Physiological Factors Concerned With the Removal of Injected Heparin From the Circulating Blood

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Recently Monkhouse, MacMillan and Brown (1) made a study of blood heparin levels and blood coagulation times in hospital patients given heparin by intravenous and intramuscular routes. The effect of heparin on the clotting time of any one person appeared to depend only on its concentration in the blood. There was considerable variation in the duration of effect and the peak levels reached with equal doses given by different routes. Much of the variation, when heparin was administered intramuscularly, was due to the difference in rate of absorption, but there was also evidence indicating variation in the rate of destruction.

In vitro experiments have suggested that heparin is not destroyed by blood itself since it can be incubated with blood for many hours without loss of potency. The rate of destruction of heparin, within the body, must therefore be influenced by the rate at which it escapes from the circulating blood and will be dependent on the uptake of heparin by the tissues. According to Piper (2) much of the heparin is taken up by the reticuendothelial system (RES), but the more recent work of Samuels and Webster (3) is open to the interpretation that heparin may escape from the vessel by passing through the intercellular substance. The latter suggestion has support in the fact that Glenn, Peterson and Drinker (4) found that lymph from the extremities became incoagulable after the intravenous injection of heparin.

The present study was undertaken in an effort to determine how much each of the above mentioned mechanisms contributed to the disappearance of injected heparin. It was hoped that such a study would provide information which would enable the clinician to select the most appropriate heparin preparation for his particular case.

METHODS AND MATERIAL

Animals. Mongrel dogs and rabbits were used. These animals were obtained locally and kept on a standard diet of Purina chow for at least 2 weeks before being used for experiments.

Chloroform Poisoning. The chloroform was injected subcutaneously. In the first experiments 1 ml was injected at a single dose, but this caused the death of three out of four rabbits within 3 days. The remaining experiments were carried out by injecting 0.5 ml for 3 succeeding days and giving the heparin injection on the fourth day. This resulted in a better survival rate, and at the same time produced evidence of severe liver damage in surviving animals.

RES Blockage. India ink (Higgins), Trypan blue and Thorotrast were used. The India ink was diluted 1:1 or 1:4 with physiological saline and the Trypan blue was prepared as a 1% solution in saline. Thorotrast was injected directly from the vials as prepared by the Heyden Chemical Company for commercial use. Dosage and combination of drugs were varied and will be discussed under the individual experiments.

Surgical Procedures. Nephrectomy in rabbits was carried out under Nembutal and ether anesthesia from a mid-line incision. In the experiments in which urine was collected, it was done by cannulating the ureters with polyvinyl tubing and bringing the cannulae to the outside through a puncture incision. The operation was carried out by a mid-line incision similar to that for nephrectomy.

Hepatectomy and eviscerated dogs were prepared by Dr. J. Markowitz and Dr. W. Lotto using a single operation technique (5).

Heparin. Heparin was supplied by Connaught Medical Research Laboratories and administered either intravenously (1,000 U/ml) or intramuscularly (10,000 U/ml).

Heparin was extracted by treating the plasma or urine with phenol, and precipitating the heparin from the aqueous phase with alcohol according to the method of Monkhouse and Jacques (6). The precipitates were extracted with saline and their metachromatic and antithrombic potencies compared with that of a stand-
TABLE I. COMPARISON OF THE AMOUNTS OF HEPARIN EXCRETED BY NORMAL RABBITS AND RABBITS RECEIVING AN INJECTION OF AN RES BLOCKING AGENT PRIOR TO AN INTRAVENOUS INJECTION OF HEPARIN

