THE VASODILATOR AND VASOCONSTRICTOR PROPERTIES OF BLOOD SERUM AND PLASMA.*

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Plate 8.

The introduction of the Loewen-Trendelenburg (1) frog preparation for the quantitative and qualitative determination of suprarenin aroused high hopes that at last a method had been discovered whereby the internal secretion of the adrenal glands could be estimated in both physiological and pathological conditions. The Ehrmann-Meltzer (2) frog's pupil preparation is not sensitive enough to detect small variations in the concentration of suprarenin in the blood; the ox carotid method of Meyer possesses the disadvantage that its application in the case of foreign sera is untrustworthy (Schlayer (3)); the virgin uterus of the rabbit may react by either contraction or dilatation (Falta and Fleming (4)); while, finally, the inhibition produced on a strip of intestine is so uncertain that this method alone is of small value.

Toward solutions of pure suprarenin the Loewen-Trendelenburg method is intensely sensitive. It will detect with ease as small a concentration as one in forty million. It is unquestionably the most accurate method for the quantitative determination of solutions of pure suprarenin. Moreover, the test is easily applied, and very small amounts of the solution are necessary. But it has been found that when used for a fluid as complex as blood serum, it is not specific for suprarenin. Its use may, however, be said to have led to the discovery of a new class of vasoconstrictor substances in blood serum.

The observation was made by O'Connor (5) and confirmed later by Falta and Fleming, that the quantitative estimation of suprarenin

* Received for publication, April 13, 1912.

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in serum, obtained by the Loewen-Trendelenburg preparation, is at variance with that given by the uterus preparation. The latter as compared with the former gave results that indicated a higher concentration of suprarenin. O'Connor therefore concluded that the discrepancy arose from the presence of an additional vasoconstrictor substance and that the uterus preparation recorded the combined action of this substance and the suprarenin. He was able to show that this newly discovered constrictor substance is not found in an active form in plasma, but is liberated during the process of coagulation.

While O'Connor's work was still unknown to us, we observed that the blood serum from a patient with Addison's disease possessed as great a constricting power on the vessels of the isolated rabbit kidney as did that of normal serum, although after the death of the patient no suprarenin could be extracted from the remains of the suprarenal gland. We interpreted this result at the time as an indication of the presence of a vasoconstrictor substance other than suprarenin in the serum of the patient with Addison's disease. We determined, therefore, to make a systematic study of the vasoconstrictor action of the serum in this disease, and we began by establishing a standard in normal serum. We have observed, however, some hitherto undescribed properties of the sera of normal animals and these observations form the basis of the present communication.

METHODS OF INVESTIGATION.

Profiting by the experience of Schlayer that foreign sera are less sensitive on a strip of artery than homologous sera, we at first so arranged our experiments that the properties of serum were tested upon the vessels of an animal of the same species. The vessels employed were those of the kidney, the hind limb, and the heart of the rabbit, dog, and cat. The principle of the apparatus is essentially that used by Trendelenburg; namely, the determination of the rate of flow of fluid perfused through the isolated organs. Since we are dealing, however, with mammalian organs, perfusion had to be carried out at the temperature approximating that of the body, and in a moist atmosphere so as to prevent drying.

We found the following device very convenient and satisfactory.

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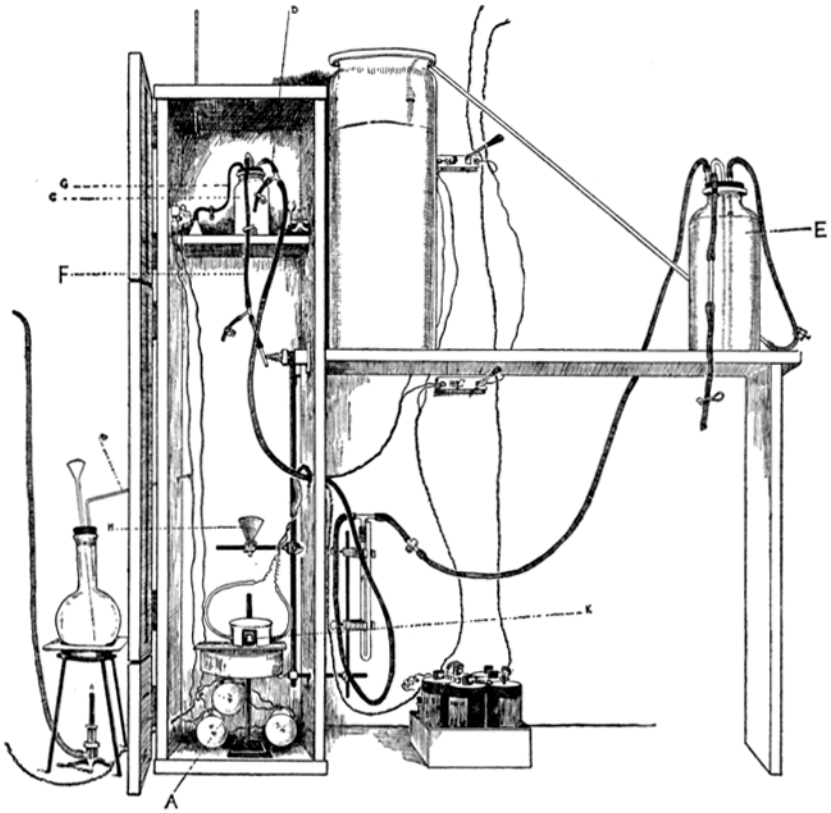
A wooden box 36 by 12 by 9 inches (text-figure 1), closed in front by three glass doors, one immediately above the other, was heated at the bottom by three Edison electric bulbs (A). When the temperature of the room is about 60° F., these will maintain a temperature within the box of about 35° C. At one side an opening admitted a glass tube 3 mm. in diameter (B) through which was passed a jet of steam from a Jena flask containing water that was kept boiling throughout the experiment. This prevented the drying of the



TEXT-FIG. 1. The perfusion apparatus.

