volume and hematocrit readings, but these were much less marked than when the vehicle also contained fat. Twenty-four hours after injection neurologic impairment was present in 3 of the 4 dogs.

Injection of Vegetable Oil Held in Stable Emulsion by the Animal's Own Plasma. These experiments were performed as follows: 80 ml. to 100 ml. of blood were withdrawn and heparinized from a pentobarbital anesthetized dog. The plasma was separated and the red blood cells and buffy coat were resuspended in normal saline and returned to the animal intravenously. An equal volume of normal saline and the oil was added to the plasma. In 2 experiments 1 Gm. of oil/Kg. body weight was added, in 3 experiments 2 Gm. of oil/Kg. body weight were added, and in the remaining 27 experiments 3 Gm. of oil/Kg. body weight were added. In the latter experiments the oil, plasma and saline were present in approximately equal proportions. This mixture was then homogenized in a Servall homogenizer at 14,500 rpm for 20 min. During emulsification it was necessary to cool the mixture to prevent harmful increases in temperature. Most of the oil globules in the emulsion varied in size up to 4 or 5 micra in diameter, although a few up to 8 or 10 micra in diameter were present in each oil immersion dark field. When the emulsion was injected slowly over a period of 1 to 2 hours the animals' respirations and reflex activity were unaffected. When the injection took less than 30 min., hyperventilation often resulted. In our 27 experiments only one animal expired during injection and in this case the injection was rapid. Very little to no visible lipemia resulted from these injections.

One experiment is shown graphically in figure 3. A slow and steady increase in viscosity was recorded. The hematocrit readings increased slightly at first, then returned to normal. The plasma volume was decreased by approximately 16 per cent 2 hours after starting the fat infusion. It gradually returned to normal or slightly above. Twenty-four hours after the injection the animal behaved

Fig. 3. Percentage changes in blood viscosity, hematocrit readings, and blood volume after intravenous injection of 3 Gm. oil/Kg. body weight emulsified in dogs' own plasma.
and performed normally. In order to record the events 10 to 16 hours after fat emulsion injection, 2 animals were injected in the early evening and then followed the following morning. One experiment is shown in figure 4. Twenty-four hours after injection the animal was quadriparietic and 48 hours after injection he was normal.

Seven dogs developed increasing tolerance for ingested and injected fat emulsified in dog's own plasma. For instance, 2 dogs received 3 injections of 3 Gm. of fat/Kg, body weight emulsified in dog's own plasma at weekly intervals. In 1 the viscosity decreased by 20 per cent during the third injection of fat rather than increased slightly as it did during the first injection. In the other the viscosity rose 40 per cent during the first experiment, but failed to increase during the third experiment. Also in both dogs, the percentage increase in viscosity exceeded the percentage increase in hematocrit readings during the first experiment, whereas during the third experiment the increase of the viscosity was less than the percentage increase of the hematocrit. Definite electrocardiogram changes were not recorded in 6 experiments in which fat emulsified in dog's plasma was injected.

