QUANTITATIVE STUDIES ON THE RELEASE OF HEPARIN IN ANAPHYLACTIC, PEPTONE, HEMORRHAGIC, AND HISTAMINE SHOCK IN THE DOG

by

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Submitted to the Department of Physiology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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May 1954

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Parke H. Woodard who suggested the problem and contributed materially to this work by constant help and encouragement. The author is also grateful to Dr. Kenneth E. Jochim for many helpful suggestions.

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INTRODUCTION

Of the varieties of shock mentioned in the title of this paper only anaphylactic shock has suffered some confusion in definition. In the literature of approximately the past twenty-five years there has been some difference of opinion as to what constitutes anaphylaxis, but in the case of the dog at least most workers have apparently agreed that anaphylactic shock occurs when there is a more or less immediate drop in blood pressure following the intravenous injection of an antigenic substance into a dog which had been previously sensitized to the same antigen. This is the criterion of anaphylaxis used in this paper.

The term anaphylactoid shock is restricted to those cases in which a blood pressure drop occurs almost immediately after the transfusion of whole blood from one animal to another without the injection of an antigen. In all instances the transfusion was from a previously sensitized dog to another normal animal in an attempt to demonstrate passive anaphylactic shock. In the literature the term anaphylactoid shock is not used with so restricted a meaning as it is used in this report.

Schmidt-Mulheim (1) reported in 1880 that a rapid intravenous injection of peptone into a dog causes a marked drop in blood pressure and a delay in the clotting time of blood. Fano (2) in 1881 demonstrated that coincident with these findings, there is usually a marked increase in the flow of lymph in the thoracic duct together with a decreased coagulability of this

fluid. He emphasized that the clotting power of lymph always parallels that of blood, and he reasoned that lymph is simply a product of the physical diffusion of blood. However, Shore (2) in 1890 reported that a slow intravenous injection of peptone renders the lymph incoagulable but not the blood, which also becomes incoagulable when large, fast peptone injections are made. Shore offered this evidence as support to Heidenhain's secretion concept of lymph formation.

In 1909 Arthus (3) reported that the blood is rendered incoagulable during anaphylactic shock. Waters and Markowitz (4) have demonstrated that anaphylaxis in the dog is possible without the liver, but that an incoagulability of the blood following shock does not occur. Menten (5) had previously reported similar findings in canine peptone shock. Howell (6) in 1925 published evidence that the release of heparin is responsible for the incoagulability of blood during peptone shock. Using more quantitative methods, Jaques and Waters (7) reported that the source of heparin in anaphylactic and peptone shock is the liver.

That the thoracic lymph might carry an anticoagulant from the liver to the blood was suggested by an experiment by Gley (8), who found that ligating the liver lymphatics prevented the incoagulability of blood following peptone shock; but Starling (8) could not confirm these results and attributed Gley's findings to an accidental aggregation of immune dogs. Dragstedt (9), while studying the release of histamine into thoracic lymph, mentioned that following shock thoracic lymph is rendered incoagulable. White and Woodard (10) reported in 1950 that the anticoagulant

released into the thoracic lymph during anaphylactic shock is heparin.

In 1918 Howell and Holt (11) obtained from the liver an anticoagulant which they called heparin. Heparin plus a cofactor (heparin-complement), found in the albumin fraction of blood, have proven to be the most potent natural anticoagulant known (12,13). Heparin has been shown to resemble mucoitin sulfuric acid in structure, having a varying number of sulfuric acid groups in ester linkage (14,15). The anticoagulant effects of heparin have been shown to be directly proportional to the number of sulfate groups present (16,17). Chargaff (12) believes that heparin acts as a prosthetic group to the heparin cofactor.

Heparin is acid in reaction and combines with basic proteins such as protamine to form salts which dissociate very slightly and, therefore, prevents the anticoagulant effect of heparin. This fact forms the basis for the protamine titration method introduced by Waters, Markowitz, and Jaques (4) to determine the concentration of heparin in blood following shock. Protamine seems to prevent the anticoagulant effect of heparin by displacing the albumin cofactor from the heparin-cofactor complex.

The neutralizing effect of protamine was first observed by Chargaff and Olson (16) quite unexpectedly. Its neutralizing effect depends on the source of heparin (7) and to a lesser degree on the source of protamine (19). Protamine sulfate itself has a slight anticoagulant effect both in vivo and in vitro (16,20). The important effect which protamine sulfate has on coagulation in vitro will be discussed later in connection with its use as a means

of estimating heparin concentration. The Connaught Laboratory (Toronto) unit of heparin was used throughout this investigation as a reference for computing heparin concentrations; one such heparin unit is equivalent to 0.01 mgm. of the barium salt.

Lison (21) has shown that toluidine blue reacts metachromatically with sulfuric acid esters of high molecular weight to give a distinct pink color. Since heparin is of this nature, it was first believed and is now widely accepted that toluidine blue or Azure A (a dye which is very similar to toluidine blue) may be used as a means of specifically determining the distribution of heparin within the body. Holmgren and associates (22) were the first to show that heparin or its precusor is produced by the Ehrlich mast cell. It is now believed that these cells are the main somatic source of heparin; and since the Ehrlich mast cells are predominantly located around blood vessels, some investigators consider these cells mainly responsible in preventing intravascular clotting (23). Recent experiments performed on rats by Bifani (24) have shown a parallelism between heparin in the blood and Ehrlich mast cell count in the mesenteries. However, studies on dogs having mast cell tumors do not lend support to the idea that heparin is released into the vascular system from these paravascular cells, even though large quantities of heparin are extractable from such neoplasm (25). The tissue culture studies of Paff and Bloom (25) show that the liberation of heparin involves degenerative changes and even death of the mast cell.

Even though heparin has a diffuse somatic distribution (23), the best evidence indicates that the only source of the heparin in

blood following shock is the liver (5,6). Whether the liver is the exclusive source of the heparin reported by White and Woodard (10) in thoracic lymph following shock is one problem which this report purports to answer.

The investigations dealing with heparin have become extensive. The anticoagulant properties of heparin have been studied in detail (12,13,17,18,23), and its clinical use in thrombic disorders is well known (23,26). Heparin has been reported to have antihistamine properties and to be capable of protecting animals against anaphylactic and peptone shock (29,30,31). The validity of the latter characteristic of heparin has been challenged by some investigations (32,33,34). An idiopathic heparinemia has been reported (35), and it is known that X-ray irradiation and mustard gas therapy may lead to heparinemia in humans (23,36). X-ray irradiation has also been shown to increase the quantities of heparin released by dogs in anaphylaxis (36). Heparin has been reported to counter the pharmacological effects of dibenamine (37) and to act as an anti-complement for sheep red cell hemolysin (40). Heparin has been used successfully to produce complete clinical remission of gout and rheumatoid arthritis (41). Knisely and associates (42) have studied the role heparin plays in preventing the formation of sludged blood and phagocytosis. Heparin is reported to inhibit cell division, frog heart rate, and protoplasmic clotting (43). Heparin is also reported to interfere with normal wound healing (44).

Many recent experiments have indicated that heparin is important in fat metabolism. Hahn (45) in 1943 quite unexpectedly

observed in the dog that the turbidity of lipemic plasm will clear when heparin is administered intravenously. This phenomenon does not occur in vitro. However, lipemic plasma clears in vitro when it is mixed with a plasma sample obtained from an animal previously injected with heparin. The antichylomicronemic substance (46) or clearing factor has been shown by Anfinsen and associates (47) to result from the interaction of a tissue factor, a factor in fraction IV-1 of plasma, and heparin. The clearing factor may be isolated from fraction III-1,2,3 of plasma. This factor has been known to reduce the turbidity of alimentary lipemia, to prevent the development of high cholesterol levels resulting from high cholesterol feeding, to shift plasma high S_f to low S_f lipoproteins, and to inhibit or prevent atherogenesis (48,49,50,51,52,53,54,55).