perfused organ and supplemented the electric bulbs in keeping the temperature at about 37° C. A small shelf was inserted inside the box ten inches from the top, on which rested a thick glass bottle containing Ringer's solution (C). Through the cork stopper of this bottle three holes were bored, and each admitted glass tubes. One tube (D), which just passed through the cork, was connected with a pressure bottle (E); a second tube (F) served as the outflow and was connected by rubber tubing with the cannula inserted in the artery;

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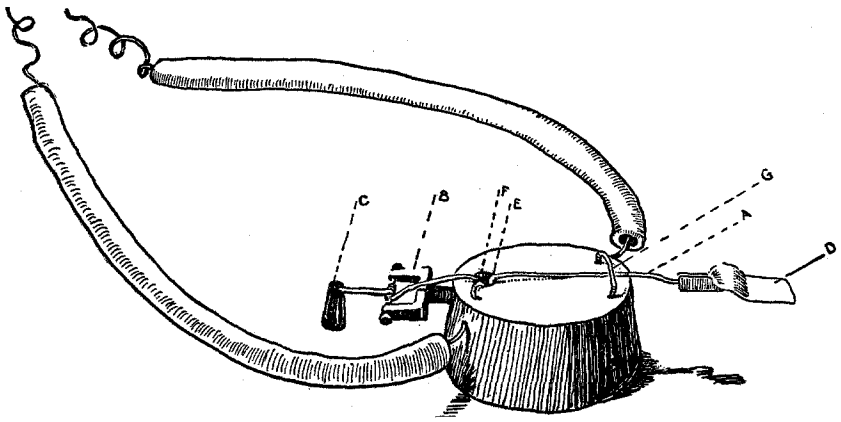
and the third tube (G), connected with a rubber tube clamped at the opposite end, served as a means for filling the reservoir. After passing through the organ, the perfusion fluid was allowed to run down the side of a filter funnel (H) and the rate of flow was determined by a drop decorder (K).

We tried many forms of drop recorder, but the most satisfactory, in that it responded to a frequency of drops just short of a constant stream, is that depicted in text-figure 2. It consists of a large rubber cork carrying a platinum wire (A). For this purpose the wire of the ordinary bacteriological platinum loop answers perfectly. Toward one end the wire passes through the pivot (B), and at (C) is attached a weight of about 5 gm. The opposite end carries a flattened piece of aluminum (D) upon which the drops fall. A small loop of the wire (E) is continuously immersed in a well of mercury (F) which is connected



TEXT-FIG. 2. Magnified drawing of drop recorder.

by a wire running to a terminal of the battery. Contact is made against a platinum cross wire (G). A constant current runs from three dry batteries, the circuit being broken by each drop. The use of platinum is necessary to prevent rusting of the contacts in a moist atmosphere.

After a few experiments we discarded the use of a cannula inserted in the vein, as we found that the least movement caused kinking and very seriously interfered with a constant rate of outflow. It was much simpler and much more accurate to allow the fluid to trickle from the vein into the funnel. Perfusion was maintained under a constant pressure of 40 mm. of mercury.

The Ringer's solution used had the following composition: sodium chlorid, 0.9 per cent.; potassium chlorid, 0.035 per cent.; calcium chlorid, 0.024 per cent.; sodium bicarbonate, 0.03 per cent. While the organ was being perfused with Ringer's solution, the serum to be tested by a hypodermic syringe was injected through the rubber tubing that conveyed the Ringer's solution to the organ. This method has the apparent objection that the serum is diluted by the perfusion fluid and does not reach the organ in a concentrated state. We found, however, that this objection is more apparent than real, for the rubber tubing is so narrow that the amount of serum used for each test, usually 2 c.c., when injected rapidly, drives the Ringer's solution backwards and reaches the organ before any great dilution has taken place. The drops were recorded

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by a signal magnet on a smoked drum on which also a Jacquet chronograph marked off the time in seconds (figure 1).

The experiments may be divided into two main groups; those that deal with the effects of serum and plasma (1) upon the renal vessels of the dog and rabbit, and (2) on the peripheral vessels of the rabbit and frog. Human, dog, rabbit, and sheep sera were tested.

In a few experiments the action on coronary arteries was tested by perfusion of the heart of the rabbit. In these experiments we used a smaller pressure in order to prevent the organ from beating. Injections were made as in the case of the kidney and limb.

EXPERIMENTS ON THE KIDNEY VESSELS.

Three distinct phases are observed when the kidney is perfused with Ringer's solution. There is a preliminary increase in rate which rises in a few minutes to a maximum while the organ is being washed free of blood. The first phase shows a fairly rapid rate of outflow and persists from seven to twelve minutes. The number of drops of outflow per minute then gradually diminishes until it is less than half that of the original rate. This period of diminishing outflow, or second phase, varies with different kidneys, and even with the kidneys of the same animal. It may last from fifteen minutes to one hour, but it is always succeeded by the third phase, a period during which the rate of outflow is constant. We have endeavored in all of our experiments to inject the plasma or serum to be tested only when the rate of perfusion had reached this period of constancy.

Fortunately, as far as the test is concerned, the effect of serum on the kidney shows itself as soon as it has reached the arterioles and lasts for a short period of time only. This stands in contrast to that which is observed in the vessels of all the other organs tested. In the case of the peripheral and coronary vessels, it sometimes takes half an hour for the outflow to return to its original level.

While the above description holds for the majority of experiments, it is to be emphasized that great variations occur and that no two kidneys exhibit phases of exactly the same duration. However, we have never failed to observe a period of constant flow, and

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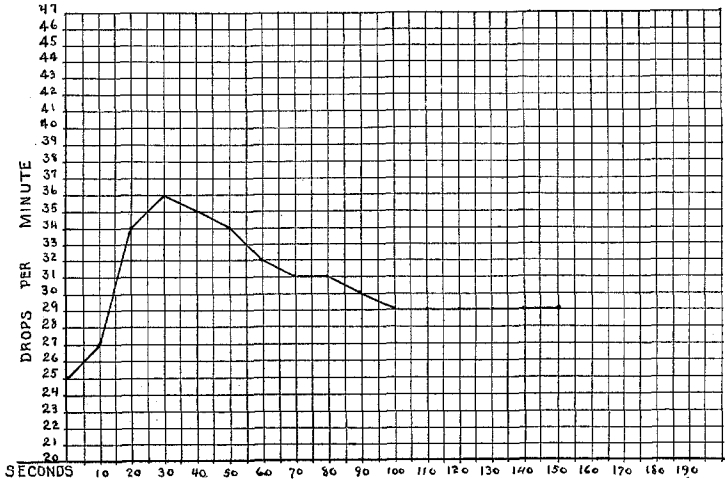
when tests are made within the limits of this phase, very clear cut and delicate reactions may be obtained.

