THE QUESTION OF EPINEPHRIN IN THE CIRCULATION AND ITS RELATION TO BLOOD PRESSURE.*

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The discovery in 1894, by Oliver and Schäfer, of the remarkable rise in blood pressure produced by the injection of suprarenal extract was naturally followed by attempts on the part of physiologists, pathologists, and clinicians to correlate the activities of the suprarenal gland with variations in blood pressure. The hypothesis of increased adrenal secretion as the cause of the high blood pressure in chronic nephritis offered a tempting explanation of this hitherto inexplicable phenomenon. This hypothesis, first proposed by Neusser, later defended by a number of French investigators, Vaquez, Aubertin, and especially Josué, on the basis of supposed anatomical changes in the suprarenal found at autopsy, has been put to the test of experimental verification by many investigators during the past five years. Since the first publication of Schur and Wiesel, who claimed to have identified epinephrin in the blood serum of nephritics by the Meltzer-Ehrmann frog pupil reaction, the results of these studies have been wholly contradictory. Schlayer, Fraenkel, Kretschmer, Trendelenburg, and others were unable to get greater vasoconstriction with the blood of patients having high pressure than with blood from normal persons. Notwithstanding this, practically all observers have believed that the substance in blood serum which produces vasoconstriction, contraction of the uterus, etc., must be epinephrin, because the effects observed appeared to be similar in character to those produced by epinephrin. The earlier studies are so completely reviewed in Biedl's book that extended citation is unnecessary.

The work here described was begun more than a year ago. It

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was undertaken in the hope of discovering the cause of the contradictory results hitherto reported. It has been conducted by a new method which is free from the objections that can be raised against methods used heretofore. Since the inception of the work five important articles, two by Stewart, two by O'Connor, and one by Schultz have appeared and have greatly elucidated the subject, so that the portion of our work dealing with the vasoconstrictor substances of normal serum is now merely confirmatory of their results, although we had been led to a similar conclusion prior to the appearance of their publications.

Stewart in his first paper criticized all previous studies concerning the epinephrin content of blood on the ground that the investigators employed biological test objects on which epinephrin produced only one of its effects, and because control experiments were omitted. He demonstrated that many substances may produce contraction of smooth muscle, of the uterus, or a blood-vessel, and that the effect is similar to that produced by epinephrin. Stewart insisted that before it could be assumed that such reactions are due to epinephrin, the substance tested must also be shown to exhibit the inhibitory effect of epinephrin. He suggested the method of Magnus as a proper control.

O'Connor, fulfilling the conditions laid down by Stewart, in parallel experiments made on rabbit intestine, and either the Fraenkel uterus preparation or the Läwen frog perfusion, showed that the substance in blood serum which exerts a vasoconstrictor effect also causes increased tonus and contraction of the intestinal muscle. He proved conclusively that the vasoconstrictor substance of blood serum is not epinephrin. By further experiments he made the important discovery that this constrictor substance is produced during the process of clotting and is not present in blood which has been obtained in an uncoagulated state. He was unable to identify epinephrin in blood from peripheral veins and arteries of rabbits when kept uncoagulated with citrate. In plasma obtained from the blood of the suprarenal vein of rabbits, however, he believed that he identified epinephrin in dilutions between 1 to 1,000,000 and 1 to 5,000,000. In his most recent article, O'Connor states that he has found the epinephrin content of blood from the suprarenal vein greatly diminished after section of the splanchnic nerves.

Schultz, studying another problem, the reaction of smooth muscle to proteins with particular reference to anaphylaxis, corroborated O'Connor's discovery that the vasoconstrictor substance appears in blood only after coagulation.

Stewart in his latest publication has failed to corroborate O'Connor's finding for blood from the suprarenal vein. He obtained epinephrin reactions constantly only when the gland was handled roughly or massaged, and regularly failed to obtain such reactions when precautions against this were taken, even though the splanchnics were stimulated. Stewart also tested on rabbit uterus and intestine the sera of a number of patients with high blood pressure and other pathological conditions, and failed to obtain evidences of epinephrin. In all this work, however, he employed serum, not unclotted blood, and attempted to detect the modifying influence of the epinephrin on the effect produced by the serum constrictor substance. Such identification of epinephrin in serum is entirely possible, as we have proved repeatedly, and can be seen from Stewart's curves; but from our experience we are convinced that only positive results obtained in this way have any value. Stewart's negative conclusions are invalidated by the fact that he worked with serum. Cannon and de la Paz, working with the Magnus intestine method, have clearly demonstrated epinephrin in the blood of the suprarenal vein in cats.