**DISCUSSION**

The ingestion of large fat meals by hamsters causes a striking increase in the blood viscosity. In dogs only a slight, and frequently equivocal increase in blood viscosity results after similar feeding, and this change is followed soon by a decrease in blood viscosity. The introduction of oil intravenously in dogs, however, produces an increase in blood viscosity. It does this in two ways. 1. If pure oil or oil held in unstable emulsion is injected rapidly, pulmonary and other embolisms occur. This is followed by a prompt and very marked decrease in plasma volume and an equally striking increase in the hematocrit readings. The blood viscosity increases also, parallel to or less sharply than the hematocrit. 2. If the oil is held in stable emulsion by blood plasma or lymph, a much larger dose can be injected without producing overt evidences of pulmonary embolism. In such cases the viscosity increases slowly and usually reaches a peak 10 to 24 hours after injection. Concurrently a slight decrease occurs in the plasma volume accompanied by an increase in hematocrit. This increase in hematocrit readings is much less striking than the increase in viscosity and
can be only partly responsible for the viscosity change. Despite an absence of respiratory evidences of pulmonary embolism after injections of oil emulsified in plasma, the decrease in plasma volume and increase in hematocrit readings which often occurs within the first hour after injection suggests that an increased permeability of blood vessels is present. The fact that the cell/plasma ratio changed little or none after injections of lipemic chyle in dogs (fig. 2), or in hamsters after feeding of fat meals suggests that this loss of plasma might be due to mild fat embolism. On the other hand, the loss of plasma volume might be due to an increased vascular permeability resulting from a slowing of the circulation consequent to an increased adhesiveness and aggregation of the red blood cells and increased blood viscosity. This mechanism seems possible particularly since the increased viscosity which follows injections of large molecular weight Dextran is also accompanied by a decrease in the plasma volume and by an increase in vascular permeability. In either event it is suggested that the basic reason for reduction in plasma volume is the same (reduced circulation and increased vascular permeability) whether the cause is embolization or an increased blood viscosity. The decrease in plasma volume was much greater when clear-cut evidences of pulmonary embolization (hyperventilation) were present. After injections of Dextran in dogs, one can predict with some degree of accuracy from the changes in blood viscosity, whether or not paralysis will be present the following day (unpublished). After injections of oil emulsified in plasma a much less direct relationship between blood viscosity and paralysis was found. Mild to severe paralysis often occurred after very slight, or even no increase or a slight decrease in blood viscosity. It seems evident that factors other than viscosity are operating which are at least partially responsible for this discrepancy. It was noted that a grayish amorphous material coated needles and pipettes after some fat injections and that this material was not soluble in ether or alcohol, but was removed by papain, a protein enzyme. One can only wonder if a similar material enveloped the red blood cells and coated the endothelium, thus interfering with oxygen exchange in the capillaries. An amorphous envelope was observed on the red blood cells in hamsters after large fat meals and a similar, although less opaque, envelope was present after injections of dextran. The fact that electrocardiographic changes were not observed after injections of emulsified oil suggests that severe fat embolization or severe ischemia for other causes was absent. However, it may be that our observations were made in too few animals, or that the heart muscle is more resistant to hypoxia than the brain.

**SUMMARY**

The ingestion of large fat meals by dogs causes little or no increase in blood viscosity. The intravenous injection of fat causes significant increases in blood viscosity. Two mechanisms are involved: Nonemulsified oil or oil maintained in unstable emulsion produces pulmonary and other embolization immediately after injection. Plasma is lost rapidly and to an alarming degree probably because of endothelial damage and tissue hypoxia due to ischemia. The hematocrit readings increase rapidly and are accompanied by a parallel or nearly parallel increase in the blood viscosity. On the other hand, oil held in stable emulsion by blood plasma or lymph is followed by a delayed and slow, but significant increase in the blood viscosity which becomes maximum 10 to 24 hours after injection, and remains elevated for 48 hours. Paralysis of varying degrees is often present after the stable fat emulsion injections. The loss of plasma is less marked than after embolization, and the increase in all plasma ratios is not sufficient to account for the increase in viscosity. It is suggested that the delayed increase in viscosity is related to the hydrolysis and metabolism of the fat and due to adhesiveness and aggregation of the red blood cells.

**SUMMARIO IN INTERLINGUA**

Le ingestion de grande repastos grasse per canes causa pane o nulle augmento del viscostate sanguine. Le injection intravenose de grassia causa significative augmentos del vis-
cositate sanguine. Duo mechanismos es involvite. Oleo non-emulsificate o oleo mantenite in emulsion instabile produce embolization pulmonar e altere immediamente post le injection. Plasma es perdite rapidemente e a grados alarmante, probablemente a causa de endothelial dannos e histohypoxia resultante de ischemia. Le valores de hematocrite se augmenta rapidemente e in parallellitate o quasi-parallellitate con un augmento del viscositate sanguine. Del altere latere, oleo que es tenite in emulsion stabile per plasma o lympha produce un lente e retardate sed significative augmento del viscositate sanguine que attinge su maximo inter 10 e 24 horas post le injection. Illo remane elevate durante 48 horas. Paralyse de varie grados es frequentemente presente post le injection de stabile emulsiones de grassia. Le perdita de plasma es minus marcate que post embolisation, e le augmento del valores de hematocrite non suffice pro explicar le augmento del viscositate. Es stipulate le possibilitate che le retardate augmento del viscositate es relationate al hydrolyse e al metabolismo de grassia e resulta del adesivitate e aggregation del erythrocytos.

REFERENCES

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