Considerable interest has been shown recently in regard to the possible role certain hormones play in antigen-antibody reactions, in the release of histamine, and, indirectly, in the release of heparin during anaphylactic shock (56,57,58,59,60,61,62). In this report experiments will be described, and the results presented to help elucidate the possible relationship, if any, between some of the hormones known to be involved in shock phenomena and the release of heparin into the lymphatic system.

The wide use of heparin and interest in this substance speak for the importance of this material in biological phenomena. Also, many phenomena with which heparin is concerned remain to be elucidated. It is the purpose of this study to provide a better understanding of the mechanism leading to incoagulability of blood and lymph following shock, to further establish the identity of the

anticoagulant in lymph following anaphylactic and peptone shock, and to determine the source of this heparin during shock.

METHODS

Dogs were used exclusively in the experiments presented in this study, and the anesthetic of choice was nembutal (Abbott, North Chicago) administered intravenously. Most of the dogs used weighed more than 15 pounds since animals of this size expedite the cannulation of lymph ducts and otherwise make experimental surgery more feasible.

Blood samples for clotting time determination as well as for other studies were withdrawn with a clean, physiological saline washed syringe. Lymph samples were collected by means of a small silicone (General Electric) treated glass cannula inserted into the lymph duct. Preceding each withdrawal of lymph, the cannula was washed with physiological saline, and the lymph samples were withdrawn from the cannula with a clean, physiological saline washed syringe. The clotting time was obtained by the use of a stop watch which was started the instant a sample of blood or lymph was withdrawn into the syringe and was stopped when the blood or lymph sample failed to run when the test tube to which it was added was inverted. This interval was recorded as the clotting time. A 2 ml. blood sample was withdrawn for this purpose. The first and last 0.5 ml. were discarded, and the intermediate 1.0 ml. was transferred into a three inch test tube. To determine the clotting time of lymph, a 0.5 ml. lymph sample was withdrawn from the cannula and transferred into a three inch test tube.

The clotting time of lymph and the clotting time of blood were compared before and after each experimental procedure. If a mixed

blood sample was sought, arterial blood, usually obtained from the femoral artery, was used. In one group of experiments the clotting times of lymph were compared to that of blood obtained from a hepatic vein.

The heparin content in blood and lymph was studied following shock. The concentration of heparin was ascertained by the protamine titration method used so extensively by Jaques and Waters (7). This method was modified here only in the amounts of protamine sulfate used. To six three inch test tubes, varying amounts of protamine sulfate in physiological saline were added. The amounts of protamine sulfate usually employed were 1.0, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.002, and 0.0 mgm. per 0.5 ml. of solution. Exactly 0.5 ml. of the above solution was placed in each tube. About 5.5 ml. of blood or lymph was withdrawn for assay; the first and last 0.5 ml. of the sample were discarded. One-half ml. of blood or lymph was added to each of the protamine tubes, and mixed by inversion. The tubes were examined every one-half minute, and the first sample to clot indicated the end-point of the titration. If the blood or lymph in any of the adjacent tubes clotted within one minute of the first, this fact was used in estimating the amount of heparin present in the first-clotted sample. This estimate of the amount of heparin was recorded in the tables as (/) when slightly more and (-) when slightly less than the value of the end-point.

Since protamine itself has a slight anticoagulant action, the amount of protamine required to neutralize heparin in blood or lymph is that amount which gives the shortest clotting time of

the blood or lymph sample (7). The protamine titration method was the only method used in these experiments to quantitatively estimate the concentrations of heparin in blood or lymph. Although this method is not quantitatively absolute, nevertheless, it gives values for comparison purposes. Other methods are available (21,63,64,65,66,67,68,69,70,71,72).

Intravenous injections of from 10 to 186 mgm. of heparin (Roche-Organon) per kilogram of body weight were made in order to determine whether heparin might be responsible for the incoagulability of lymph following shock and also to ascertain the degree of capillary permeability to heparin. The femoral artery and vein were exposed in normal dogs for blood sample withdrawal and for heparin injections respectively. The thoracic duct was cannulated. Following the intravenous injection of heparin, protamine titrations were made at various intervals on thoracic duct lymph and arterial blood for comparison. In this series of experiments the rate of thoracic duct lymph flow was recorded to determine if an increase might follow the injection of heparin resulting from an altered capillary permeability.

The heparin employed was obtained from beef (Roche-Organon) whose Connaught Laboratory heparin unit strength is 110 per ml. (7). Because the salt forming strength of protamine with heparin depends on the source of both, an attempt was made to standardize the Bios Laboratory (New York) protamine used in these experiments with the Roche-Organon heparin by mixing a known amount of heparin in a blood sample with a known amount of protamine sulfate. The result was about a one to one ratio; consequently any correction for the

amount of heparin with respect to the amount of protamine used was not necessary. However, since dog heparin is nearly two times greater in strength than beef heparin (7,73), it was necessary in those experiments where the release of heparin in canine shock was quantitated to divide the protamine titration amounts by two to obtain mgm. of heparin.

The term shock in this report is mainly employed to denote a marked, sustained drop in blood pressure. While many organs may be deranged by experimental shock, most authorities consider circulatory impairment or failure as constituting the main criterion of shock (34,74). One of the larger arteries, usually the right carotid, was cannulated with a silicone treated cannula, and the blood pressure was recorded by the use of the conventional mercury manometer and kymograph.

Heparin concentrations of thoracic duct lymph and arterial blood were compared at various intervals following canine anaphylaxis. These animals were actively sensitized by a subcutaneous injection of 0.75 ml. of horse serum (Jensen-Salsbery Lab., K.C., Mo.) per kilogram of body weight, followed two days later by an intravenous injection of the same amount. These animals were then shocked about 14 days later by an intravenous injection of a shock dose which ranged from 1.0 ml. to 0.004 ml. per kilogram body weight.

Similar studies were performed using hemocyanin* as the antigen. However, in this series the initial challenge dose was usually much less than the amount employed in the group sensitized to horse serum. If this initial dose did not result in a fatal reaction and

*Courtesy Dr. C. Leone, K. U. Zoology Department.

the animal recovered, then the challenge dose was progressively increased at various intervals until either a fatal reaction resulted or the animal proved to be refractory. Heparin concentrations of lymph and blood were determined and compared in these experiments. Also a study was made to determine if a correlation existed between the size of the challenge dose of antigen and the amounts of heparin released.

The left cervical lymph duct was cannulated in four animals to compare the heparin content of cervical lymph with that of thoracic duct lymph and arterial blood following shock.

A group of dogs were passively sensitized by injecting 20 ml. of sensitized dog blood per kilogram of body weight. These animals were shocked the same day by administering a shock dose of horse serum. Also, four dogs that gave anaphylactoid response as a result of an injection of donor blood from sensitized dogs are included with this group in Table III. Following shock, heparin protamine titrations were made at various intervals on both thoracic lymph and arterial blood for comparison.

Peptone shock was produced in a series of animals, and the content of heparin in thoracic lymph and blood following shock was studied. The injection was made by means of a femoral vein cannula. In seven of these animals from 0.25 to 0.3 gram per kilogram of Witte's peptone, which was first boiled and filtered, was used to produce shock. When Witte's peptone became unavailable, Bactoprotone (Difco Laboratory), similarly treated, was used in its place. Not all commercial peptones will produce shock.