METHODS EMPLOYED IN THESE EXPERIMENTS.

Arteries.—The method used in the experiments here reported is a modification of the Meyer ox carotid strip method. It fulfills the conditions laid down by Stewart and it has, we believe, distinct advantages over that used by him or by previous investigators. Every experiment was performed in duplicate, one portion of the substance being tested with a carotid or mesenteric artery preparation, which is constricted by epinephrin; the other with a preparation of the coronary, which is relaxed by epinephrin. Instead of dividing the strip of artery, as recommended by Meyer, and attaching the parts to the chamber and the lever by ligatures placed around their ends, two rings of artery were cut and ligatured together. When working with the coronary artery, this procedure is essential

because of the smaller size of the vessel. The results obtained are exactly similar to those obtained from a single, divided ring. The lower ring was secured to the floor of the chamber by a ligature and a hook; the upper one, at a point exactly opposite, was connected by a thread or wire with the writing lever at a point behind its fulcrum. The lever used was a special muscle lever of light construction, so arranged that the magnification could be varied by the distance of the attachment of the preparation from its fulcrum. The loads were hung on the long arm of the lever, which wrote upon a revolving drum making one revolution in four hours. The weight of the lever was carefully eliminated by counterbalancing. The time was marked in one minute intervals. Stretching the artery to overcome its initial tonus was carried on for from twenty to thirty minutes; the load was then reduced to the permanent lifting load, and the artery allowed to come to equilibrium before further steps were taken. The cylinders containing each artery preparation were immersed in water baths maintained at a constant temperature of about 38° C., and the fluid bathing them was oxygenated constantly by a stream of oxygen introduced at the bottom of the cylinder. Locke's solution of uniform composition was used during all preliminary steps, and for washing out the fluids tested.

Certain precautions in technique are necessary. It is most important to maintain a uniform temperature, as close as possible to 37° C. Variations in temperature produce marked alterations in the contraction of the vessel. A uniform oxygen flow is equally necessary, as variations in the stream produce striking changes in the tonus of the vessel. Vessels which have been frozen must not be used.

Proper selection of the loads for stretching and for permanent lifting is important. Accuracy in this direction can only be achieved by practice in estimating the size of the segments used. The protocols hereafter given show the loads employed in a series of experiments. An overloading of the peripheral vessel may result in failure to respond to small doses of epinephrin. Underweighting is apt to keep the vessel in unstable equilibrium so that it responds inordinately to such mechanical stimuli as changing the solutions in the chamber, and produces curves difficult of interpreta-

tion. The effects of varying loads upon the coronary have been described in a separate article by one of us (Park). In working with large magnifications, a proper adjustment of the loads is of great significance.

Care must be taken when changing the solutions in the chamber. This was accomplished by syphoning out from the bottom the contents of the chamber, through a tube which perforated its floor, and introducing the fluid to be tested slowly by means of a pipette from the top. This method obviated any mingling of solutions and all unnecessary jarring of the preparation. Each fluid before being introduced into the chamber was brought to the temperature of the solution which it replaced.

We believe that the use of coronary and peripheral artery preparations, as controls one for the other, has the following advantages for the detection of epinephrin, and particularly in the investigation of the problem of hypertension.

1. The test objects employed are parts of the mechanism under investigation.

2. The test objects are highly sensitive; epinephrin can be identified in a dilution of 1 to 50,000,000 in Locke's fluid and 1 to 20,000,000 in blood.

3. The test objects are not affected by the viscosity of the blood as O'Connor found the frog perfusion preparation to be. Hence dilution is unnecessary in the attempt to detect exceedingly small amounts of epinephrin. This fact makes the negative results of these experiments more valuable than those reported previously. O'Connor was obliged to work with blood diluted 1 to 4.

4. Arteries may be kept for at least six days at a temperature just above freezing; they are, therefore, available for tests at any time.

5. The sensitiveness of the artery preparations does not seem to vary materially during experiments lasting for a number of hours, as is the case with the frog perfusion.

