PRESSOR SUBSTANCES FROM THE BODY FLUIDS OF MAN IN HEALTH AND DISEASE*

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It has been a theory of long standing that high blood pressure is due to circulation of a pressor substance acting on the peripheral blood vessels. Convincing proof either for or against this conception does not appear to have been established. It seemed to us, therefore, that the problem was worthy of intensive study.

Much has been written on the subject of pressor substances in blood, but due to the fact that the methods employed in the preparation and testing of the extracts are so divergent, only certain of them need be considered here.

Vasoconstrictor substances in the alcoholic extract of blood from hypertensive patients have perhaps been demonstrated (1, 3, 9, 10), but their presence has been denied (11–13).

Bohn (9, 10) prepared alcoholic extracts of blood and tested them on curarized cats, anesthetized with ethyl urethane. He concluded that a pressor substance was present in the blood of patients suffering from nephritic hypertension, from eclampsia, and from malignant nephrosclerosis, but, without exception, blood from patients suffering from essential hypertension or normal subjects contained none. The substance was found to be ultrafiltrable and rendered inactive by exposure to ultraviolet irradiation or to alkali. It also acted as an antidiuretic. Bohn believes that the material is pituitrin. These experiments were taken as proving Volhard's hypothesis that the mechanisms of nephritic and of essential hypertension are fundamentally different, the former depending on the presence of a chemical substance and the latter being predominantly of a nervous origin.

Marx and Hefke (14) also found that alcoholic extracts of blood from nephritic hypertensive patients induced a prolonged rise of blood pressure level in non-anesthetized dogs. Ultrafiltrates were not active, contrary to Bohn's results. Extracts from the blood of normal persons were inactive, while those from epileptic patients, shortly before or during convulsions, were strongly positive. No relationship between the height of the pressure and the amount of pressor substance was found.

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Plasma of eclamptic patients acidified (pH 4 to 4.5) with acetic acid and passed through an ultrafilter was studied by Anselmino and Hoffmann (15–17) in anesthetized rabbits. They appeared able to demonstrate the presence of a substance in the blood of eclamptic patients and those suffering from toxemia of pregnancy that they considered identical with the pressor hormone of the posterior pituitary body. They obtained active antidiuretic ultrafiltrates only when edema was present in the patient, and active pressor extracts only when the blood pressure was over 180 mm. of mercury. The amount of the substance in the blood appeared to parallel the severity of the symptoms. Byrom and Wilson (18) have failed to find antidiuretic hormone in ultrafiltrates of plasma of ten cases of preeclamptic toxemia and three cases of eclampsia with edema.

Many investigators have presented evidence which suggests that the pressor hormone of the posterior pituitary body may be in part responsible for the maintenance of vascular tone (20–33). Others have denied that the evidence is convincing (34–38). Hoyle (37) has clearly demonstrated that normal spinal fluid contains, at most, only a trace of pituitrin, nor is it increased in fluid from patients suffering from nephritis or essential hypertension.

Efforts to detect adrenalin or adrenalin-like substances in the blood by the use of the intestinal segment method or the Trendelenburg frog preparation have led to contradictory results (39–51). No proof has yet been offered that the blood *in vivo* contains such substances in active form.

Chemical Qualities of Pressor Extracts

Extraction and Concentration.—Blood was drawn into a syringe containing heparin from the antecubital vein of the patient. Moderate compression of the arm by the use of a tourniquet did not affect the amount of pressor substance. Both sodium citrate and potassium oxalate have been employed as anticoagulants and, while it could not be definitely proven that they interfered with the animal testing, heparin seemed less likely to complicate the pharmacological assay.

The blood was immediately centrifuged (3 minutes) and the plasma pipetted into 95 per cent ethyl alcohol in the proportion of 10 cc. plasma to 90 cc. alcohol. 10 minutes were usually required for the whole procedure. The flasks were then placed in the ice box for from 2 to 24 hours, the precipitated protein and lipid filtered off, employing strong suction, and the clear filtrate concentrated to one-half the original plasma volume. The removal of the alcohol was carried out in a Claissen flask with water-cooled condenser under vacuum (1 to 2 mm.). The temperature in the flask did not rise above 20°C.