An attempt was made to reproduce Shore's finding (2) that a slow peptone injection causes an incoagulability only of the lymph;

and, in contrast, a rapid injection renders both lymph and blood incoagulable. The former finding seemed to demonstrate a preferential release of heparin into the lymphatics and was restudied for this purpose. A slow, constant infusion of four per cent peptone into the femoral vein was employed to study this phenomenon. The rate and length of time that this slow infusion was carried out varied, with negative results. These same animals were subsequently subjected to a rapid infusion of peptone.

Since histamine is known to be released during anaphylaxis (34), a series of experiments were devised to see if histamine per se might be responsible for the release of heparin during shock or, secondarily, if the marked drop in blood pressure caused by histamine might be responsible for such a release. Approximately 100 gamma per kilogram of histamine was administered intravenously to these animals with no demonstrable release of heparin. These animals were subsequently peptone shocked to demonstrate a capacity to release heparin.

Another series of animals were bled fatally from the femoral artery to determine whether hemorrhagic shock and the resultant derangement of organ function might be an important cause of heparin release.

A group of animals were hepatectomized to ascertain whether the liver was the entire source of the heparin in thoracic lymph following peptone shock. Since compressing the abdomen of the dogs after hepatectomy caused an increase in thoracic lymph flow, it was reasoned that the lymph collected from the thoracic duct was derived from tissue other than the liver which normally contributes to thoracic duct lymph. The animals were immediately

peptone shocked after being hepatectomized, and the blood and lymph were titrated for heparin content. The experimental work must progress rapidly after hepatectomy since removing the liver causes an abrupt decrease in concentration of many substances responsible for normal clotting (75). After hepatectomy, a constant infusion of a 400 mgm. per cent glucose solution in Ringer's solution was used on several of these animals to satisfactorily maintain blood pressure before shock.

Several experiments were devised to determine whether the adrenal gland, in particular the adrenal cortex, might be significantly involved in the release of heparin into the lymphatic and circulatory systems since this gland seems to be of paramount importance in stress reactions (76). Adrenalectomy was performed on several animals which were subsequently subjected to peptone shock. Cortisone acetate (Merk) was administered intramuscularly several hours prior to peptone shock in one animal, and in another cortisone was administered intravenously ten minutes prior to peptone shock. In one experiment 20 mgm. of adrenocorticotropin (Armour Laboratory) was first administered intramuscularly the day before, and a second 20 mgm. given intravenously 3½ hours prior to peptone shock.

In addition to protamine sulfate, toluidine blue (National Aniline Co.) was used to identify heparin as the anticoagulant present in lymph following shock. Lymph was collected from one animal subjected to anaphylactic shock and from another during peptone shock. The method of analysis used was that devised by Gibson (77) and is based upon the colorimetric reaction between

heparin and toluidine blue. This method is presented in detail as an appendix to this work. Other methods that use toluidine blue (or Azure A) to identify heparin are available (63,64,67,71, 72).

RESULTS

The challenge dose of horse serum is expressed in Tables II and III as grams of serum protein per kilogram of body weight. This value is calculated by assuming the total protein concentration of horse serum to be 7 grams per 100 ml. of serum. The challenge dose of hemocyanin is also expressed in Table II as grams of protein per kilogram of body weight and this value is obtained as with horse serum except that the total protein concentration of hemocyanin is assumed to be 4 grams per 100 ml. of solution.

Table I shows that heparin administered intravenously is subsequently detectable in thoracic lymph but is found in greater concentration in the blood than in the lymph. Furthermore, this heparin does not increase the capillary permeability as evident by the negligible change in thoracic lymph flow in most of the animals.

Table II shows, however, that following anaphylactic shock the concentration of heparin in thoracic lymph is usually greater (never less) than that of blood, and that an increase in the flow of lymph might be expected. This increase in thoracic lymph flow during anaphylaxis is a common finding (1,6,23). A heparin release was not evident in 6 of the 49 dogs of this series, and in 12 dogs heparin was evident only in the thoracic duct lymph. In 5 animals comparable concentrations of heparin were detected in the blood and lymph, but in three of these animals it was shown that at one interval following shock the concentration of thoracic lymph heparin was greater than that of blood. The maximum concentration of thoracic lymph heparin detected was 1.0 mgm. per ml.

and in blood 0.5 mgm. per ml.

Animals 1, 3, 6, and 35 in Table II show that although one shock agent (antigen) may fail to cause the release of heparin, a second shock agent (Bactoprotone) may cause such a release. Also, Bactoprotone is seemingly capable of causing a subsequent release of heparin in some animals in addition to that which was released during anaphylaxis as seen with animals 8, 35, 47, and 48. On the other hand, dogs 10 and 19 showed a release of heparin during anaphylaxis but did not subsequently release heparin in peptone shock. Dog 36 proved to be refractory to the release of heparin in both anaphylactic and peptone shock.

Since 6 of the 49 dogs in Table II did not release heparin during anaphylaxis, it is evident that the release of heparin during shock is not an invariable concomitant of shock. There is no absolute relationship shown between antigen dose and the concentration of heparin, but it is evident that the animals showing the greatest release of heparin were subjected to antigen injections of 0.07 mgm. per kilogram of body weight or greater.

Table III shows that the heparin release during passive transfer anaphylactic shock and during anaphylactoid shock is similar. In each animal of this group the concentration of heparin in thoracic lymph was greater than that of blood. In animals 1 and 5 heparin was evident only in the thoracic lymph.

The animals presented in Table IV were subjected to peptone shock, and all but one showed a greater concentration of heparin in thoracic lymph than in blood. The thoracic lymph flow was unusually slow in this particular dog. The thoracic lymph of dog 3

contained heparin after shock, whereas the blood did not. No relationship was found between the shock dose of peptone used and the release of heparin in this particular series.

Table V shows that heparin was not evident in thoracic lymph, arterial blood, and hepatic venous blood after shock in 4 out of 16 animals. Comparable concentrations were evident in all three places in one dog, and in dogs 3 and 12 heparin was evident only in the thoracic lymph. The most striking result presented in this table was that in two dogs the concentrations of heparin in hepatic venous blood was greater than that found in thoracic lymph. Possible reasons for this finding will be discussed later.

Table VI presents evidence that the liver is probably the only source of the heparin released into thoracic duct lymph following shock since hepatectomy in these animals prevented the appearance of heparin in the thoracic lymph and blood.

Table VII presents evidence that hemorrhagic shock does not lead to a release of heparin into the thoracic lymph and blood. Even death due to hemorrhage failed to result in a release of heparin in these dogs.

Table VIII shows that histamine shock failed to result in a heparin release either by direct injury to mast cells or indirectly by lowering the blood pressure. Peptone shock, however, did give rise to a release of heparin in the same animals previously subjected to histamine shock. Therefore, histamine probably plays no role in the release of heparin during shock.

Table IX shows that adrenalectomy immediately before peptone shock does not prevent the release of heparin, whereas adrenocorticotropin and cortisone administration prior to peptone shock

evidently prevents such a release.

Dogs 3, 4, 7, 8, 9, and 10 in Table X demonstrate that a slow constant infusion of even large quantities of peptone will not give rise to a heparin release (i.e., have an accumulative effect), whereas a rapid single injection of comparable amounts of heparin into the same animal will cause a release of heparin. Dogs 1 and 2 proved refractive to the release of heparin, and dog 10 shows that a slow single injection of small amounts of peptone might cause a release of heparin into the thoracic lymph but not into the blood. Dogs 5 and 6 also indicate that this last finding may not happen. The importance of these findings will be discussed later in relation to those reported by Shore (2).

Dogs 2 and 3 in Table XI show that heparin may be present in thoracic lymph and not appear in cervical lymph or blood after peptone shock. Dog 4 shows clearly that heparin appears in cervical lymph after it appears in blood. No release of heparin following shock was evident in animal 1.