Blood.—The technique for obtaining uncoagulated blood from patients was as follows: Where venesection was performed therapeutically, a paraffined cannula was introduced into the vein. The blood was collected in large paraffined tubes which contained the

anti-clotting solution, kept in ice so that the blood was immediately chilled to a temperature near the freezing point. Where large quantities of blood were drawn, ice and salt were used. When venesection was not desirable, it was possible to obtain from forty to eighty cubic centimeters of blood by means of an ordinary blood culture needle, fitted to a specially constructed tube so curved as to hang below the arm when the needle was inserted into the vein. The needle was boiled in oil and the tube coated with a film of sterile oil before using. The non-clotting solutions used were Locke's fluid with sodium citrate, 1 per cent., to which an equal amount of blood was added; and hirudin dissolved in a small amount of Locke's fluid, four milligrams of hirudin being taken for each seven cubic centimeters of blood. The hirudin blood was practically undiluted; the citrated blood was diluted one half.

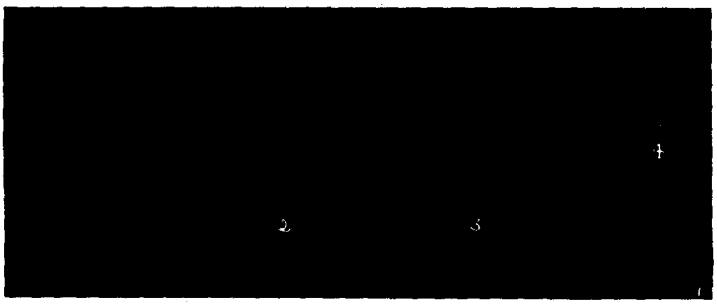
The most essential part of the technique is the chilling of the blood. In most experiments the test was begun within an hour of the time of drawing the blood. It was impossible to have the artery preparations in the best condition for the experiment earlier without running the risk of their too long exposure to body temperature. In experiments with unclotted blood, all ligatures were coated with vaselin after the method of Carrel.

EXPERIMENTS PERFORMED.

The experiments fall into two groups.

1. *Defibrinated Blood.*—The earlier experiments, those made prior to the appearance of O'Connor's publication, were performed with defibrinated blood or serum. It was obvious at once that the vasoconstrictor substance present was not epinephrin, as the coronary artery was constricted equally with the peripheral vessel. The subsequent experiments with defibrinated blood were made with a view to elaborating a method for the detection of epinephrin in the presence of the serum constrictor substance. We attempted destruction of the epinephrin in the serum by protracted oxygenation under pressure, with shaking, etc., but arrived at no satisfactory method. The results of these attempts at destruction are of some interest. While the bulk of the epinephrin was evidently destroyed quickly by oxygen at body temperature, some of it seemed to per-

sist for an extraordinary length of time, in one experiment for eighteen hours. These results are in contrast to those reported by Emden and von Fürth, and O'Connor. We do not feel, however, that our experiments are conclusive on this point. O'Connor's discovery that the vasoconstrictor substance appears only after coagu-

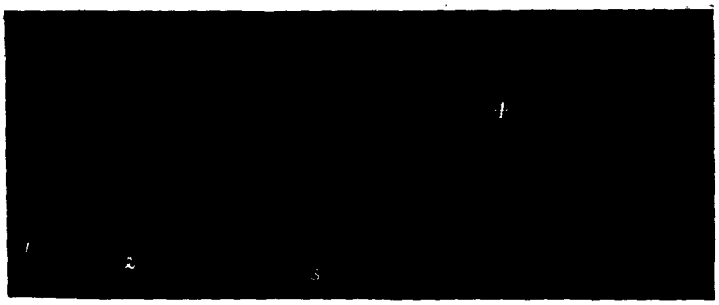


A

TEXT-FIG. 1*A*. Ox carotid, 2 rings. Loads: stretching, 90 gm.; lifting, 60 gm. 1. Locke's fluid containing hirudin, 10 mg. in 20 c.c., introduced. 2. Ox blood containing hirudin, 10 mg. in 20 c.c. 3. Defibrinated ox blood. 4. About 1 c.c. epinephrin (adrenalin chlorid 1 : 1,000) added to blood in chamber. Magnification, × 20½ (half size reproduction).

lation of the blood opened the way to a more profitable study of blood from patients showing high pressure, and our destruction experiments were discontinued.