In our earlier experiments the changes in ureter flow were recorded in addition to the outflow from the vein. Since, however, we soon noticed that the flow from the ureter was the same under all observed conditions we confined our subsequent observations to the venous outflow.

Text-figure 3 is a composite curve obtained from the venous flow



TEXT-FIG. 3. Effect of dog serum on rabbit kidney.

through rabbit kidneys after injections of dog serum. The abscissæ represent time in seconds, and the ordinates the change in the number of drops of outflow from the vein per minute.

The essential effect produced by the injection of two cubic centimeters of serum into an excised kidney perfused with Ringer's solution is, first, a rapid increase in the velocity of outflow, amounting in many cases to 150 per cent. The reaction begins as soon as the serum enters the kidney vessels, and gradually reaches a maximum which is attained in from twenty to thirty seconds. The maximum rate of outflow is maintained for only a few seconds. In fact, in many experiments the curve begins to recede immediately after the highest point is reached. The return to the original rate is much more gradual and not infrequently shows an irregularity for which

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it is difficult to find an explanation. The fall in rate after the maximum dilatation is rapid during the first twenty seconds. Thereafter, the flow is much more gradual. A point slightly in excess of the original rate is reached usually in about two minutes. It is not unusual to find that this slightly increased rate may persist for several minutes before the normal outflow occurs. We are inclined to attach some importance to the fact that after the maximum has been reached the rate with which the curve falls to normal is faster at first than it is later, and to this we shall have occasion to refer in another part of this paper.

Figure 1 is a representative tracing which is charted in text-figure 4.

The ureter outflow shows a fall coincident with the arterial dila-

TEXT-FIG. 4. Effect of dog serum on rabbit kidney.

tation, but in contrast to the venous outflow, recovery is much delayed and frequently the outflow ceases entirely. We have not been able to determine the factors that influence ureter flow even when the kidney is perfused with Ringer's solution alone, for we have seldom been able to obtain a constant rate for any length of time. After the injection of serum into a kidney, the flow through the ureter rarely returns to normal.

The sera of different animals vary markedly in their vasodilator effect on the vessels of the rabbit kidney. Of those tested, we have found that the serum of the dog acting either on dog or on

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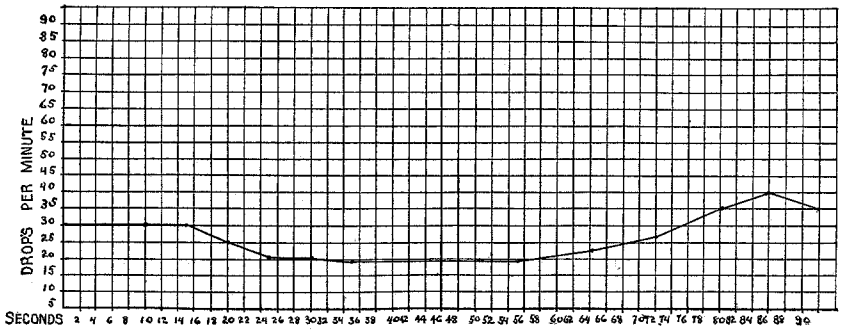
rabbit kidney is always the most efficient in causing vasodilation. It causes a much more abrupt response than do other sera, and the increased outflow frequently amounts to three times the original. Return to normal is more gradual than with rabbit serum, but otherwise the sera are essentially similar in their mode of action. Human serum also possesses a vasodilator action which varies in different individuals, but is, as a rule, less potent than the serum of the dog or the rabbit. Sheep serum is the least active of those we have examined.

While in the majority of experiments rabbit serum when tested on rabbit kidney reacts in the typical manner already described, we have observed in eight experiments out of a total of thirty-four that the reverse result may be obtained; namely, either a vasoconstriction alone or a vasoconstriction followed by a vasodilatation. An example is seen in text-figure 5 (experiment 28). In this figure the



TEXT-FIG. 5. Effect of rabbit serum on rabbit kidney.

injection of two cubic centimeters of rabbit serum caused a fall in outflow amounting to 45 per cent. and was followed in the course of a minute by an increased outflow of 22 per cent.

In another experiment (experiment 20) the diminished outflow was the dominant feature and was not followed subsequently by an increased outflow.

The reversed action is to be attributed to the state of the kidney rather than to the serum, for the same serum tested on dog kidney produces the usual vasodilator effect. Unfortunately, the kidneys that did not react in the usual manner to serum were not examined histologically for the presence of pathological changes.

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The kidney of the rabbit alone exhibits this reversal. We have not observed it in the dog or cat.

Repeated injections of serum tend to establish a condition of insensitiveness of the kidney vessels. It is probable that this is the result of a partial exhaustion of the vasodilator mechanism, since a kidney rendered inactive by repeated injections of rabbit serum will not respond further to dog or human serum.

To all the sera tested the kidney of the dog reacts in identically the same manner as does that of the rabbit except that no instance of reversed action has been observed. Nevertheless, we have found dog kidney less sensitive as a routine test for the vasodilator substance, because it very readily becomes edematous and soon ceases to respond to vasomotor influences.

It may be mentioned at this point that the vasodilator power of serum is specific for the renal vessels. When serum is tested on the peripheral vessels of both warm and cold blooded animals, the opposite effect, namely, a pronounced vasoconstriction, is always produced. Separate constituents of the blood serum are responsible for the different effects on the two sets of vessels, and the causal factor cannot be ascribed to one substance acting differently on different arteries.