Table XII presents a series of 6 unshocked animals that showed a prolongation of the clotting time in thoracic lymph as compared to normal values. In 4 of these animals heparin was evident only in thoracic lymph. Heparin was evident in both blood and lymph in the remaining 2 animals. All dogs of this group except dog 5 were previously sensitized to horse serum before anesthetization. Dog 5 was a normal anesthetized dog selected at random.

The average clotting time of blood of 131 dogs was found to be 6'51" and for thoracic duct lymph 5'57". The clotting time

value for lymph reported here is less than that reported in the literature (6,77). Possible reasons for this discrepancy will be discussed later. Seventeen dogs out of 131 showed thoracic lymph clotting times exceeding 10 minutes and 12 dogs showed blood clot-time time exceeding 10 minutes.

The finding that 21 out of 116 dogs failed to release heparin during shock (7 during anaphylaxis, 6 after histamine shock, 5 after peptone shock, and 3 after hemorrhagic shock) is evidence that heparin release is not an invariable concomitant of shock.

By use of the procedure reported by Gibson for the extraction of heparin from plasma (77), it was shown that the material extracted from incoagulable thoracic lymph after anaphylactic and peptone shock gave a color reaction with toluidine blue which was indistinguishable from that given by a toluidine blue-heparin reaction. Therefore, it is reasonable to consider that this material extracted from lymph after shock contains heparin.

DISCUSSION

Since the concentration of heparin in the thoracic lymph was found to be greater than that present in arterial blood in most of the dogs reported here, and since Jaques and Waters (7) have shown that the liver is the source of heparin during anaphylaxis or peptone shock, it is reasonable to postulate that after shock the thoracic duct serves as an important transport for heparin from the liver into the blood stream. This contention is supported by the findings reported in Table VI and also by the fact that in an experimentally produced obstructive jaundice, bile is detected earlier in the thoracic duct lymph than in blood (79), indicating that the hepatic lymphatics are more accessible to certain materials than is blood. Furthermore, many of the animals reported here showed a heparin release only into the thoracic lymph, and many more showed a much more prolonged release of heparin into the thoracic lymph than into blood following shock.

This conclusion is essentially the same as that reached by Chittenden and associates (80), who discovered that diverting the lymph flow from the general circulation by cannulation of the thoracic lymph prevented peptone injection from rendering the blood incoagulable, while the coagulation of lymph was delayed. Spiro and Ellinger (80) had demonstrated the same phenomenon earlier. Chittenden considered these findings as supporting the results of Gley (2), who found that ligation of the liver lymphatics prevented the ordinary clot-retarding action of albumose when injected into

dogs. This led Chittenden to conclude that probably the anti-clot substance formed in the liver reaches the blood mainly through the lymph. Starling, on repeating the experiments on ligation of the liver lymphatics of Gley, failed to confirm this experimental finding and believed Gley's results due to the use of immune dogs. Since this last contention is unlikely (81), another possible explanation of these results based on the evidence presented here exists.

It is possible that the animals used by Gley, Spiro, Chittenden, and their associates may have released heparin only into the liver lymphatics, and that the animals used by Starling may have released heparin both into the liver lymphatics and blood; consequently ligation of the liver lymphatics by Starling failed to render the blood incoagulable.

Jaques and Waters (4,7) reported that maximum values for heparin in the blood were detected from 6 to 12 minutes following shock. They favor the explanation of their titration curves that heparin is initially released rapidly from the liver during shock, but that maximum heparin values in the blood are delayed because a drop of blood pressure of 25-35 mm. Hg causes a lengthening of the circulation time of the liver. However, considering the thoracic duct a primary portal of entry for heparin into the systemic circulation would explain, because of the comparatively slow lymph flow, the delay in the maximum heparin values in blood shown by their titration curves.

Jaques and Waters (7) have shown by direct extraction methods that the amount of heparin released from the liver during anaphylaxis may be from 53.0 to 98.8 per cent of the total heparin content

of the liver, although they state that their method is perhaps not without error. This finding would explain why some of the animals reported here showed an additional release of heparin with a second shock and others did not.

Heparin has been reported to protect animals against fatal shock (29,30,31). Since heparin is known to have antihistamine properties, this protective action might be expected. However, Haughey (32) and others (33,34) were unable to demonstrate such protection. This last finding is supported in the experimental studies presented here by a lack of correlation between the concentration of heparin release into lymph or blood and the degree of recovery from shock.

The experimental data presented by Arbesman (61), Criep (62), and their coworkers show that adrenalectomy increases the immunologic reaction of hypersensitiveness, whereas cortisone and adrenocorticotropin confer some protection against anaphylaxis. They did not study heparin release during shock. The studies presented here suggest that cortisone or adrenocorticotropin administration in some manner raises the threshold for the release of heparin. Perhaps the reason why some of the experimentally shocked dogs shown in Table II and V did not release heparin was because of a hyperadrenocortical function.

The hepatectomized animals reported in Table VI and dogs 3 and 12 in Table V lend support to the evidence presented by Jaques and Waters that the only source of heparin during shock is the liver.

The demonstrable existence of accessory lymphaticovenous communications in some dogs (82) may explain the relatively low

concentration of thoracic lymph as compared to hepatic venous blood shown in dogs 4 and 13 in Table V. Hemodilution of the hepatic venous blood as a result of entering the systemic circulation as well as urinary excretion (63) would also explain this relatively high concentration of heparin in the hepatic venous blood as compared to arterial blood. Manipulation of the liver while obtaining the venous sample of blood may have decreased significantly the quantity of lymph entering the thoracic duct from the liver in these two animals. In this regard, it is significant that the thoracic duct lymph flow was unusually slow in these dogs.

The data presented in Table X do not support Shore's contention (2) that the slow administration of peptone selectively renders the thoracic lymph incoagulable, whereas a rapid injection renders incoagulable both lymph and blood. Shore used Grubler's (83) peptone, and in the studies reported here Bactoprotone was used. It is not known whether he used different animals for slow and rapid injection. If the latter is true, Shore may have inadvertently discovered one of the findings reported here that some dogs may release heparin only into the thoracic lymph during shock.

Howell (84) reported in 1914 that thoracic duct lymph collected from fasting dogs clotted between 10 and 20 minutes, and that the lymph collected from well-fed dogs containing much chyle-fat coagulates much more slowly (50-60 minutes). Shore in 1890 reported somewhat lower coagulation times (5-15 minutes) for thoracic lymph. Perhaps the use of a vaselined cannula to obtain the thoracic lymph accounts for the coagulation times reported by

Howell as compared to those reported here which were collected by means of a silicone-coated cannula. Since about 16 per cent of the dogs reported here had control clotting times for lymph exceeding 10 minutes, it is possible that the dogs used by Howell in his studies represent an accidental aggregate of dogs showing a longer than average clotting time for thoracic lymph. Howell's finding that the presence of chyle-fat prolongs the clotting time of thoracic lymph presents a problem worthy of investigation.

Since the addition of suitable amounts of protamine will cause incoagulable lymph resulting from shock or an intravenous injection of heparin to coagulate, and since extracts of incoagulable lymph obtained from anaphylactic and peptone shocked dogs give a typical heparin color reaction with toluidine blue, it is reasonable to identify the anti-clot substance present in thoracic lymph following shock as heparin.

SUMMARY

1. The thoracic duct has been shown to be an important, if not the main, portal of entry of an anti-clot substance into the general systemic circulation during peptone, anaphylactic, anaphylactoid, and passive transfer anaphylactic shock.

2. This anti-clot substance has been identified as heparin.

3. The liver is the source of this heparin in dogs.

4. If heparin is released during shock, its concentration is greater in thoracic duct lymph than in blood.

5. The maximum concentration of heparin in the thoracic lymph following shock was found to be 1.0 mgm. per ml. and in arterial blood 0.5 mgm. per ml.

