The Alteration of the Lipemia-Clearing Effect of Heparin Following the Intravenous Injection of Thorium Dioxide (Thorotrast)

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The lipemia-clearing response to intravenous heparin in the normal dog has been compared with that in dogs injected intravenously with Thorotrast. In normal dogs, intravenous heparin caused clearance of the lipemia (optical density) of approximately 77 per cent of the total amount possible. Dogs receiving low total amounts of Thorotrast (50 to 100 ml.) responded to heparin with a reduced ability to clear an alimentary lipemia, while dogs receiving large total amounts of Thorotrast (150 ml. or more) responded to heparin by becoming even more lipemic. The lipemia inducing effect of protamine was similar in both normal and Thorotrast-injected animals.

Intravenous injection of heparin and certain other anticoagulants will clear an alimentary lipemia in vivo. Recent work suggests that a tissue substance, found primarily in abdominal and thoracic viscera, catalyzes the conversion of heparin and a plasma component to “clearing factor,” and that “clearing factor,” in the presence of a coprotein, causes a reorientation in the distribution of the plasma lipoproteins in such a way that the optical density of the plasma is decreased. Once produced in vivo this factor is active in vitro, and clearing of lipemic optical density can be obtained by incubating plasma from heparinized animals with lipemic plasma, fat emulsions or milk. The total plasma lipid level is not altered during the reaction, but there is a change in the physico-chemical state of the blood lipids as determined with the ultracentrifuge. The lipemia-clearing effect of heparin can be reversed in vivo and the lipemia restored with intravenous injection of protamine.

The report of Anfinsen, Boyle and Brown, that only the abdominal and thoracic regions produce high levels of “clearing factor,” suggested the possibility that the reticulo-endothelial system might be a source of some part or parts of the “clearing factor” system. In the work to be presented, this possibility has been studied by comparing the lipemia-clearing response to heparin of the normal dog with that of dogs that had received intravenous injections of thorium dioxide (Thorotrast, Heyden).

Procedure

Dogs weighing between 10 and 14 Kg. were used for these experiments. A majority of the experiments were performed on unanesthetized dogs, but in a few instances an anesthetic was necessary and small doses of Evipal (Winthrop) were used.

The lipemia clearing effect of heparin in the normal dog was studied in 23 experiments using 17 different dogs. An alimentary lipemia was produced by feeding 100 cm. of olive oil or soya bean oil; corn oil was fed in smaller quantities. Four to five hours after a fatty meal, a 5 ml. blood sample was obtained by venipuncture. Heparin (Abbott) was then injected intravenously. In one instance 60 mg. of heparin was administered, but in the rest, a standard dose of 30 mg. was used. Additional 5 ml. blood samples were obtained at 5, 10 and 30 minute intervals after the heparin was injected. At the termination of the experiment each dog received 50 mg. of protamine sulfate (Lilly) intravenously. A final blood sample was taken 5 to 10 minutes after the injection of protamine. All blood samples were drawn into sodium citrate and centrifuged within five minutes. The optical density of the plasma samples was measured with an Evelyn Photoelectric Colorimeter using a 660 m\(\mu\) interference filter. This measurement was used as an indication of the amount of lipemia. The galvanometer reading produced by a 1:10 dilution of plasma was compared with that produced by a blank of saline alone. Photometric readings of the diluted plasma were made within 10 minutes after withdrawing the blood sample.

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The lipemia-clearing effect of heparin in the dog after intravenous injection of Thorotrast was studied in 18 experiments using eight different dogs. In this series of experiments the dogs first received 50 ml. of Thorotrast intravenously. Eighteen hours after injection of the Thorotrast the lipemia-clearing effect of heparin was tested in a manner identical with that described for the normal animal. Additional injections of Thorotrast were given these same animals at intervals of time varying from one week to one month, but no more than 50 ml. of Thorotrast was given at any one time. Experiments testing the

lipemia-clearing response of these animals to heparin were done 18 hours after the last injection of Thorotrast, except for one instance to be described later.