As far as we have seen, the first work in the literature dealing with the vasomotor effect of blood serum is that of Brodie which appeared in 1900, although it must be mentioned that Stevens and Lee (6) had previously encountered great difficulty in perfusing the frog and terrapin with defibrinated blood. Brodie (7) found that the injection of blood serum, from whatever source, into the external jugular vein of a cat caused an immediate depression of the blood pressure. In addition there was also produced cardiac and respiratory inhibition. He gives clear proof that the fall in blood pressure is not explained by the cardiac inhibition alone. Thus the fall can occur in some animals in which there may be no noticeable inhibition, and section of the cardiac branches of the vagus does not prevent its occurrence. By plethysmographic records he also eliminated the possibility of a direct action of the serum on the heart muscle. The effect is almost entirely a reflex through the vasomotor center from excitation of the pulmonary nerves.

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Although in the cat the general effect is a dilatation of the systemic arterioles, the kidney forms an exception. According to Brodie, the injection of serum in the cat causes a constriction of the renal vessels. This conclusion, however, is probably erroneous. Brodie considers the action on the kidney to be due to a reflex stimulation of the vasomotor center for the vessels supplying the kidney. Since experiments on the dog and rabbit were negative, it would seem that the cat is possibly the only animal which reacts in this manner to injections of serum.

While Brodie's experiments deal essentially with a substance acting by reflex inhibition of the vasomotor center and in that respect differ from the effects we have described on the isolated organ, he must be given the credit for having given the first demonstration of the fact that serum and plasma are not identical in their physiological activity. Since the publication of O'Connor's work, to which reference has already been made, this difference has received much attention. Brodie discovered the following facts concerning the nature of the substance that acts through the vasomotor center: first, that it is a proteid of the albumin class, coagulable by boiling, and formed during the process of clotting; and, secondly, that the corpuscles take an active part in its formation.

We have not repeated Brodie's experiments, but on the isolated cat kidney we have found that serum produces a pronounced vasodilatation and that the kidney of this animal differs in no way from that of the rabbit and dog.

Comparing Brodie's experiments with our own it would seem difficult at first sight to reconcile the facts that serum acting peripherally causes a dilatation of the kidney vessels, but acting centrally leads to a constriction of these vessels through a reflex stimulation of that part of the vasomotor center that supplies the kidney. It might of course be argued that two distinct substances are present in the serum, each having its selective action and affinity. It is, however, unnecessary to make this assumption in view of the work of Sollmann (8) which appeared six years later.

Sollmann, to whom apparently Brodie's work was unknown, described in the serum a vasodilator substance for the renal vessels acting on the isolated kidney. He also observed that the substance

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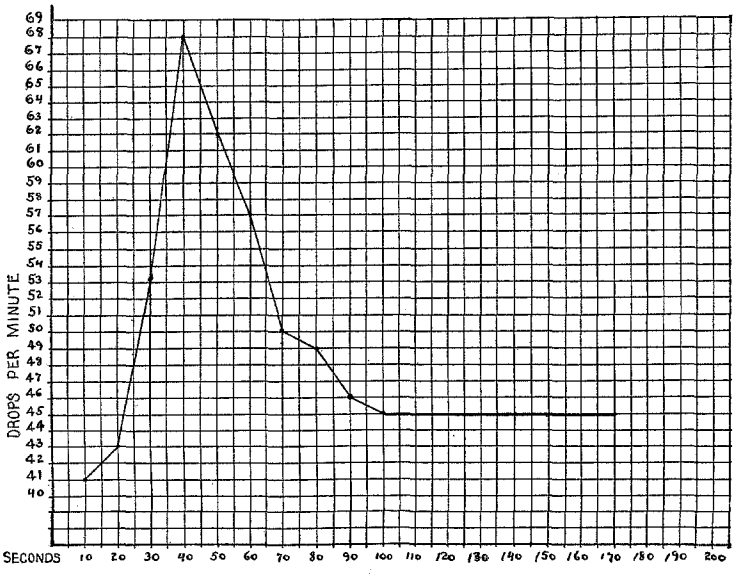
was a coagulable proteid, but did not extend his investigation beyond the determination of this fact. He shows, however, that the increased flow from the vein is accompanied by a diminution in kidney volume, the vasodilator effect being therefore limited to the efferent vessels.

This probably accounts for Brodie's misinterpretation that there was a vasoconstriction of the kidney vessels and a vasodilatation of the peripheral vessels, when in reality there is a dilatation of both peripheral and renal vessels. The difference lies in the fact that in the case of the kidney it is the efferent vessels which are dilated, while in the limb the afferent vessels are more especially dilated.

NATURE OF THE DILATORY SUBSTANCE.

It is evident at the outset that the dilatation of the vessels of the perfused kidney must be regarded as the result of the activity of a substance other than that described by Brodie. His dilatory substance is potent only by reflex inhibition of the vasomotor center and is specific for the cat's vessels. The vasodilator substance described by us acts on the periphery and is not specific for the vessels of any one animal, and is found in the serum of all the laboratory



TEXT-FIG. 6. Effect of dog serum on dog kidney.

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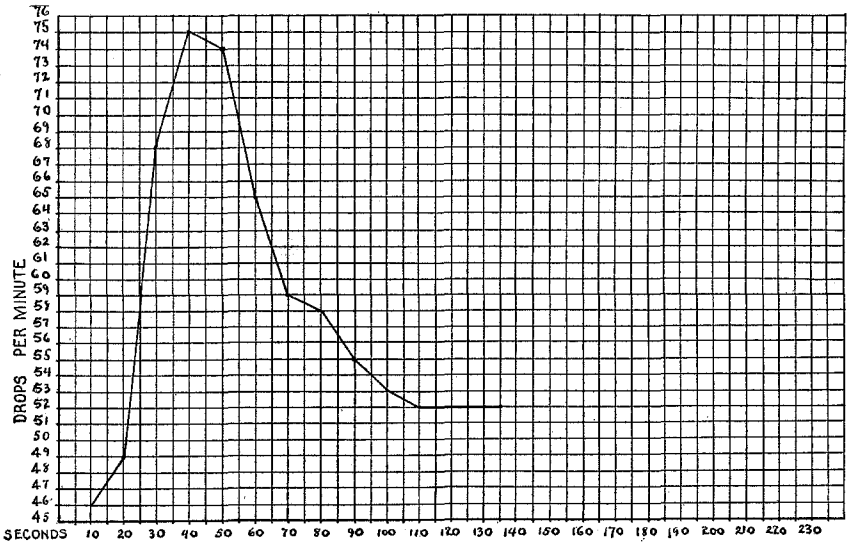
animals tested. We have found, further, that it is a constituent of the plasma and is not liberated during the process of clotting. This fact is brought out by the following experiments.