In all, we have tracings from fifteen tests made with defibrinated



B

TEXT-FIG. 1*B*. Ox coronary, 2 rings. Loads: stretching, 30 gm.; lifting, 20 gm. All other conditions as in text-figure 1*A*.

ox blood or serum, one with defibrinated rabbit blood, and three with defibrinated human blood. In every experiment, constriction of the coronary was as marked as constriction of the peripheral vessel (text-figure 1, *A* and *B*). We are, therefore, able to confirm completely the conclusions of O'Connor, Stewart, and Schultz, that the vasoconstrictor substance found in blood serum is not epinephrin. We would go further and take exception to O'Connor's statement in his first conclusion that it is an "epinephrin-like" substance. Judged by its action on the coronary artery and on the intestinal muscle, this substance is antagonistic to epinephrin. Further, the contraction curve produced with a peripheral artery preparation differs distinctly from the curve produced by epinephrin, particularly in the length of time during which a high degree of contraction persists. It is similar to the contraction produced by barium chlorid and to contractions which we have observed when a hypotonic solution of sodium chlorid was substituted for the Locke's fluid bathing the vessel.

We are in entire agreement with Schultz that the substance is one which acts directly upon the smooth muscle of the artery and not, as seems to be the case with epinephrin, upon the so called receptive substance or neuromuscular junction. We are unable to offer a hypothesis as to the exact nature of this constrictor substance.

3. *Whole Blood.*—Twelve experiments were performed with uncoagulated, whole blood.

Of these, two experiments were made with ox blood containing hirudin, four milligrams to each seven cubic centimeters. In one, the coronary test failed through technical error, but the carotid showed no vasoconstriction whatever and subsequently responded promptly to epinephrin. The other experiment is conclusive; neither the coronary nor the carotid showed any change on substituting the blood for Locke's fluid; both contracted promptly upon the introduction of the same blood defibrinated, and finally the carotid showed further constriction and the coronary prompt dilatation upon the introduction of epinephrin (text-figure 1, *A* and *B*).

One experiment was made with rabbit blood diluted one half with Locke's fluid containing 1 per cent. of sodium citrate. In this

experiment, again, there was no sign of an effect upon either the carotid or the coronary.

Nine experiments were made with human blood. The full details of these tests are given in the following protocols.

A

TEXT-FIG. 2*A*. Experiment 1, series *B*. Carotid tracing. For details see protocol. Magnification, × 15½.

B

TEXT-FIG. 2*B*. Experiment 1, series B. Coronary tracing. For details see protocol. Magnification, × 15½.

PROTOCOLS OF EXPERIMENTS WITH HUMAN BLOOD, SERIES B.

In each case the first load given is the preliminary or stretching load, the second the permanent or lifting load. Mag. indicates lever magnification. The epinephrin used was Parke, Davis and Company's adrenalin chlorid.

Experiment 1.—(Text-figure 2, *A* and *B*). May 2, 1912. Young adult male, robust health. Venous blood by needle, diluted with equal parts of Locke's fluid containing sodium citrate, 1 per cent. Loads: carotid 130 gm., 70 gm.; coronary 25 gm., 20 gm. Mag.: × 15½. Steps of experiment: (1) Locke's fluid plus sodium citrate, 0.5 per cent.; no effect. (2) Citrated blood. Carotid, transient constriction; coronary, no effect. (3) Same blood plus epinephrin, 1 : 1,000,000. Carotid, moderate constriction; coronary, moderate dilatation. (4) Same blood, defibrinated, diluted four times with Locke's fluid and containing epinephrin, 1 : 1,000,000. Carotid, further constriction; coronary, slight constriction.

Experiment 2.—May 7, 1912. Normal human placental blood, Sloane Maternity Hospital, diluted with equal parts of Locke's fluid containing sodium citrate, 1 per cent. Loads: carotid not recorded, substantially as in experiment 1; coronary (rather small) 25 gm., 15 gm. Mag.: × 15½. Steps of experiment: (1) Locke's fluid plus sodium citrate, 0.5 per cent. Carotid, slight dilatation; coronary, no effect. (2) Citrated blood. Carotid, slight dilatation; coronary, no effect. (3) Epinephrin to make 1 : 20,000,000 introduced by pipette. Carotid, slight constriction; coronary, slight dilatation. (4) Epinephrin again introduced in same amount. Carotid, slight further constriction; coronary, no clear effect. (5) Defibrinated ox blood plus Locke's fluid containing 1 per cent. citrate, equal parts. Moderate constriction of both arteries. (6) Washing with Locke's fluid. Carotid, slight dilatation; coronary, arrest of constriction. (7) Undiluted defibrinated ox blood. Carotid, arrest of dilatation; coronary, moderate constriction. (8) Epinephrin, 1 : 1,000, large dose introduced by pipette. Carotid, moderate constriction; coronary, moderate dilatation.