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Amount Heparin Inj.</th>
<th>RES Blocking Agent</th>
<th>Urine Vol., ml</th>
<th>Units Heparin Recovered in 1st Hr.</th>
<th>A/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>1000</td>
<td>India ink</td>
<td>10.0</td>
<td>5.6</td>
<td>60</td>
</tr>
<tr>
<td>32</td>
<td>1000</td>
<td>None</td>
<td>5.5</td>
<td>20.0</td>
<td>90</td>
</tr>
<tr>
<td>29</td>
<td>1000</td>
<td>India ink</td>
<td>4.0</td>
<td>3.0</td>
<td>40</td>
</tr>
<tr>
<td>30</td>
<td>1000</td>
<td>India ink</td>
<td>5.5</td>
<td>6.0</td>
<td>70</td>
</tr>
<tr>
<td>33</td>
<td>1000</td>
<td>None</td>
<td>4.5</td>
<td>80.0</td>
<td>240</td>
</tr>
<tr>
<td>34</td>
<td>1000</td>
<td>India ink</td>
<td>4.5</td>
<td>80.0</td>
<td>300</td>
</tr>
<tr>
<td>35</td>
<td>1000</td>
<td>India ink</td>
<td>4.5</td>
<td>75.0</td>
<td>30</td>
</tr>
<tr>
<td>66</td>
<td>1000</td>
<td>Repeated India ink</td>
<td>3.0</td>
<td>55.0</td>
<td>100</td>
</tr>
<tr>
<td>67</td>
<td>1000</td>
<td>Repeated India ink</td>
<td>7.0</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>1000</td>
<td>Thorotrast</td>
<td>12.3</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>500</td>
<td>None</td>
<td>2.0</td>
<td>&lt;0.5</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>500</td>
<td>None</td>
<td>2.0</td>
<td>&lt;0.3</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>500</td>
<td>Thorotrast</td>
<td>3.5</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

A/M = ratio of antithrombic to metachromatic potency.

ard solution of beef heparin. The reader should consult the above reference for further details of these methods.

With the exception of table 1, where comparisons are made, the heparin levels indicated are based on antithrombic potencies and expressed in terms of standard beef heparin.

EXPERIMENTAL RESULTS

In the first group of experiments a total of 11 rabbits were treated with chloroform. Of these only four survived 4 days or more. In two of these at the time of the second injection of heparin the plasma was brown with excess bile pigment and the liver sections showed fatty infiltration and cellular damage. Each animal received an intramuscular injection of 0.2 ml (2000 u) of heparin. As shown in figure 1B, there was no difference in the average blood levels of these animals when compared to a similar group of control animals. It appears that either the liver is not important for the destruction of heparin or the damage due to chloroform poisoning does not affect its ability to take up heparin. Results of experiments on hepatectomized and eviscerated (kidneys intact) dogs shown in figure 2 suggest that the latter is the more likely. It is evident that both the liverless dog and the eviscerated dog have lost their ability to inactivate heparin at anything like the normal rate. These animals received adequate intravenous glucose and their blood pressure and pulse remained good until toward the end of the experiment when they began to deteriorate rapidly. Urine flow appeared adequate in the eviscerated animal but was almost completely suppressed in the two hepatectomized dogs. The much higher levels of heparin in the eviscerated animal can be attributed in part to the decreased circulating volume of blood.

Experiments on rabbits in which an attempt at blocking the RES was made using India ink, Trypan blue or Thorotrast produced variable results. These results are illustrated in figure 3. In general little effect was obtained with India ink or Trypan blue, either alone or combined, with doses up to 2 ml India ink or 5 ml Trypan blue. With such doses the plasma was still blue up to 30 hours after injection. Some prolongation of the heparin effect was obtained in three out of four rabbits by the use of Thorotrast injected 18 hours before the heparin in doses of 9 ml to a rabbit. This was the dose used by Beeson (7).

It was felt that failure to obtain a significant
FIG. 2. Blood heparin levels after intravenous injection in normal, eviscerated and hepatectomized dogs. Animals ranged in weight from 5.5 to 12.8 kg, and the dose varied from 150 to 200 u heparin/kg body weight.

FIG. 3. Effect of RES blocking agents on the heparin levels in rabbits receiving 500 u of heparin intravenously. Rabbits ranged in weight from 2.8 to 3.5 kg. The dose of India ink was 2 ml diluted with an equal volume of saline. Five ml. of 1% Trypan blue was given and 9 ml of Thorotrast.

change in the blood heparin level under the above circumstances could be due, in some part, to a compensatory increase in excretion by the kidneys. According to Jaques and Kuri-Szanto (8) uroheparin has a low antithrombic to metachromatic ratio. Consequently if failure of the blood levels to be influenced by RES blockage is due to greater excretion one might expect to find more heparin in the urine of such animals. Also, that more of this heparin would be unaltered and the antithrombic to metachromatic ratio would be increased.