OBSERVATIONS

1. Experiments Demonstrating the Lipemia Clearing Effect of Heparin in the Normal Dog

This series of experiments can be divided into three groups based on the type of fat employed.

a. Olive Oil. In 17 experiments done on 12 different dogs intravenous injection of heparin caused clearing of the lipemia within five minutes.

b. Soya Bean Oil. In two experiments performed on two different dogs intravenous heparin caused clearing of the lipemia within five minutes.

c. Corn Oil. During the course of these experiments, there came to our attention a report by Waldron and Friedman that, "If corn oil is administered in an amount which, in itself, does not produce lipemia, the blood will become lipemic within five minutes after the intravenous injection of heparin or the other anticoagulants." This procedure was tried in six experiments on four different dogs. In one dog there was an increased lipemia after intravenous injection of heparin. However, on repeating this procedure in three different dogs and again on the same dog, clearing of even the slight lipemia present was observed.

![Graph](attachment:graph.png)
Figure 1 demonstrates the response of 20 lipemic dogs five minutes after the intravenous injection of heparin. It can be seen that the greater the initial lipemia (R1) the greater the difference between the photometer reading of the initial sample and that of the five minute post-heparin sample (R2). There appears to be a constant relationship between the amount of the initial lipemia (R1) and the degree of clearing (R2 — R1) which can be expressed by the equation:

\[
R_2 - R_1 = b(98 - R_1)
\]

After substituting various readings in this formula and solving for the constant b, it was found that the value for the points falling on the line is 0.77, which indicates that regardless of the amount of the initial lipemia, the dosage of heparin used in these experiments will cause the optical density of the plasma to be decreased approximately 77 per cent of the total amount possible within five minutes. The figure 98 was used as the highest possible value obtainable as that was the highest reading obtained with clear plasma.

Data obtained from measuring the optical density of 10 and 30-minute post-heparin samples cannot be made to fit into any one pattern. Using data obtained from 18 experiments on 12 different dogs, four patterns can be found (fig. 2).

a. The lipemia continued to clear slowly or, apparently having reached a maximum, remained unchanged for at least 30 minutes in nine instances (six different dogs).

b. The plasma became more lipemic at some point between 10 and 30 minutes after injection of heparin in three instances (three different dogs).

c. The plasma became more lipemic between 5 and 10 minutes after injection of heparin. It is of interest that all three of these experiments were done on the same dog.

d. There was an increase in the lipemia of the 10 minutes post-heparin sample but then clearing again at the time of the 30 minutes post-heparin sample in three instances (three different dogs).


In a series of 19 experiments using 14 different dogs, it was found that in almost all instances the heparin effect could be reversed. It was occasionally observed that the lipemia failed to reappear after injection of protamine. The amount of increase in lipemia after injection of protamine varied. Often the lipemia was restored to a degree as great or greater than that present before clearing with heparin. These data can be divided into three groups based on the type of fat employed.

a. Olive Oil. After feeding olive oil and then causing the resulting lipemia to be cleared with heparin, 50 mg. protamine was given intravenously in 12 experiments done on eight different dogs. In 10 instances the lipemia returned to the plasma while in two instances it was unchanged.

b. Soya Bean Oil. After feeding soya bean
oil and causing the resulting lipemia to be cleared with heparin, 50 mg. protamine was given intravenously in two experiments on two different dogs. In both instances the protamine caused the lipemia to return.

c. Corn Oil. After feeding corn oil and causing the resulting lipemia to be cleared with heparin, 50 mg. protamine was given intravenously in five experiments on four different dogs. The protamine caused the return of the lipemia in four instances but failed to alter it in one instance. In the one dog fed corn oil where heparin caused the lipemia to be increased, protamine caused the lipemia to be still further increased.

This decrease and increase in lipemia can be produced repeatedly in the same animal by injecting first heparin and then protamine. In a typical experiment, an alimentary lipemia was produced by feeding olive oil; the lipemia was cleared with intravenous heparin; protamine was injected and the lipemia returned. Another injection of heparin was given and the plasma cleared again. One hour later the plasma was still clear. It is of interest to note that in dogs with no visible lipemia the intravenous injection of protamine will produce lipemia. This phenomenon has been observed in rats by Brown.6