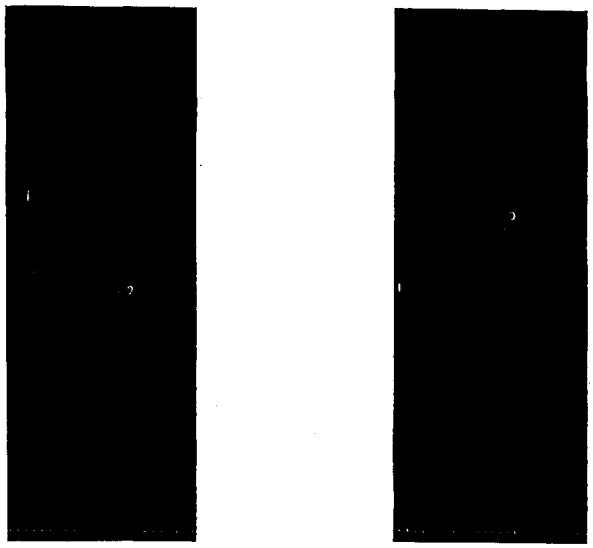
Experiment 3.—May 9, 1912. M.C., female, age 46, Presbyterian Hospital, Ward VII, admitted April 16. Clinical diagnosis: chronic nephritis, cardiac insufficiency. Blood pressure before bleeding, 210 systolic, 150 diastolic; after bleeding, 240 systolic, 150 diastolic. Blood from vein by needle diluted with equal parts of Locke's fluid containing sodium citrate, 1 per cent. Loads: carotid, 160 gm., 80 gm.; coronary, 35 gm., 20 gm. Mag.: × 15½. Steps of experiment: (1) Locke's fluid containing sodium citrate, 0.5 per cent. No effect on either vessel. (2) Citrated blood. Carotid, no definite effect; coronary, no definite effect. (3) Epinephrin to make 1 : 30,000,000 by pipette. Carotid, no effect; coronary, very slight dilatation. (4) Epinephrin to make 1 : 15,000,000. Carotid, no effect; coronary, very slight dilatation. (5) Epinephrin to make 1 : 1,500,000. Carotid, slight constriction; coronary, moderate dilatation. (6) Washing with Locke's fluid. Carotid, slight dilatation; coronary, moderate constriction with some clotting on vessel. (7) Undiluted, defibrinated blood from this patient. Carotid, no effect; coronary, extreme constriction. (8) As the carotid acted like an overweighted vessel, it was then tested with a large dose of epinephrin. The resulting constriction was slight.

Experiment 4.[1]—May 20, 1912. Patient with nephritis and high tension. Blood from arm vein diluted with equal parts of Locke's fluid containing sodium citrate, 1 per cent. Loads: carotid, 160 gm., 80 gm.; coronary, results invalidated by binding of lever. Mag.: × 41. Steps for carotid: (1) Locke's fluid

[1] This experiment suffers from serious technical errors, probably overweighting.

plus sodium citrate 0.5 per cent.; no effect. (2) Citrated blood; no appreciable change. (3) Washing with citrated Locke's fluid; very slight dilatation. (4) Citrated normal placental blood; slight temporary arrest of dilatation. (5) Citrated placental blood plus epinephrin, 1 : 10,000,000; dilatation continued. (6) Washing with citrated Locke's fluid, no effect. (7) Epinephrin in Locke's fluid, 1 : 1,000,000; no definite effect. (8) Defibrinated ox blood; slight constriction.

Experiment 5.—(Text-figure 3, *A* and *B*). J. N. H., male, age 39. Presbyterian Hospital, Ward XI, admitted May 17. Clinical diagnosis: chronic ne-



A *B*

TEXT-FIG. 3*A*. Experiment 5, series *B*. Carotid tracing. For details see protocol. Magnification, × 20½ (half size reproduction).