In an initial experiment a group of seven rabbits of approximately equal size was chosen. Five of these were given two injections of 0.5 ml of India ink in 4 ml of saline 10 minutes apart, the last one being 15 minutes prior to a heparin injection of 1000 u. The other two served as controls. The ureters were cannulated and urine collected for 1 hour after the heparin injection. All animals received an intravenous injection of 15 ml of saline to ensure a good flow of urine. The results are shown in the first seven items of table 1. In contrast to expectations, three of the rabbits receiving India ink excreted less heparin than normal rabbits and the antithrombic/metachromatic ratio was reduced. Such results could be explained if India ink interfered with secretion of heparin (2) more than it was interfering with the uptake of heparin by the tissues. Some support for this concept is found in the experiments of Altschul and Hummason (9), who brought about severe renal damage in rats by repeated injections of India ink.

To test the effect of repeated injections of India ink on the blood level and excretion of heparin, two rabbits were injected intravenously with 1 ml of India ink in 4 ml of saline every other day for 10 days. Twenty-four hours after the last injection they were given an intravenous injection of heparin. Their ability to excrete heparin was not impaired (see rabbit 66 and 67, table 1) and there was no significant difference in the blood heparin curve over that of normal rabbits given a similar injection of heparin. The experiment was repeated on four more rabbits treated similarly, but intramuscular injections of heparin were given. These were injected just 15 minutes after the last India ink injection. The average results for the four animals are shown in figure 1B and give evidence of some increase in blood heparin levels over a similar group of normal animals. If we interpret this latter result as indicating an effect on the kidney, it must be a consequence of the presence of India ink in the kidney interfering with glomeruli permeability to, or tubular secretion of, heparin for when 24 hours were allowed to elapse after the last injection of India ink there was no decrease in excretion of heparin given intravenously. It was difficult to understand why interference with the kidney should affect the blood levels more after intramuscular than after intravenous injection, since in the latter case the peak heparin levels were several times higher than in the former. The total dose, however, was much greater with the intramuscular injections, and this raised the question whether or not excretion of heparin was a function of the total dose rather than the blood level per se. In an attempt to find an answer to this question a series of experiments were carried out on bilateral nephrectomized rabbits.

As shown in figure 1B, nephrectomy had a
pronounced effect on the average blood levels after intramuscular injections of 2000 U (0.2 ml) of a concentrated aqueous solution of heparin, both as to peak levels and duration of effect. It should also be noted that the average peak level of heparin in the blood of the intact animals was less than 0.6 u/ml. This is far below what has been considered by Jaques and his colleagues (8) to be the renal threshold. Since these authors had used intravenous injections, we carried out experiments in which nephrectomized rabbits were given single intravenous doses. Typical results are shown in figure 4. It is obvious that the size of the dose is an important factor to consider. In agreement with Jaques and Ricker (10), we found that single intravenous doses giving initial blood levels of between 2 and 3 u/ml of blood were not significantly influenced by nephrectomy, but higher doses were. Furthermore the injection of India ink plus nephrectomy did not prolong the effect of a heparin injection to any greater extent than nephrectomy alone. It did influence the initial rate of decrease, especially with the high doses. These results suggest that

the importance of the kidney on heparin levels depends not so much on blood levels per se, but rather on the total dose given. This is made more evident by the experiment illustrated in figure 1A. This graph shows the levels obtained when repeated small intravenous doses are given to normal and nephrectomized rabbits. With the dose given (250 U/hr.), the heparin in the normal rabbit remained low, while in the nephrectomized animal it continued to increase to several times that of the normal.

**DISCUSSION**

This study was begun with the working hypothesis that heparin could be lost from the blood stream by one or more of three ways, a) excretion by the kidneys, b) ingestion by the reticulo-endothelial system or, c) elimination by way of the intercellular cement, either by sticking to it as the work of Samuels and Webster (3) would suggest, or by passing through it in a manner outlined by Chambers and Zweifach (11) for other substances.

An attempt has been made to evaluate the importance of each of these ways by studying the effect on blood heparin levels of (1) interference with the RES, (2) total nephrectomy, and (3) combined RES blockage and total nephrectomy.