Two 20 c.c. samples of blood were taken from the same animal. To one was added 5 c.c. of a 0.2 per cent. solution of hirudin in Ringer's fluid. To the other was added 5 c.c. of Ringer's fluid alone. After the sample to which Ringer's solution alone was added had clotted, the serum was pipetted off and injected into the perfused kidney. It produced the typical dilatory effect (text-figure 6).

The sample of blood that was prevented from clotting by the addition of hirudin was immediately centrifugalized, the plasma filtered, and 2 c.c. were injected as before. An identical result was obtained; namely, a pronounced vasodilatation which equalled that of the serum alone (text-figure 7).



TEXT-FIG. 7. Effect of dog hirudin plasma on dog kidney.

There remains the possibility that in the plasma sample the dilatory effect was produced by the hirudin used to prevent clotting. This point was readily settled by the injection of hirudin alone in the concentration used in the plasma. No change was produced in the rate of flow.

Having thus found that the vasodilator substance is a constituent of normal plasma, we next attempted to determine its origin and chemical properties.

Recent work on the internal secretion of the pituitary gland has

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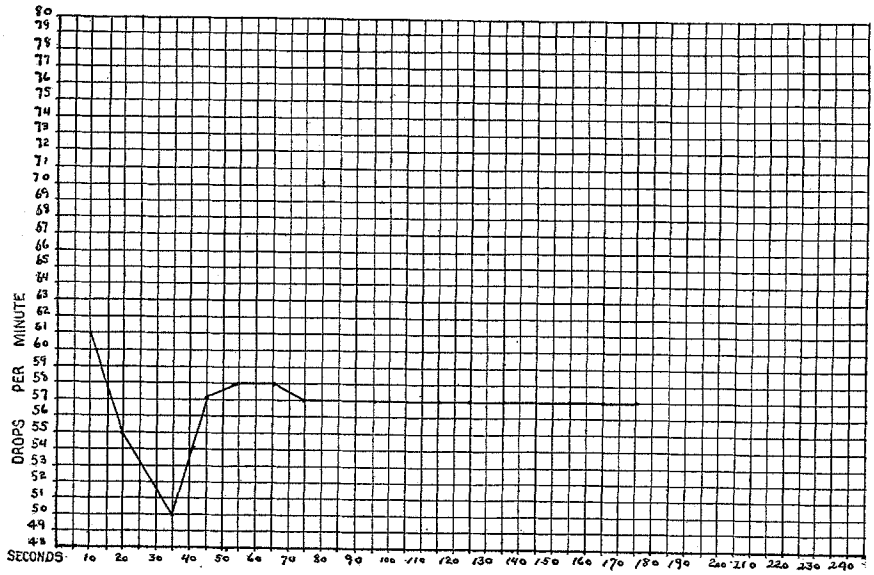
indicated that in this gland a substance is present that causes an increased flow of blood from the renal vessel in addition to an increase of the kidney volume as measured oncometrically (Magnus and Schäfer (9)). This substance is present only in the posterior lobe and can be extracted either by water or glycerin. It produces, in addition, a constrictor action on the vessels of the extremity as was shown by Oliver and Schäfer. They found that it diminishes the flow through a perfused frog.

Can the dilator effect of normal plasma on the kidney be attributed to the presence of this substance? It can be satisfactorily shown that the active substance obtained from the pituitary gland is not responsible for the dilatory effect on the renal vessels, for it has been found that pituitary extracts retain their activity after boiling, whereas we have observed that plasma or serum after coagu-



TEXT-FIG. 8. Effect of dog serum (boiled) on dog kidney.

lation by boiling loses its dilator property. The following experiments bear this out.

The serum was diluted three times with Ringer's solution, brought to the boiling point, and filtered through a Berkefeld filter. Two c.c. were injected and the result was compared with that obtained after the injection of a similar

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amount of normal serum diluted to the same extent. The normal diluted serum caused a dilatation, but in text-figure 8 it will be seen that the boiled diluted serum produced, not a vasodilatation, but a vasoconstriction.

While these experiments serve to differentiate the two substances, they introduce a new factor that will be discussed later.

A recent work by Ogawa (10) dealing with the dilator effects of suprarenin makes it clear that suprarenin stimulates both the vasoconstrictor and the vasodilator nerve terminations, and the weaker the solutions, the more pronounced, relatively, is the dilator action. At a certain concentration no primary constrictor effect may be elicited, the only observable result being dilatation. He has proved that this holds good for the vessels of the intestine, and in some experiments he has found it to occur also in the kidney.

That a dilator action such as that described by Ogawa should account for our results seems *a priori* highly improbable, but the fact that suprarenin, like pituitary extract, resists boiling, indicates at once that we are not dealing in our experiments with suprarenin.

Since the vasodilator substance is precipitated by boiling, we infer that it belongs to the proteids. The filtrate, after precipitation by alcohol and dilution with Ringer's fluid, is inactive, indicating that the substance is present in the precipitate. We have not, however, been able to recover the active substance when once precipitated. It is not identical with the complement of the blood serum, since heating to 70° C. for four hours does not destroy it, although there is a noticeable reduction in its activity.

The vasodilatin described by Popielski (11), which he regards as the depressor substance of Witte's peptone, differs both chemically and physiologically from the dilator substance that we have found in plasma and serum. This vasodilatin has been carefully studied by Popielski and his pupil, and it has been shown by them to be identical with the depressor substances that can be extracted from the brain, intestines, and other organs. It is not a proteid and resists boiling in alkaline solutions. It does not act specifically on the kidney, but exercises its influence on the peripheral vessels as well.

As we have already pointed out, boiled serum loses its vasodilator property, but it is not inactive, for it can cause a well marked vasoconstriction (text-figure 8).