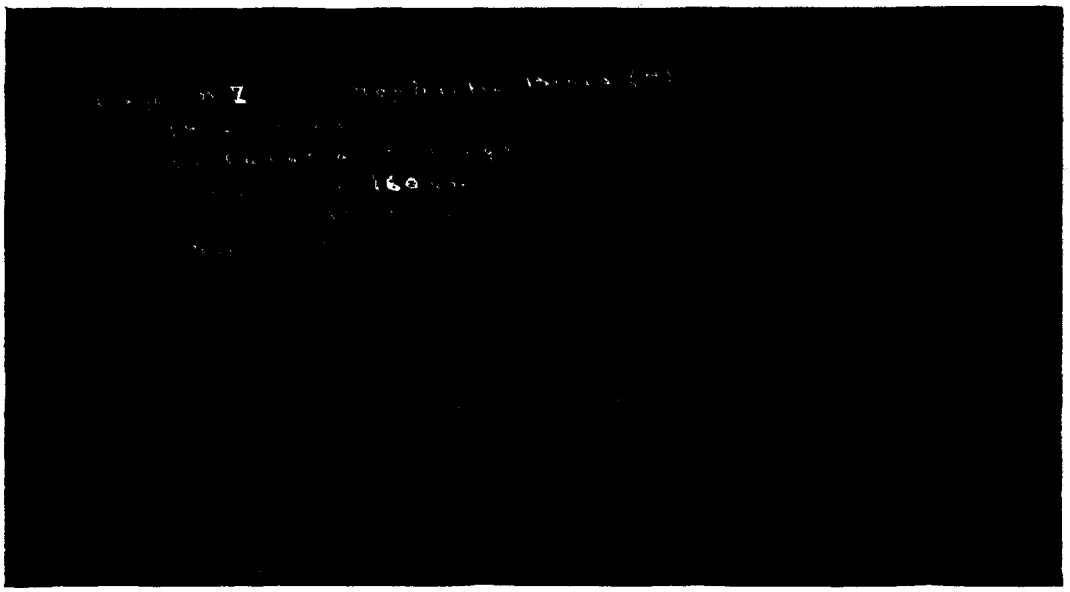
TEXT-FIG. 3*B*. Experiment 5, series *B*. Coronary tracing. For details see protocol. Magnification, × 20½ (half size reproduction).

phritis, cardiac insufficiency, auricular fibrillation. Average blood pressure, 180–190 systolic; 110–120 diastolic. Blood from vein by needle, 20 c.c., diluted with Locke's fluid, 1 c.c., containing hirudin 12 mg. Loads: carotid 140 gm., 80 gm.; coronary, 35 gm., 20 gm. Mag.: × 41. Steps of experiment: (1) Hirudin blood. Carotid, well marked dilatation, then equilibrium; coronary, well marked constriction, then equilibrium. (2) Epinephrin to make 1 : 200,000 by pipette. Carotid, extreme constriction; coronary, extreme dilatation.

Experiment 6.—May 24, 1912. E. V., male, age 50, patient of Dr. Victor Agostini. Clinical diagnosis: chronic nephritis, slight cardiac insufficiency, mild uremia. Blood pressure, 200–215 systolic; 150 diastolic. Pulse rate, 100–108. Blood from venesection, with hirudin 4 mg. to each 7 c.c. of blood, dis-

solved in Locke's fluid, about 1 c.c. to 20 c.c. of blood. Loads: carotid, 160 gm., 80 gm.; coronary, 35 gm., 20 gm. Mag.: × 41. Steps of experiment: (1) Cold Locke's fluid. Sharp constriction of both arteries, with rapid dilatation and resumption of equilibrium near former level; coronary, slight subsequent constriction. (2) Locke's fluid at 36° C.; no effect on either vessel. (3) Locke's fluid containing hirudin, 4 mg. to 7 c.c. Carotid, slight temporary dilatation; coronary, slight continued constriction. (4) Hirudin blood. Carotid, slight temporary constriction; coronary, transient constriction followed by marked dilatation, resuming equilibrium. (5) Epinephrin to make 1 : 180,000, by pipette. Carotid, sharp constriction; coronary, sharp dilatation.