To be complete such a study should include, for each of the above circumstances, a range of dose levels with all known methods of administration in a number of different species of animal. Such a program, however, would require an enormous number of experiments. We chose for this present study the two most common methods of clinical administration, intramuscular and intravenous, and used rabbits and dogs as experimental animals. By far the greater number of experiments were carried out on the rabbit. In a large part, however, results secured in the rabbit were confirmed in the dog.

The amount of chloroform necessary to cause severe liver damage was variable and the loss of animals was high. Nevertheless rabbits which survived and exhibited a high degree of liver damage showed no change in their blood levels after standard doses. On the other hand, we have shown that hepatectomy caused a pronounced prolongation of effect of heparin in the dog. These results suggest that the liver
plays a part in the removal of heparin from the blood but that the cells responsible for this are not severely damaged by chloroform. The increased 'heparin-sensitivity' reported by Schwartz, Usteri and Koller (12) in patients with liver damage, and the contrasting 'relative heparin resistance' claimed by Studer (13) in experimental liver damage in rabbits may indicate a change in factors which influence the anticoagulant or antithrombic effect of heparin rather than its level in the blood. Such a concept would mean that while a patient with liver damage might require a lower blood heparin level to secure the desired therapeutic effect (and thus a smaller dose per injection), the rate of removal of heparin from the blood would not be impaired, and the frequency of injection should be the same as for a patient with a healthy liver.

The experiments designed to test the effect of RES blockage were difficult to interpret. Thorotrast was the only agent used that caused any change in the blood level of heparin when the kidneys were intact. This may not be surprising as Barrow, Tullis and Chambers (14) and Gordon and Katsh (15) found Thorotrast to be the most effective RES blocking agent. In nephrectomized rabbits Trypan blue and India ink did interfere with the initial drop in blood levels after intravenous injection, especially with the higher doses, but did not prolong the over-all effect beyond that in nephrectomized rabbits not receiving the blocking agents. We would interpret such results as indicating that the RES does function in the removal of heparin from the blood stream. Though our inability to demonstrate increased quantities of heparin in the urine after the injection of RES blocking agents fails to give positive support to such an hypothesis, this failure may be due to the use of the wrong dose level of heparin and of a poor RES blocking agent. The only rabbit to show any anti thrombic material in the urine (rabbit 64, table 1) with a dose of heparin as low as 500 U was one which had received Thorotrast.

A comparison of the effect of nephrectomy on the heparin levels in rabbits after intramuscular and intravenous doses of heparin indicate that the role played by the kidney is dependent on the total dose of heparin given per unit time rather than the blood level per se. For example, an intramuscular dose of 2000 U of heparin, which will give a peak level of less than 0.6 U/ml in an intact rabbit, will give a level 2-3 times this amount after nephrectomy. On the other hand, a single intravenous dose of 500 U will give a peak blood level of 2 U/ml, and the rate of decrease is not altered by nephrectomy. Our experimental results agree, at least in part, with the clinical results of McCleery, Artz and Sirak (16) who found that caronamide (4'-carboxyphenylmethanesulfonanilide) increased the therapeutic effectiveness of heparin in a gelatin menstruum.

Such results suggest that intramuscular injections of large amounts of heparin, may not be the best way of giving the anticoagulant in cases of kidney disease.

SUMMARY

The average blood heparin levels in rabbits receiving intramuscular injections of heparin are not altered by chloroform poisoning. Hepatectomy or evisceration greatly alters the shape of the heparin curve and prolongs the effect of intravenous heparin in the dog. Results on normal rabbits receiving Thorotrast, and nephrectomized rabbits receiving India ink indicate that the RES plays a role in the elimination of heparin from the blood. Experiments on nephrectomized animals indicate that the part played by the kidney in the elimination of heparin is dependent on the total dose given per unit time rather than on the blood levels per se. A single intramuscular dose of heparin is influenced more by nephrectomy or kidney damage than a single intravenous dose required to produce a comparable level of heparin in the blood.

We wish to express our gratitude to Dr. Charles H. Best for his continued interest, to Dr. W. S. Hartroft for his work on the liver and kidney sections and to Mr. J. M. O. Wheatley for his careful and patient technical help.

REFERENCES