6. Peptone and anaphylactic shock can occur without a concomitant release of heparin.

7. The intravenous injection of heparin results in a concentration of heparin in the blood which is proportionately greater than that found in thoracic duct lymph.

8. Heparin administered intravenously does not increase capillary permeability.

9. It has been shown that occasionally when one shock agent fails to cause a release of heparin in an animal, a different shock agent may cause such a release in the same animal.

10. Heparin is not released during histamine or hemorrhagic shock.

11. Cortisone and adrenocorticotropin administration inhibits the release of heparin during peptone shock, whereas adrenalectomy prior to peptone injection has no effect on the release

of heparin in shock.

12. Six animals showing an idiopathic release of heparin are reported. In each case the concentration of heparin in thoracic duct lymph was greater than that in blood.

13. The average clotting time of blood in 131 dogs was found to be 6'51" and for thoracic duct lymph 5'57".

14. This report did not confirm the finding reported by Shore that a slow injection of peptone selectively renders the thoracic lymph incoagulable, whereas a rapid injection renders incoagulable both thoracic lymph and blood.

APPENDIX

PHOTOMETRIC ASSAY OF LYMPH HEPARIN

Add 5 ml. of lymph to a 15 ml. test tube, dilute with an equal volume of M/15 phosphate buffer pH 6.2, and precipitate by repeated addition of octylamine hydrochloride in 0.5 ml. amounts every 5 minutes with immediate mixing until a total 2 ml. of octylamine is used. If floculation is delayed for more than 10 minutes, warm the tube to 40 degrees centigrade. Centrifuge down the precipitate and discard the supernatant. Wash the precipitate with distilled water and pour off the supernatant. Moisten the precipitate with exactly 0.5 ml. of N/2 NaOH and mix with 10 ml. of 0.9 per cent saline. Heat this mixture in a water bath at 65-70 degrees centigrade for 15 minutes. After cooling, add 0.5 ml. of 10 per cent $ZnSO_4$. Mix and let stand for 15 minutes. Heparin is in the precipitate that forms.

Extract this heparin precipitate with 5 ml. saline and 5 ml. of 7.4 phosphate buffer. Heat at 65-70 degrees centigrade. Centrifuge down the precipitate, transfer the supernatant to another test tube, and add 1 ml. of toluidine blue O solution (1 to 1,000 solution). Let this mixture stand for several hours or preferably over night. A red precipitate will form. Centrifuge down this precipitate, wash with water, and dissolve in alkaline alcohol (4 ml. of alcohol plus 1 ml. of N/10 NaOH).

Prepare a heparin standard and a dye blank reference. Read the heparin content spectrophotometrically at 530 m . Transmittancy plotted semi-logarithmically gives a straight line graph.

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TABLE I

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING INTRAVENOUS INJECTION OF HEPARIN

Dog No.	Clotting time before injection		Amt. heparin injected in mgm. per kg.	Clotting time after injection		Mgm. heparin per ml.		Time interval following injection		Rate of thoracic lymph flow in % of control
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood	
1	6'30"	7'00"	20.00	8'00"	24 hr.+	none	0.04	5'00"	60'00"	98.
						none		60'00"		
2	3'40"	4'20"	10.00	2'30"	24 hr.+	none	0.20-	20'00"	10'00"	50.
						0.10		45'00"		
3	5'00"	10'00"	20.00	7'00"	24 hr.+	none	0.20	5'00"	25'00"	107.
						0.10		30'00"		
4	7'00"	8'00"	40.00	6'00"	24 hr.+	none	0.24	15'00"	25'00"	96.
				24 hr.+		0.20		35'00"		
				24 hr.+		0.08		140'00"		
5	2'00"	3'00"	10.00	24 hr.+	24 hr.+	0.04-	0.04	20'00"	10'00"	102.
6	2'30"	2'30"	23.00	24 hr.+	24 hr.+	0.04	0.04	15'00"	10'00"	100.
7	4'30"	7'00"	186.00	48 hr.+	48 hr.+	0.50	1.00	2'00"	2'00"	
				48 hr.+	48 hr.+	0.75	1.00	32'00"	37'00"	
				6.5 hr.+	6.5 hr.+	0.50	0.50	1 hr.	1 hr.	
				5 hr.+	5 hr.+	0.25	0.25	2.5 hr.	2.5 hr.	

TABLE II

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING ANAPHYLACTIC SHOCK*

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock		Rate of thoracic lymph flow in % of control
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood	
1	6'00"	3'00"	0.77	1'00"	11'00"	none	none	6'00"	6'00"	
			0.33p	24 hr.-	24 hr.-	0.50	0.05	9'00"	9'00"	
2	3'00"	5'00"	0.004	2'00"	2'00"	none	none	6'00"	6'00"	
				24 hr.≠	12'00"	0.10	none	30'00"	30'00"	
	24 hr.≠	12'00"	0.12p	24 hr.-	4 hr.-	0.25	0.05	5'00"	10'00"	
3	2'30"	3'30"	0.0064	4'30"	3'30"	none	none	5'00"	0'30"	
				7'00"	4'00"	none	none	12'00"	12'00"	
	7'00"	4'00"	0.15p	17 hr.≠	32'00"	0.05	0.002	3'00"	3'00"	
4	6'30"	5'00"	0.0082	9'00"	3'00"	none	none	3'30"	3'30"	
5	6'00"	3'30"	0.07	3'00"	1'30"	none	none	7'00"	7'00"	
6	3'00"	3'00"	0.21	14'00"	2'00"	none	none	6'00"	6'00"	
	14'00"	2'00"	0.75p	4 hr.≠	4 hr.≠	0.50	0.05	11'00"	11'00"	
7	7'00"	3'30"	0.0052	27'00"	3'20"	0.002	none	3'00"	3'00"	
8	6'00"	10'00"	0.0068	5 hr.≠	7'00"	0.25	none	10'00"	13'00"	
	5 hr.≠	7'00"	0.07	4 hr.≠	5'00"	0.01	none	3'30"	5'00"	
	5 hr.≠	13'00"	0.25p	2 hr.≠	2 hr.≠	0.50	0.25	7'30"	9'00"	
9	0'30"	2'00"	0.01	6 hr.≠	0'45"	0.25	none	17'00"	13'00"	
			0.06	6 hr.≠	0'45"	0.50	none	3'00"	3'00"	
10	1'10"	0'45"	0.017	3 hr.≠	2'00"	0.25	none	3'00"	3'00"	
				3 hr.≠	1'30"	0.10	none	11'30"	11'30"	
	3 hr.≠	1'30"	0.026	3 hr.≠	0'40"	0.05	none	6'00"	2'30"	
	12'00"	2'00"	0.115p	8'00"	2'00"	none	none	5'00"	7'00"	

TABLE II

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING ANAPHYLACTIC SHOCK (cont.)