Experiment 7.—(Text-figure 4, *A* and *B*.) Same blood as in experiment 6, after refrigeration for eighteen hours. Loads and magnification as in experiment

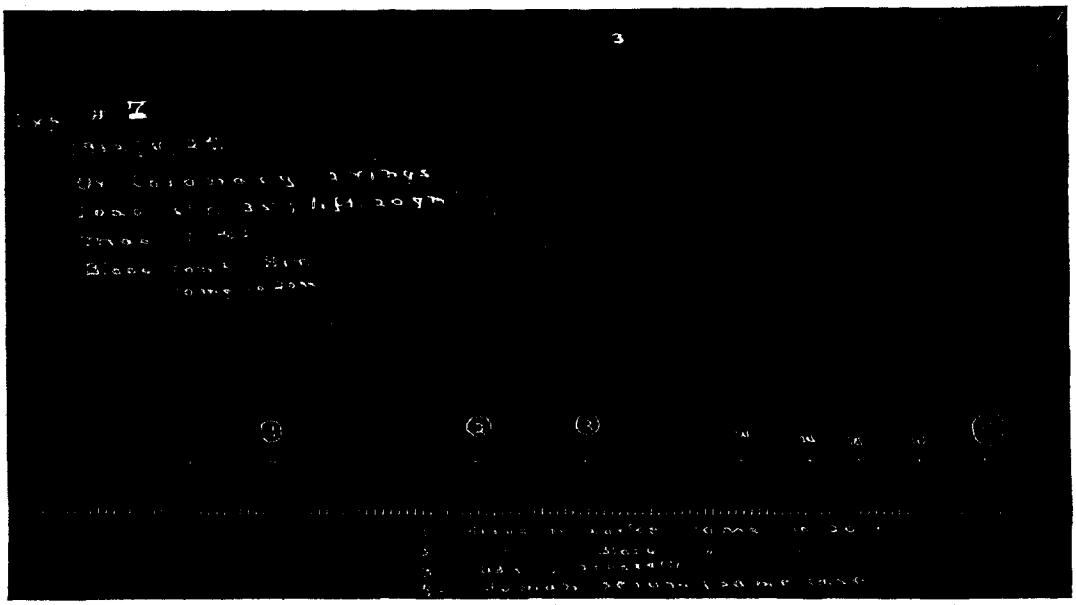


A

TEXT-FIG. 4*A*. Experiment 7, series *B*. Carotid tracing. For details see protocol. Magnification, × 20½ (half size reproduction).

6. Steps of experiment: (1) Locke's fluid containing hirudin. Carotid. transient dilatation, followed by slight continuing constriction; coronary, marked constriction continued. (2) Hirudin blood. Carotid, momentary slight constriction, followed by continuing dilatation; coronary, moderate dilatation, followed by sudden marked constriction. (3) Epinephrin to make 1 : 200,000. Carotid, extreme constriction; coronary, marked dilatation. W = washing with Locke's fluid. Dilatation of both vessels, with resumption of equilibrium. (4) Defibrinated blood from above case; extreme constriction of both vessels.

Experiment 8.[2]—May 25, 1912. D. S., male, age 64, private patient. Clinical diagnosis: chronic nephritis, moderate polycythemia. Occasional observations during 4½ years. Blood pressure, 170–185 systolic; 95–110 diastolic: before bleeding 170, after bleeding 160. Blood by venesection with hirudin, 10 mg. in 1 c.c. of Locke's fluid to 20 c.c. of blood. Loads: carotid, 150 gm., 80 gm.; coronary, 40 gm., 25 gm. Mag.: ×41. Steps of experiment: (1) Locke's fluid with hirudin, 10 mg. to 20 c.c. Carotid, little effect; coronary, slight dilatation. (2) Blood with hirudin. Carotid, marked dilatation con-



B

TEXT-FIG. 4*B*. Experiment 7, series *B*. Coronary tracing. For details see protocol. Magnification, ×20½ (half size reproduction).

tinuing; coronary, slightly accelerated dilatation. (3) Epinephrin to make 1:18,000,000 by pipette. Carotid, slight check of dilatation; coronary, no clear effect. (4) Epinephrin to make 1:9,000,000. Carotid, further check of dilatation; coronary, no effect. (5) Same blood defibrinated. Carotid, extreme constriction; coronary, no effect.