<table>
<thead>
<tr>
<th>Animals having received</th>
<th>Difference in the Galvanometer Reading of Control Plasma Samples and Plasma Samples Taken 5 Min. after Intravenous Heparin (R² - R₁)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ml. of Thorotrast</td>
<td>48</td>
</tr>
<tr>
<td>100 ml. of Thorotrast</td>
<td>36</td>
</tr>
<tr>
<td>150 ml. of Thorotrast</td>
<td>24</td>
</tr>
<tr>
<td>200 ml. of Thorotrast</td>
<td>12</td>
</tr>
<tr>
<td>250 ml. of Thorotrast</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 3. The five minutes post-heparin responses of eight dogs that had received varying amount of Thorotrast previous to being made lipemic. The heavy line represents the lipemia clearing responses of the normal dog five minutes post-heparin (the same line represented in fig. 1). Note that some animals which had received 50 ml. of Thorotrast cleared within the range of normal but that all of these animals fell below the normal mean. All animals that had received 150 ml. of Thorotrast or more responded to heparin by becoming more lipemic instead of clearing.


Figure 3 demonstrates the five minutes post-heparin responses of eight different dogs that had receiving varying amounts of Thorotrast before being made lipemic with olive oil. The heavy line represents the lipemia-clearing response of the normal dog after intravenous injection of heparin (the same line presented in figure 1). This series of experiments can be
described in three groups on the basis of the total amount of Thorotrast given the animal prior to the time of the experiment.

a. Animals that Had Received 50 ml. of Thorotrast. It can be seen from figure 3 that the six dogs receiving 50 ml. of Thorotrast 18 hours prior to being made lipemic and then receiving heparin did not respond by clearing to as great an extent as the normal dog. Several of the dogs cleared within the range of normal, but it is of significance that all points fall below the normal line.

b. Animals that Had Received a Total of 100 ml. of Thorotrast. The lipemia-clearing responses of four dogs that had received a total of 100 ml. of Thorotrast were tested. Three of these dogs responded to intravenous heparin by a decreased lipemia, but not to as great an extent as would have been expected in the normal dog. One animal not only failed to clear in response to heparin, but instead, the plasma became more lipemic.

c. Animals that Had Received a Total of 150 ml. or More of Thorotrast. Two dogs that received a total of 150 ml. of Thorotrast both responded to heparin by becoming more lipemic, one of them to a much greater extent than the other. The response of one of these dogs was tested after a total of 200 ml. of Thorotrast and again after a total of 250 ml. of Thorotrast. In both instances the lipemias were increased in response to heparin.

The response of Thorotrast-treated animals 10 and 30 minutes after injection of heparin varies in the same manner as that of normal animals.

I. In animals in which low total amounts of Thorotrast caused only a diminution in lipemia-clearing ability five minutes after injection of heparin three patterns are found: (a) The plasma continued to clear slowly or, apparently having reached a maximum, remained unchanged for at least 30 minutes in six instances (five different dogs). (b) The plasma became more lipemic at some point between 10 and 30 minutes after injection of heparin in one instance. (c) The plasma became more lipemic between 5 and 10 minutes after injection of heparin in one instance.

II. In animals in which large total amounts of Thorotrast caused an increase in lipemia five minutes after injection of heparin two patterns are found: (a) The lipemia began to clear at some point between 5 and 10 minutes after injection of heparin in one instance. (b) The lipemia began to clear at some point between 10 and 30 minutes after injection of heparin in one instance.

Figure 4 shows the responses of one dog (F-16) after increasing amounts of Thorotrast. Before receiving Thorotrast, this dog responded to heparin by clearing 70 per cent of the total amount possible in five minutes. After 50 ml. of Thorotrast the dog responded to heparin by clearing as in the normal, but after a total of 150 ml. of Thorotrast, this animal responded by becoming more lipemic five minutes after
LIPEMIA-CLEARING EFFECT OF HEPARIN AFTER THOROTRAST

Heparin. Subsequent samples showed progressive clearing of the enhanced lipemia. After a total of 200 ml. of Thorotrast and again after 250 ml. of Thorotrast this animal responded by becoming much more lipemic within five minutes after intravenous heparin.