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It might be contended that the change in the concentration of the inorganic elements of the serum incidental to boiling may have given rise to the constrictor effect that was evident after coagulation of the proteid. It is now well known, especially from the work of Hooker (12), that an increase in the sodium salts tends to cause dilatation of the blood-vessels, while an increase in the calcium salts produces constriction. Although boiling does not alter the relative concentration of the salts in blood serum, the possibility that an increase in the concentration of these salts might cause the constrictor effect was considered, and several injections of serum were made in which the original volume was restored by the addition of distilled water, but decreasing the concentration of the salts altered in no way the intensity of the reaction.

A more likely explanation is that there are two substances in serum, one of which, the dilator, is precipitated by boiling, while the constrictor remains in the filtrate. If this is the case, the two substances are so apportioned that the dilator is the more powerful. What one really measures after the injection of normal serum is the resultant of the activities of two substances that oppose each other.

If a vasoconstrictor substance is present in serum before boiling, it may be the normal suprarenin of the serum or it may be a substance liberated during clotting.

That the vasoconstriction is not produced entirely by suprarenin is proved by experiments with apocodeine to be described later. It seems highly probable, therefore, that a true vasoconstrictor substance is actually present in the serum and that its existence becomes manifest only after the removal of the vasodilator.

In this opinion we are strengthened by the study of the form of the plotted curve obtained by injecting unboiled normal serum. This curve (text-figure 3) has a sharp apex formed by the quick rise to a maximum and the sudden fall toward normal. At first the curve descends very rapidly but soon more gradually. We have evidence that the vasoconstriction (represented by the drop in the curve) begins before the serum has passed through the organ, for in many experiments in which the serum was blood-tinged due to the laking of a little blood in defibrinating it, this color of the serum has enabled us to recognize it in the fluid escaping from the vein. Our

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interpretation of the curve is that the dilator substance, on account of its shorter latent period, is unrestrained in its action for the first few seconds after it reaches the vessels and thus causes the very sudden increase in the flow. Soon, however, the constrictor substance becomes active and rapidly diminishes the flow until an equilibrium is established, after which the outflow gradually drops to normal as the serum is washed out.

THE SEAT OF ACTION OF THE VASODILATOR AND THE VASOCONSTRICTOR SUBSTANCES.

The work of Dixon (13) has furnished a method whereby the action of a vasodilator or vasoconstrictor substance can be localized either on the sympathetic nerve terminations or on the muscle coat. Apocodeine hydrochlorid injected into the intact animal paralyzes the sympathetic endings in the arterial coat with the result that a subsequent injection of suprarenin is without effect. We therefore added to the stock Ringer's solution a sufficient amount of apocodeine to make a strength of 0.1 per cent., and perfused the kidney as before. In a few minutes the outflow from the vein was uninfluenced by the injection of even 1 to 10,000 adrenalin chlorid solution (Parke, Davis and Company). Very strong doses of the drug may, however, produce some slight constriction, but the marked difference in response of the vessels before and after the action of apocodeine is so pronounced that for concentrations of suprarenin approximating that of the blood serum it may be held that apocodeine completely abolishes its constrictor effect.

Effect of Serum after Apocodeine.—If two cubic centimeters of normal serum be injected into the vessels of a kidney perfused with Ringer's solution containing apocodeine, the effect is to produce the vasodilatation that is usual when the organ is perfused with Ringer's solution alone.

In an average of five experiments the increased outflow amounted to 120 per cent. In text-figure 9 the three phases previously described are to be distinguished. We have received the impression that paralysis of the sympathetic nerve terminations results in a greater percentage of increase in flow for the same amount of the serum injected. The experiments, however, are insufficient in num-

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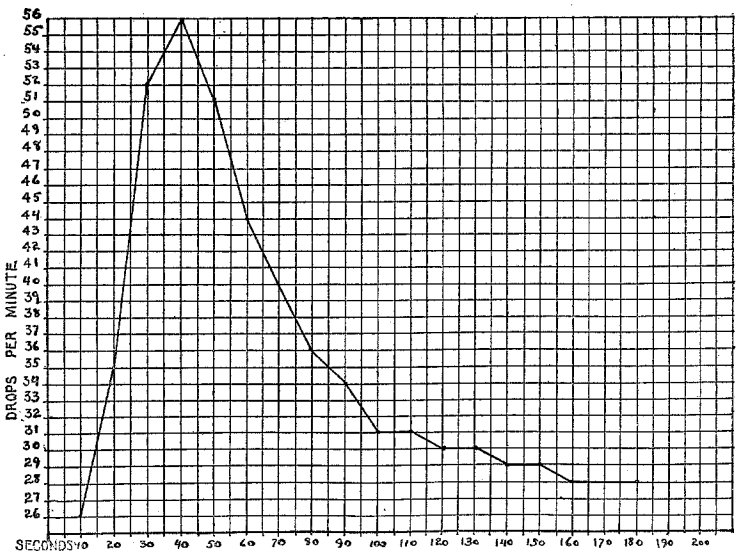
ber to justify a positive statement, since very great variations are observed normally both in regard to the sensitiveness of the kidney and to the potency of serum. *A priori* a greater percentage increase in the outflow after apocodeine is to be anticipated since the adrenalin content of the serum tends to lessen the degree of dilata-



TEXT-FIG. 9. Effect of rabbit serum and apocodeine on rabbit kidney.

tion produced. We are concerned, however, in the present work more with the qualitative than with the quantitative effect, and the results are so distinct as to warrant the conclusion that the dilator substance in normal serum acts directly on the muscle coat.

In a similar manner it is easy to determine that the vasoconstrictor substance has an analogous mode of action, for boiling destroys the vasodilator substance, while the vasoconstrictor remains active. The injection of boiled serum into the vessels of the kidney whose vasomotor endings are put out of function by apocodeine produces a well marked diminution in the flow from the organ, indicating that the vasoconstrictor substance also acts directly on the muscle coat.

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THE RESPONSE OF PERIPHERAL VESSELS TO INJECTIONS OF BLOOD SERUM AND PLASMA.