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock		Rate of thoracic lymph flow in % of control
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood	
11	4'00"	4'00"	0.021	24 hr.+	11'00"	0.10	none	8'00"	8'00"	
	24 hr.+	10'00"	0.23	24 hr.+	4'00"	0.10	none	30'00"	30'00"	
			0.06	24 hr.+	2'30"	0.05	none	2'00"	2'00"	
12	2'20"	3'00"	0.024	24 hr.+	3'00"	0.05	none	3'00"	3'00"	
				24 hr.+	24 hr.+	0.25	0.025	17'00"	17'00"	
	24 hr.+	9'00"	0.15p	24 hr.+	4'00"	0.25	none	5'30"	5'30"	
13	4'00"	5'30"	0.052	82'00"	5'00"	0.005	none	5'30"	7'30"	
				2'30"	3'00"	none	none	15'00"	17'00"	
14	6'30"	2'20"	0.013	2 hr.+	9'00"	0.05	0.0008	2'00"	2'00"	
	1.5 hr.+	4'30"	0.105	2 hr.+	15'00"	0.10	0.025	7'00"	7'00"	
15	2'30"	4'00"	0.07	19'00"	21'20"	0.02-	0.005	5'00"	10'00"	105.
16	3'00"	3'40"	0.07	24 hr.+	27'00"	0.10	0.005	5'00"	3'00"	
				1.5 hr.	2'00"	0.005	none	40'00"	40'00"	
			0.10p	4 hr.-	2'20"	0.01	none	3'00"	4'00"	
17	4'00"	4'00"	0.07	24 hr.+	24 hr.+	0.10	0.005	12'00"	20'00"	100.
18	2'10"	5'00"	0.07	24 hr.+	24 hr.+	0.50	0.005+	8'00"	12'00"	
19	1'20"	2'45"	0.0068	2 hr.+		0.005+		4'00"		
				2 hr.+	2 hr.+	0.005	0.005	11'00"	9'00"	
				2 hr.+	2'20"	0.05	none	32'00"	26'00"	
			0.05p	6'00"	1'20"	none	none	8'00"	6'00"	
20	6'30"	4'30"	0.0038	22'00"	14'00"	0.01	0.0004	7'00"	6'00"	
				25'00"	4'00"	0.01	none	20'00"	20'00"	

TABLE II

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING ANAPHYLACTIC SHOCK (cont.)

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock		Rate of thoracic lymph flow in % of control
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood	
21	2'00"	6'00"	0.0005	24 hr.+	115'00"	0.25	0.05	7'00"	7'00"	
	2'00"	2'00"	0.13p	24 hr.+	3'00"	0.25	none	5'00"	5'00"	
22	4'30"	2'00"	0.0082		24 hr.+		0.05		2'00"	
				2 hr.+	2 hr.+	0.10	0.05	8'00"	8'00"	
				2 hr.+	2 hr.+	0.50-	0.025	20'00"	20'00"	
	17'00"	8'00"	0.0205	15'00"	8'00"	none	none	14'00"	14'00"	
23	3'00"	4'30"	0.042	24 hr.+	26'00"	0.50	0.05	4'00"	4'00"	
24	2'00"	1'30"	0.07	24 hr.+	24 hr.+	0.50	0.05	10'00"	10'00"	270.
				24 hr.+	24 hr.+	0.50	0.05	13'00"	27'00"	
25	15'00"	3'00"	0.07	24 hr.+	24 hr.+	0.50	0.05	5'00"	5'00"	100.
				24 hr.+	24 hr.+	0.10-	0.05-	10'00"	8'00"	
						0.10	0.02+	5'00"	7'00"	
26	14'00"	13'00"	0.07	10 hr.+	1 hr.+	0.25	0.05	3'30"	3'30"	
27	7'00"	1'30"	0.076	10 hr.+	10 hr.+	0.05	0.025	5'00"	4'00"	
28	3'00"	3'00"	0.07	24 hr.+	24 hr.+	0.50	0.10-	5'00"	10'00"	250.
29	9'00"	2'30"	0.016	24 hr.-	2 hr.+	0.50	0.05	7'00"	15'00"	
30	7'20"	6'00"	0.07	24 hr.+	24 hr.+	0.50	0.10-	15'00"	10'00"	500.
31	3'00"	11'00"	0.088	24 hr.+	24 hr.+	1.00	0.50	5'00"	5'00"	
32	8'00"	6'45"	0.15	24 hr.+	24 hr.+	1.00	0.50	8'00"	10'00"	
33	10'30"	5'00"	0.00043	4'30"	2'30"	none	none	5'00"	7'30"	
	7'30"	4'00"	0.07	24 hr.+	2.5 hr.	0.50	0.025	4'30"	7'30"	

TABLE II

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING ANAPHYLACTIC SHOCK (cont.)

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock	Rate of thoracic lymph flow in % of control
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood
34	9'00"	3'30"	0.0031	7'00"	3'30"	none	none	5'30"	5'30"
	7'00"	3'00"	0.07	24 hr.+	24 hr.+	1.00	0.50	4'30"	7'00"
35	6'00"	1'45"	0.07	5'00"	5'00"	none	none	3'30"	3'30"
	4'00"	4'00"	0.07	2'00"	2'30"	none	none	15'00"	15'00"
	2'00"	2'30"	0.17p	65'00"	45'00"	0.10	0.10	19'00"	19'00"
36	2'30"	5'00"	0.08	3'00"	2'30"	none	none	6'00"	6'00"
	6'00"	3'00"	0.29p	7'00"	4'00"	none	none	7'00"	7'00"
37	7'30"	3'00"	0.0003	10 hr.+	3'30"	0.025	none	17'00"	21'00"
	6'00"	4'00"	0.07	24 hr.+	24 hr.+	0.50	0.05	4'30"	6'00"
38	4'00"	7'00"	0.0015	1 hr.+	5'00"	0.25-	none	16'00"	16'00"
	4'30"	2'00"	0.07	1.5 hr.+	2'00"	0.25	none	6'00"	6'00"
39	2'00"	7'00"	0.0015	4 hr.+	1.5 hr.+	0.25+	none	4'30"	4'30"
40	2'00"	5'00"	0.002	5 hr.+	5'30"	0.01	none	3'00"	6'00"
	10'00"	1'30"	0.10"	8'30"	1'30"	none	none	5'00"	9'00"
41	3'00"	3'00"	0.07	6 hr.+	8'00"	0.01	none	8'00"	11'00"
42	13'00"	3'00"	0.07	24 hr.+	10'00"	0.10	none	3'00"	3'00"
43	9'00"	10'00"	0.00017	6 hr.+	6 hr.+	0.01	0.01	4'00"	4'00"
	24 hr.-	1 hr.-	0.0085	24 hr.-	1 hr.-	0.10-	0.01	29'00"	29'00"
				2 hr.-	50'00"	0.01+	0.01	7'00"	7'00"
44	10'00"	6'00"	0.0007	24 hr.+	35'00"	0.025	0.01	5'00"	7'00"
	8'00"	2'00"	0.07	2 hr.+	1 hr.+	0.01	0.01	4'30"	7'30"

TABLE II

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING ANAPHYLACTIC SHOCK (cont.)

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock	Rate of thoracic lymph flow in % of control
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood
45	6'00"	11'00"	0.0015	2 hr.+	50'00"	0.10	0.10	2'30"	2'30"
	4'00"	2'00"	0.015	2 hr.+	2'00"	0.50	none	5'00"	3'00"
46	3'00"	8'00"	0.0034	24 hr.+	45'00"	0.05	0.01	5'00"	5'00"
	3'00"	2'00"	0.035	3 hr.+	6'00"	0.05	none	3'30"	5'30"
47	4'30"	3'00"	0.07	25'00"	25'00"	0.01	0.01	7'30"	9'30"
	2'30"	5'00"	0.25p	24 hr.+	6'30"	0.10	none	10'00"	10'00"
48	2'00"	4'30"	0.07	3 hr.+	19'00"	0.01+	0.01-	6'00"	9'00"
	3 hr.+	19'00"	0.14	3 hr.+	8'00"	0.025	none	3'00"	4'00"
	3 hr.+	8'00"	0.25p	24 hr.+	4'30"	0.25	none	3'30"	4'00"
49	3'30"	6'00"	0.072	24 hr.+	24 hr.+	1.00	0.50	2'00"	2'00"

* Horse serum was used as the antigen in the first 32 animals, and hemocyanin for the remaining 17.
p Bactoprotone shock.