After a period of rest varying from two weeks to one month, an animal that has received enough Thorotrast to cause reversal of the clearing effect will respond to heparin by clearing a lipemia like a normal animal (fig. 4). This has been observed in three different dogs. However, this recovery is not complete, for if yet another 50 ml. of Thorotrast is administered and then the lipemia-clearing response tested, the animal does not respond with only a somewhat lowered ability to clear the lipemia, but again responds by becoming more lipemic.

In order to test whether there is any relationship between the time of injecting the Thorotrast and the lipemia-clearing response to heparin, the following experiment was performed. A normal dog was given an injection of 50 ml. of Thorotrast. The lipemia-clearing response to heparin was tested at three and again at 12 hours after the injection of Thorotrast. The animal responded both times by clearing slightly, but to a degree well below the normal. From this observation it is felt that the reversal effect is not related to the time after the injection of the Thorotrast as much as it is related to the accumulated amount of Thorotrast given the animal.

The injection of varying amounts of Thorotrast did not alter the response of the animals to protamine. After clearing an alimentary lipemia with heparin and then injecting protamine the lipemia was increased, but to variable degrees as was found in the normal dog.

DISCUSSION

The data in this report concerning the lipemia-clearing response to intravenous heparin in the normal dog show that regardless of the degree of the initial lipemia, a standard dose of 30 mg. of heparin will cause a decrease in plasma optical density of approximately 77 per cent of the total amount possible within five minutes. A few observations indicate that a high degree of clearance is reached within one and two minutes which suggests that the reaction depends on a uniform distribution of the heparin throughout the vascular bed.

Variations in the response at 10 and 30 minutes after the injection of heparin could be the result of many factors such as individual animal or sex variations. It is possible that a component of the clearing factor system could become exhausted during the clearing reaction as is suggested by the in vitro results of Anfinsen, Boyle and Brown. Other possibilities are the continued absorption of fat from the intestine, the inactivation of heparin, and the possible competition for heparin by other heparin-binding substances.

Increased plasma optical density after intravenous injection of protamine in nonlipemic dogs might be explained by assuming that the injected protamine would neutralize all available heparin and thus inactivate the available "clearing factor." The variable responses to protamine that were observed in heparin-cleared dogs may be due to the amount of heparin available. The amount of heparin in the blood of heparin-cleared dogs may range from a minimal amount required for clearing the lipemia to a considerable excess. If the excess is sufficient to neutralize the injected protamine, a return of the lipemia would not be expected. This is in agreement with the suggestion of Anfinsen, Boyle and Brown that substantial quantities of tissue factor and "clearing factor precursor" exist in normal animals, and that "clearing factor" may exist normally in plasma in low concentrations dependent upon the available heparin supply.

It was repeatedly observed that the magnitude of the increase in optical density following intravenous injection of protamine can be greater than the magnitude of the decrease due to the preceding heparin injection. This is in agreement with the observation of Brown, utilizing the chylomycin count.

The affinity of reticuloendothelial cells for lipids and the appearance of fat droplets in both normal and abnormal reticuloendothelial cells have led to the assumption that in some manner this system participates in fat metabolism. If it is assumed that the main site of removal of the Thorotrast is the reticuloendo-
thelial system, that system may be implicated as having a rôle in "clearing factor" production since injection of Thorotrast does alter the lipemia-clearing response to heparin. However, interference with a possible enzymatic reaction involving either the formation or activity of "clearing factor" is suggested by the fact that Thorotrast will prevent the conjugation of sulfanilamide.15

The fact that sufficient Thorotrast can cause the lipemia-clearing response to be reversed after an injection of heparin suggests that there may be a competitive system involving heparin.

Conclusions

1. In the normal dog made lipemic by feeding olive oil, soya bean oil or corn oil, an intravenous injection of heparin causes the plasma to clear approximately 77 per cent of the total amount possible.

2. In the majority of instances intravenous protamine will restore the lipemia in a dog that has been cleared by a preceding heparin injection. The degree to which the lipemia is restored varies, at times it becomes greater than the initial lipemia.

3. Thorotrast alters the response of the lipemic dog to heparin. Low total doses of Thorotrast (50 to 100 ml.) result in a diminished ability to clear a lipemia. Large total doses of Thorotrast (150 ml. and over) cause the lipemia to be increased rather than diminished when heparin is administered.

4. The response to intravenous protamine is not altered by Thorotrast.

References


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