Experiment 9.—June 8, 1912. H. G., female, age 60, Presbyterian Hospital, Ward VII. Clinical diagnosis: osteophytes of *os calcis*, arterial hypertension without proof of nephritis. Highest pressure, 220 systolic, 110 diastolic; lowest, 190 systolic, 110 diastolic. Blood by venesection with hirudin, 10 mg. in 1 c.c. of Locke's solution to 20 c.c. of blood. Loads: carotid, 160 gm.,

[2] Coronary vessels possibly not living at end of experiment.

90 gm.; coronary, 35 gm., 20 gm. Mag.: ×41. Steps of experiment: (1) Locke's fluid with hirudin, 10 mg. to 20 c.c. Carotid, no definite change; coronary, slight constriction with subsequent equilibrium. (2) Blood with hirudin. Carotid, slight dilatation; coronary, slight dilatation. (3) Blood with hirudin and epinephrin to make 1:20,000,000. Carotid, dilatation checked; coronary, dilatation slightly accelerated. (4) Blood with hirudin and epinephrin to make 1:2,000,000. Carotid, moderate constriction; coronary, moderate dilatation.

From these experiments it is clear that normal human blood preserved from clotting by the addition of sodium citrate, 0.5 per cent., shows no trace of a vasoconstrictor substance. It is also clear that epinephrin added to such blood can be identified readily.

The seven experiments with blood from patients showing high blood pressure represent a study of six cases, five patients with chronic nephritis, and one without evident kidney lesion. In experiment 4 the result is of relatively small value, as the lever attached to the coronary preparation was not working freely. There remain six satisfactory experiments performed with blood from five patients. In none of these is there evidence of a simultaneous epinephrin effect upon both carotid and coronary. At times changes were observed in one artery, which, without the control of the other, might have been interpreted as due to the presence of epinephrin. These changes are difficult to explain. Different bloods have seemed to affect quite markedly the subsequent sensitiveness of the preparations to defibrinated blood and to epinephrin. The substance used to prevent clotting also appeared to have possible toxic effects upon the artery. Sodium citrate in protracted experiments diminished the subsequent response to epinephrin quite definitely. For this reason, and because its use required half dilution of the blood, it was abandoned and hirudin used in all later experiments.

In the first experiment with hirudin blood (experiment 5), a rather striking effect, exactly opposite to that of epinephrin, was produced on each vessel (text-figure 3, *A* and *B*). Upon the subsequent introduction of epinephrin, both vessels gave an unusual response, suggesting that they had been rendered peculiarly sensitive to it. This question of the sensitization of the artery to epinephrin is discussed by one of us (Park) in a separate article dealing with

the coronary. In the six subsequent experiments in which hirudin was used, the arteries were treated with hirudin in Locke's solution before introducing the hirudin blood, in the attempt to eliminate any possible influence of the hirudin itself. Upon such introduction there was no change in the carotid in four instances, and in the coronary in two. There was a slight relaxation of the carotid in two experiments, a slight constriction of the coronary in three experiments, and a moderate constriction in one.

In experiment 8 the substitution of the hirudin blood for Locke's solution containing an identical concentration of hirudin resulted in as striking a relaxation of the carotid as in experiment 5, with the same extreme subsequent response to epinephrin. The coronary effect was negative in this instance, but the coronary preparation was probably overweighted.

It seems probable, therefore, that these effects were due only in small measure to the hirudin itself, and were probably produced by the combination of the hirudin with some protein of the blood, possibly by substances preformed in the blood itself.

CONCLUSIONS.

1. The modified Meyer method here proposed, of parallel tests upon segments of surviving carotid and coronary arteries from the ox, is a satisfactory means for detecting epinephrin in complex body fluids like blood.

2. At the present time there is no evidence that epinephrin, in amounts sufficient to produce its physiological effects upon any hitherto used test objects, exists in the circulating blood, with the exception of blood from the suprarenal vein.

3. The examination of uncoagulated blood from six persons with high blood pressure has failed to show the presence of epinephrin or other constricting substances.

4. The constrictor substance in defibrinated blood and serum is not an epinephrin-like substance. In its point of action and its effects it is similar to barium chlorid. It is a direct stimulant to smooth muscle and seems to have no relation to the sympathetic innervation of muscle.

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