TABLE III

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING PASSIVE TRANSFER ANAPHYLACTIC AND ANAPHYLACTOID SHOCK*

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock	
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood
1	4'30"	5'30"	0.059	3 hr.+	12'00"	0.10	none	3'00"	3'00"
2	4'30"	7'00"	0.08	40'00"	9'00"	0.005	0.0004	4'00"	5'00"
3	4'00"	8'00"	0.07	2 hr.+	8'30"	0.01	0.0004	15'00"	15'00"
				24 hr.+	7'00"	0.025	none	6'00"	6'00"
4	2'00"	4'30"	0.044	2 hr.+	60'00"	0.10	0.05	2'00"	2'00"
	5'00"	0'30"	0.025	3 hr.+	1'40"	0.01	none	8'00"	8'00"
	3'00"	13'00"	0.014	2 hr.+	14'00"	0.01	none	22'00"	22'00"
5	2'10"	11'00"	23 ml.	22'00"	11'00"	0.005	none	5'00"	5'00"
6	9'00"	6'00"	50 ml.	1 hr.	23'00"	0.01	0.01	8'00"	5'00"
				2 hr.	28'00"	0.10	0.05	27'00"	27'00"
7	2'40"	5'00"	14 ml.	2 hr.+	18'00"	0.05	0.01	5'00"	3'00"
8	15'00"	12'30"	14 ml.	24 hr.+	24 hr.+	0.50	0.50	5'00"	7'00"
				24 hr.+	12'00"	0.10	none	80'00"	80'00"

* The last 4 dogs gave an anaphylactoid response when injected with the amounts of blood indicated in ml. per kilogram.

TABLE IV

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING PEPTONE SHOCK

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock	
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood
1	8'30"	8'00"	0.12	1 hr.+ 1 hr.+	9'00" 9'30"	0.01 0.10	none none	5'00" 25'00"	5'00" 25'00"
2	3'00"	2'30"	0.22	24 hr.+ 24 hr.+	9'00" 24 hr.+	0.05 0.10	none 0.025	3'30" 12'00"	3'30" 12'00"
3	1'25"	8'30"	0.15	2 hr.+	7'00"	0.25	none	8'00"	5'00"
4	2'00"	2'00"	0.18	24 hr.+ 8'30"	3'00" 4'00"	1.00- 0.05+ none	none 0.02 none	8'00" 35'00" 65'00"	3'00" 21'00" 55'00"
5	4'00"	1'20"	0.15	7 hr.+	32'00"	0.005	0.002	3'00"	3'00"
6	4'00"	10'00"	0.12	24 hr.+	3 hr.+	0.50	0.01	6'00"	6'00"
7	8'30"	9'00"	0.34	16 hr.+	16 hr.+	0.05 0.50	0.025 0.50	5'30" 16'00"	5'30" 16'00"
8	4'00"	5'00"	0.37	24 hr.+	24 hr.+	1.00	0.025	4'00"	4'00"
9	10'00"	2'00"	0.075	4 hr.+	4.5 hr.+	0.50	0.05	11'00"	11'00"
10	3'00"	5'00"	0.61	24 hr.+	24 hr.+	0.25	0.05	6'00"	6'00"
11	11'00"	8'00"	3.85	24 hr.+	24 hr.+	0.05	0.05	6'30"	10'00"
12	5'00"	2'00"	0.19	19'00"	2 hr.	0.025	0.05	6'30"	6'30"
13	2'30"	3'00"	0.115	24 hr.+ 4'00"	16 hr.+ 4'30"	0.50 none	0.05 none	2'40" 11'00"	4'00" 11'00"

TABLE IV

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING PEPTONE SHOCK (cont.)

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock	
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood
14	2'30"	5'30"	0.10	24 hr.+	24 hr.+	0.50	0.05	4'00"	4'00"
				24 hr.+	24 hr.+	0.50	0.05	15'00"	15'00"
15	7'30"	1'00"	0.10	24 hr.+	24 hr.+	0.50	0.10	5'00"	15'00"
16	6'00"	5'00"	0.10	1 hr.+	1 hr.+	0.50+	0.10	3'30"	3'30"
17	3'00"	2'00"	0.13	24 hr.+	24 hr.+	0.50	0.10	3'00"	3'00"
				24 hr.+	24 hr.+	0.25	0.025	13'00"	13'00"
18	1'30"	9'00"	0.40	24 hr.+	24 hr.+	0.50	0.10	9'30"	13'00"
19	8'30"	6'00"	0.12	24 hr.+	24 hr.+	1.00	0.10	5'00"	5'00"
20	7'00"	3'30"	2.70	24 hr.+	20'00"	1.00	0.10	3'00"	3'00"
21	6'30"	1'10"	0.12	24 hr.+	24 hr.+	1.00	0.10	6'00"	6'00"
22	2'30"	6'30"	0.14	24 hr.+	24 hr.-	1.00	0.50	7'00"	7'00"
23	8'00"	5'30"	0.22	24 hr.+	24 hr.+	0.50	0.50	5'00"	3'00"
						0.25	0.05	18'00"	16'00"

TABLE V

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH, ARTERIAL BLOOD, AND HEPATIC VENOUS BLOOD FOLLOWING SHOCK*

Dog No.	Clotting time before shock			Challenge dose in gm. per kilo.	Clotting time after shock			Mgm. heparin per ml.		
	Lymph	Arterial Blood	H.V.Bl.		Lymph	Arterial Blood	H.V.Bl.	Lymph	Arterial Blood	H.V.Bl.
1	7'00"	4'00"	6'30"	0.081	12'00"	3'45"	5'00"	none	none	none
2	3'00"	3'00"	3'00"	0.07	3'00"	3'00"	3'00"	none	none	none
3	7'30"	6'30"	4'20"	0.07	5 hr.+	11'00"	12'30"	0.01	none	none
4	4'00"	3'30"	3'30"	0.07	4 hr.+	4 hr.+	4 hr.+	0.01	0.01	0.01
5	3'30"	4'00"	6'00"	0.07	24 hr.+	24 hr.+	24 hr.+	0.025	0.05	0.10
6	14'30"	5'00"	6'00"	0.07	24 hr.+	24 hr.+	24 hr.+	0.10	0.05	0.10
7	7'00"	4'30"	4'30"	0.14	24 hr.+	24 hr.+	24 hr.+	1.00	0.25	0.50
8	10'00"	8'00"	8'00"	0.07	24 hr.+	6 hr.+	6 hr.+	1.00	0.025	0.25
9	3'00"	4'00"	2'00"	0.07	3 hr.+	1 hr.+	1 hr.+	0.10	0.01	0.01
10	11'30"	6'00"	7'00"	0.14	11'00"	7'00"	8'00"	none	none	none
11	7'30"	3'50"	7'30"	0.30	2'00"	6'30"	4'00"	none	none	none
12	8'30"	7'00"	3'00"	0.10	24 hr.+	6'30"	3'20"	1.00	none	none
13	12'00"	2'00"	2'00"	0.27	20 hr.+	1.17 hr.	1.25 hr.	0.025	0.01	0.01
14	5'00"	2'00"	4'00"	0.37	1 hr.+	25'00"	1 hr.+	0.01	0.01	0.025
15	8'30"	8'30"	3'00"	0.087	24 hr.+	24 hr.+	24 hr.+	0.25	0.05	0.10
16	8'00"	8'00"	8'00"	0.22	24 hr.+	24 hr.+	24 hr.+	0.50	0.25+	0.50

* The first 9 animals were sensitized and shocked with horse serum, the remaining were normal dogs shocked with Bactoprotone.