The posterior extremities of the frog and rabbit were used to investigate this point—the technique for the rabbit's limb being essentially the same as that used for the kidney. The limb was severed from the body in such a way that the attachments of the muscle of the thigh were left intact, the object being to prevent oozing of the perfusion fluid from cut muscle surfaces. The limb was perfused through the femoral artery and the flow from the vein allowed to drop on the drop recorder. For the frog we used the method described by Trendelenburg.

The injection of two cubic centimeters of rabbit serum into the vessels of the extremity produces an immediate constriction, the maximum of which was obtained in from one to three minutes. The interval between the time of injection and the attainment of the full constrictor effect varies with the amount of serum injected, and especially with the sensitiveness of the preparation. The smaller the dose, the sooner is the point of maximum constriction obtained.

Great variations are found in the constrictor effect of the same serum on different preparations. While the frog's vessels are generally more sensitive than those of the rabbit, it is not unusual to find two cubic centimeters of rabbit serum in one case producing a marked diminution of outflow amounting almost to complete stoppage, while the same serum in the other limb of the same animal may produce only a moderate slowing.

The plotted curve after the point of maximum intensity is reached, apart from its inversion, differs markedly from the corresponding curve of the dilator substance acting on the kidney. In the latter two phases are present, and in one the return to the normal flow is more rapid than in the other. The apex of the constrictor curve is never pointed as in the renal vasodilator. There is a gradual and regular return to normal following a period in which the flow has remained for a number of minutes at a constant low level. The effect of the constrictor is more lasting than that of the kidney dilator, and it may sometimes last as long as twenty minutes before the original flow is established.

The coronary vessels respond in a similar manner. O'Connor in

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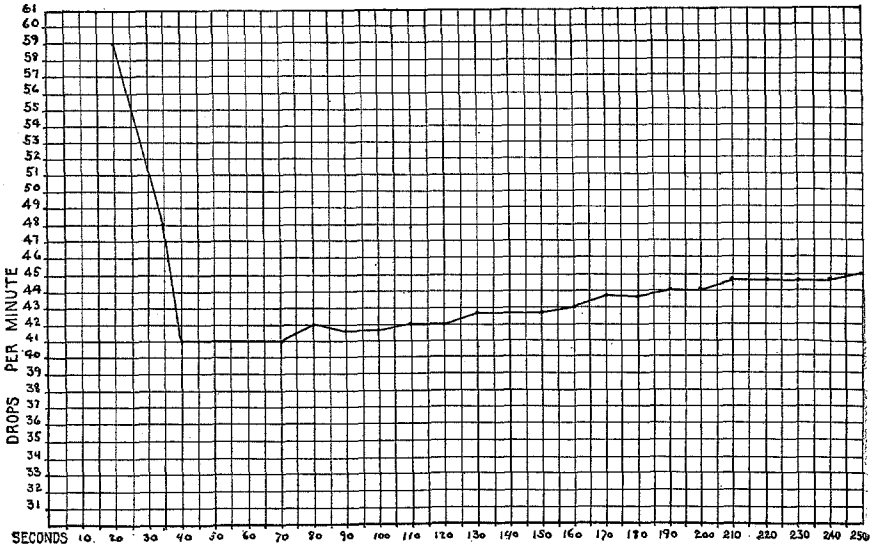
analyzing the vasoconstriction of peripheral vessels found that it equalled that produced by a concentration of 1 to 10 to 1 to 5 of pure suprarenin in the Trendelenburg frog preparation. The same serum when tested on the uterus preparation gave a suprarenin equivalent of 1 to 300,000 to 1 to 200,000. The conclusion drawn from the variability of these results was that other vasoconstrictor



TEXT-FIG. 10. Effect of rabbit serum on rabbit's leg.

substances were present in the serum which acted more powerfully on the uterus than on the Trendelenburg preparation. That this conclusion was correct he proved by destroying the suprarenin of serum by the method of Embden and von Furth. This method consists in the passage of a stream of oxygen for two to six hours through the serum at body temperature. Serum thus treated still retains the power of constricting the vessels of the frog's extremities. O'Connor further showed that this constrictor substance is found in serum and not in plasma, and that it is formed during the process of clotting.

We have repeated O'Connor's experiments on the relative activity of serum and plasma on the vessels of the rabbit's limb and on the frog, and our results confirm his. Thus in text-figure 11 are given

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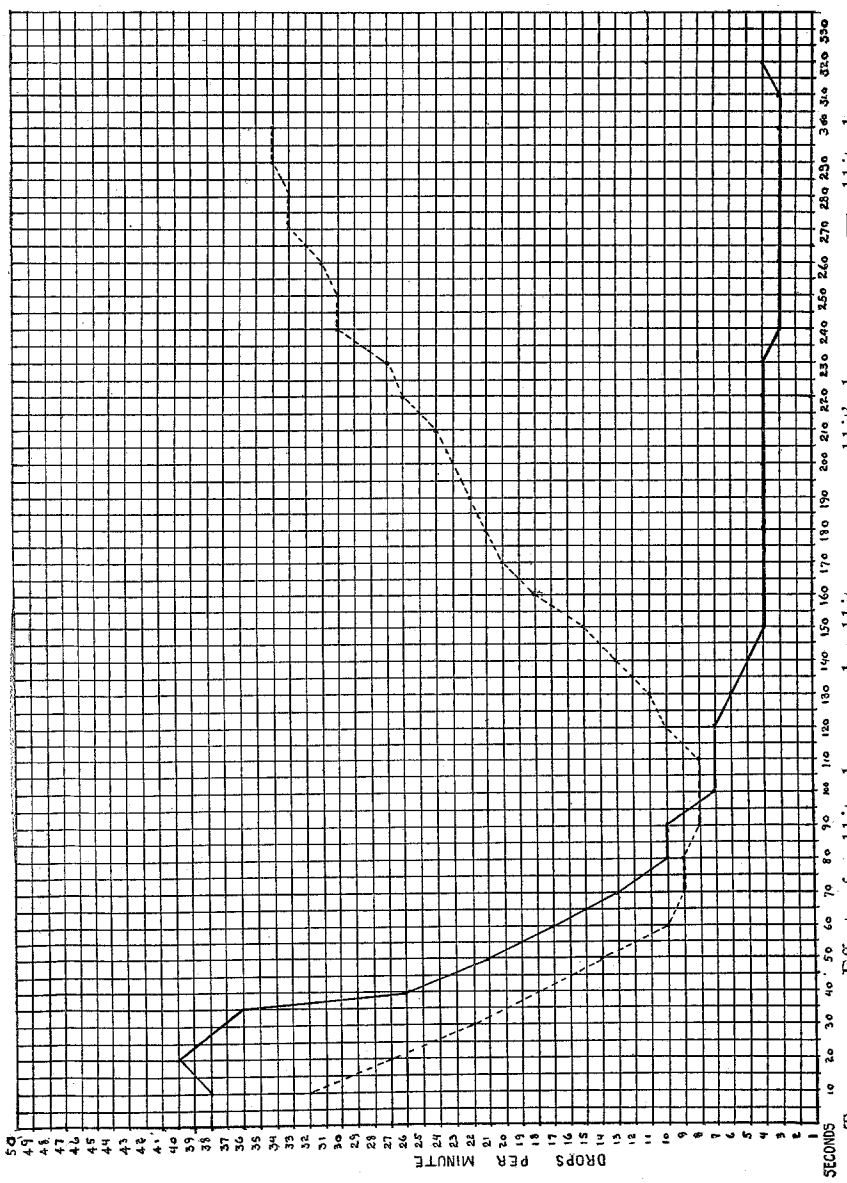