TABLE VI

EFFECT OF HEPATECTOMY ON THE CONTENT OF HEPARIN IN THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING PEPTONE SHOCK

Dog No.	Clotting time before shock		Challenge dose in gm. per kilo.	Clotting time after shock		Mgm. heparin per ml.		Interval following shock	
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood
1	6'00"	8'30"	0.67	5'30"	11'00"	none	none	6'30"	2'30"
2	4'30"	5'00"	0.71	6'00"	4'00"	none	none	7'00"	4'00"
3	7'00"	5'30"	0.64	4'30"	8'30"	none	none	2'00"	3'00"

TABLE VII

COMPARISON OF HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD FOLLOWING HEMORRHAGIC SHOCK

Dog No.	Clotting time before shock		Per cent drop in blood pressure from control	Clotting time after shock		Mgm. heparin per ml.		Interval following shock	
	Lymph	Blood		Lymph	Blood	Lymph	Blood	Lymph	Blood
1	4'30"	6'00"	66.00	3'00"	3'30"	none	none	2'00"	3'00"
				4'30"	4'00"	none	none	12'00"	14'00"
				4'30"	3'45"	none	none	23'00"	24'00"
2	7'30"	7'30"	100.00	3'30"	3'30"	none	none	3'30"	2'00"
3	10'00"	8'30"	100.00	6'00"	3'30"	none	none	5'00"	7'30"

TABLE VIII

EFFECT OF HISTAMINE AND PEPTONE SHOCK ON HEPARIN CONTENT OF THORACIC LYMPH AND ARTERIAL BLOOD

Dog No.	Clotting time before shock		Amt. Histamine in gamma per kilogram	Shock dose in gram peptone per kilogram	Clotting time after shock		Mgm. heparin per ml.	
	Lymph	Blood			Lymph	Blood	Lymph	Blood
1	7'00"	9'30"	100.		10'30"	12'30"	none	none
	8'00"	8'00"		0.55	2 hr.+	23'00"	0.10	0.025
2	8'00"	6'30"	100.		9'00"	4'00"	none	none
	9'00"	4'00"		0.45	27'00"	3'00"	0.01	none
3	8'15"	7'30"	105.		8'30"	7'00"	none	none
	8'30"	7'00"		0.45	18 hr.+	18 hr.+	0.25	0.05
4	15'00"	6'30"	122.		25'00"	3'30"	none	none
	18'00"	7'00"		0.61	24 hr.+	24 hr.+	0.25	0.10
5	8'30"	5'00"	125.		7'00"	6'00"	none	none
	7'00"	6'00"		0.62	30'00"	22'00"	0.025	0.01
6	11'30"	12'00"	130.		15'00"	14'00"	none	none

TABLE IX

EFFECT OF ADRENOCORTICAL HORMONES OF THE RELEASE OF HEPARIN DURING PEPTONE SHOCK

Dog No.	Pre-shock treatment	Clotting time before shock		Shock dose in gram peptone per kilogram	Clotting time after shock		Mgm. heparin per ml.	
		Lymph	Blood		Lymph	Blood	Lymph	Blood
1	Adrenal-ectomy	9'30"	3'00"	0.34	2 hr.+	2 hr.+	0.01+	0.01
2	Adrenal-ectomy	12'30"	10'00"	0.074	1 hr.+	4'00"	0.01	none
3	100 mgm. cortisone I.V.	12'00"	9'30"	0.025	14'00"	9'00"	none	none
4	150 mgm. cortisone I.V.	4'30"	4'00"	0.033	10'00"	11'30"	none	none
5	20 mgm. I.M. plus 20 mgm. I.V. of ACTH	5'00"	5'30"	0.04	3'30"	5'00"	none	none

TABLE X

COMPARISON OF SLOW AND RAPID ADMINISTRATION OF PEPTONE ON THE RELEASE OF HEPARIN

Dog No.	Clotting time before injection		Rate of injection in gm. per min.	Total amt. injection in grams	Clotting time after injection		Mgm. heparin per ml.	
	Lymph	Blood			Lymph	Blood	Lymph	Blood
1	6'00"	3'00"	0.06**	2.4	5'30"	2'30"	none	none
	5'30"	3'00"	Rapid*	2.4	8'00"	6'00"	none	none
2	7'00"	6'00"	0.004**	0.12	1'30"	1'20"	none	none
	1'30"	1'20"	Rapid*	0.50	2'00"	0'45"	none	none
3	4'00"	4'00"	0.10**	5.00	2'30"	0'20"	none	none
	2'30"	0'25"	Rapid*	5.00	45'00"	0'25"	0.01	none
4	7'00"	5'00"	0.015**	0.32	6'00"	6'00"	none	none
			0.04**	0.72	2'30"	4'00"	none	none
			0.04**	3.00	2'00"	2'00"	none	none
	2'00"	2'00"	Rapid*	3.00	3 hr.	4'00"	0.05	none
5	4'00"	4'30"	1.0*	2.00	8'30"	5'00"	none	none
			Rapid*	2.00	24 hr.+	5'00"	1.0	none
6	4'30"	4'00"	0.05*	0.25	2'00"	6'30"	none	none
			Rapid*	0.40	2 hr.+	3'00"	0.50	none
			Rapid*	1.50	2 hr.+	2 hr.+	0.50	0.10
7	11'00"	2'30"	0.106**	3.20	2'45"	2'00"	none	none
			Rapid*	3.20	14 hr.+	10 hr.+	0.01	0.01
8	5'30"	5'00"	0.24**	0.20	7'00"	6'00"	none	none
			Rapid*	6.50	24 hr.+	2 hr.+	0.05	0.01
9	7'00"	3'30"	0.10**	0.40	7'00"	4'00"	none	none
			Rapid*	0.40	2 hr.+	2 hr.+	0.025	0.025
10	6'00"	7'30"	0.125*	0.25	6 hr.+	8'00"	0.01	none
	6'30"	7'00"	0.09**	1.50	6'30"	7'00"	none	none
			Rapid*	6.00	18 hr.+	16'00"	0.01	0.01-

* Single injection
**Infusion

TABLE XI

COMPARISON OF HEPARIN CONTENT OF CERVICAL LYMPH, THORACIC LYMPH, AND ARTERIAL BLOOD FOLLOWING SHOCK

Dog No.	Clotting time before shock			Dose in Gm. per kilo.	Clotting time after shock			Mgm. heparin per ml.		
	Cervical lymph	Thoracic lymph	Arterial blood		Cervical lymph	Thoracic lymph	Arterial blood	Cervical lymph	Thoracic lymph	Arterial blood
1	4'00"	7'00"	5'00"	0.001*	1'30"	6'00"	8'00"	none	none	none
2	2'00"	8'00"	2'00"	0.07**	3'30"	25'00"	4'00"	none	0.025	none
3	7'00"	9'00"	3'30"	0.37***	3'00"	7 hr.+	4'00"	none	0.50	none
4	2'00"	8'00"	5'30"	0.13**	1'30"	2 hr.+	2'00"	none	0.10	none
				0.04***	2'30"	24 hr.+	24 hr.+	none	0.50	0.25
					2 hr.-	24 hr.+	24 hr.+	0.002	0.50	0.25

* Hemocyanin (death resulted
** Horse serum
***Bactoprotone

TABLE XII

STUDIES ON ANIMALS SHOWING IDIOPATHIC RELEASE OF HEPARIN

Dog No.	Control clotting time		Mgm. heparin per ml.	
	Lymph	Blood	Lymph	Blood
1	75'00"	3'30"	0.002	none
2	6 hr.+	11'00"	0.025	none
3	24 hr.+	3'00"	0.10	none
4	5 hr.+	13'00"	0.10	none
5	85'00"	30'00"	0.025	0.001
6	2 hr.+	2 hr.+	0.02	0.005