TEXT-FIG. 11. Effect of rabbit plasma and rabbit serum on rabbit's leg. ..... = rabbit plasma. ——— = rabbit serum.

the effects of hirudin plasma and of serum on the same preparations.

It will be noticed that the former produces much less constriction than the latter. In none of our experiments have we observed any

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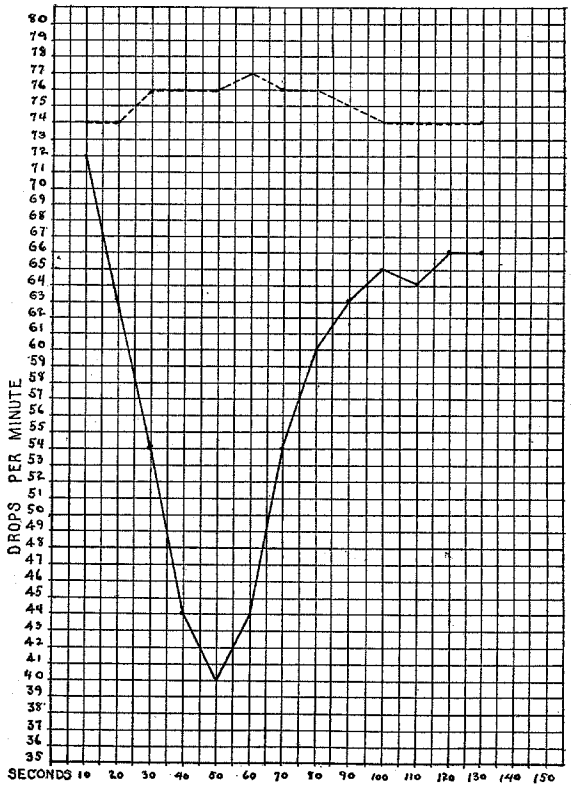
indication of a vasodilator substance for peripheral vessels. The same serum which produces a vasodilatation of the renal vessels causes a constriction of the vessels of the limb.

By the use of apocodeine we have been able to differentiate more exactly between suprarenin and the vasoconstrictor substance liberated during clotting. After the vasomotor terminations have been paralyzed by apocodeine, injections of serum still produce a well defined reduction in the outflow from the veins of the extremities, proving, in the first place, the presence of a constrictor substance other than suprarenin; and, secondly, its direct action on the muscle coats.

A further proof of the presence of a vasoconstrictor substance produced by coagulation of the blood is furnished by the following



TEXT-FIG. 12. Effect of rabbit plasma and rabbit serum on rabbit's leg paralyzed by apocodeine. ..... = rabbit plasma. ——— = rabbit serum.

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experiment. If suprarenin is the only active constrictor present in plasma, then the injection of plasma into the vessels of the limb perfused with Ringer's solution containing apocodeine should produce no change in the rate of flow, while serum should produce a diminution in the rate provided the substance given off during clotting acts by direct stimulation of the muscle coats of the arteries.

Text-figure 12 shows that such is indeed the case. Diminution in the flow was produced by serum, but not by plasma.

The constrictor substance for peripheral vessels is not influenced by boiling and is soluble in alcohol. It is therefore not a proteid of the albumin class, as is the dilator substance acting on the kidney vessels, but beyond this we have not attempted to define its chemical composition.

CONCLUSIONS.

1. In plasma there exists a vasodilator substance specific for the vessels of the kidney.
2. This substance is a proteid of the albumin class and is precipitated by boiling and by alcohol.
3. It is present also in the serum.
4. It acts directly on the muscle coats of the arteries.
5. The process of clotting of the blood liberates a constrictor substance that acts on the renal vessels and also on the vessels of the limb.
6. This constrictor substance is not a proteid; it resists boiling, is soluble in alcohol, and acts directly on the muscle coat.

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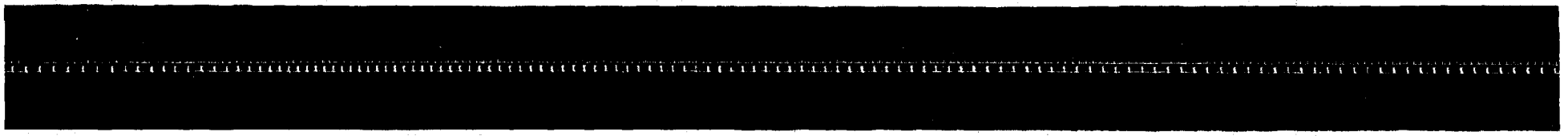

FIG. 1.

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EXPLANATION OF PLATE 8.

FIG. 1. Effect of dog serum on rabbit kidney.

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