It is essential that all alcohol be evaporated from the extract. Small amounts allowed to remain in solution markedly accentuate the action of the pressor substance. It seemed desirable as a control whenever a great rise had been observed in the level of the blood pressure of the test animal to evaporate the extract still further under vacuum. The type of blood pressure curve resulting from injection of extract containing alcohol is similar to that from alcohol-free extract except

that the level to which the pressure rises is much greater. This precaution must be rigidly observed.

An alternative method was also employed, in which, after removal of the alcohol, absolute alcohol was added in the proportion of 40 cc. for every 1 cc. of concentrate. The mixture was quickly chilled to 4°C., and after standing 6 hours filtered, and the alcohol removed under vacuum.

Other solvents than alcohol have been tried. Acetone extracts were in almost every case either moderately or strongly depressor in action. Ether extracts were indifferent, being neither pressor nor depressor.

Extracts prepared by the ordinary Folin-Wu phosphotungstic acid precipitation were inactive. Trichloroacetic acid dissolved more depressor than pressor substance and, furthermore, the sodium acetate formed on adjusting the pH to that of the body interfered with the animal testing.

Precipitation by tannic acid at pH 3.0 proved an effective protein precipitant, but the removal of the excess tannic acid by the addition of barium hydroxide was troublesome. Excess barium was removed with sulfuric acid. While a small amount of residual tannic acid does not interfere with animal testing, any residual barium may seriously disturb the results. The pressor substance does not appear to be destroyed by the treatment.

Shifting the hydrogen ion concentration of plasma to pH 4.5 by the addition of normal acetic acid had no effect on the efficiency of the alcohol extraction. The concentration of the alcohol employed in the extraction, as long as the protein precipitation was complete, also affected the results insignificantly.

The extract prepared by the alcohol method was usually protein-free as measured by the sulfosalicylic acid method. Often relatively large amounts of lipids were present, and while it was demonstrated that they did not interfere in the animal testing, the lipids were usually removed by cooling the extract to 8°C. and decanting the clear fluid. The extracts were tested usually within 12 to 24 hours, but often immediately after concentration. Serial experiments showed that the time of extraction with alcohol in the refrigerator made little difference in the amount of pressor material extracted. After removal of the alcohol, however, the extracts, especially if kept at room temperature, tended to lose their pressor quality and become progressively more depressor. If extracts were not tested immediately, they were kept at 8°C. It has been our experience that the technique for the preparation of extracts must be carried out with exactness and rapidity, otherwise depressor substances are liberated. These substances may be fatal to the test animal unless caution is used.

Coagulation as a Factor in the Production of Pressor Substance.—

Every care was taken to prevent coagulation by the use of the anticoagulant heparin, but the necessary manipulations during extraction might have allowed some alteration to occur. After it had been shown that ascitic fluid also contained pressor substance a way was open for excluding coagulation as a factor in the production of the blood pressure-elevating principle. During a paracentesis ascitic fluid was allowed to flow directly from the trocar into alcohol. The extract of this fluid was neither more nor less active than heparinized or citrated ascitic fluid. It seems reasonable therefore to suppose that the substance is present in plasma and is not produced as the result of coagulation.

Ultrafiltration.—After exhaustive investigation it became evident that ultrafiltration could not be utilized for the separation of pressor substance from plasma.

Various methods for the preparation of ultrafiltrates have been employed. Collodion sacs of various porosity (100 cc. capacity), and pear-shaped porcelain thimbles on which acetic acid collodion (6 to 8 per cent Shering-Kalbaum[1]) was deposited as a membrane, were found convenient. Ultrafiltrates were also prepared by high pressure (150 kilos per sq. cm. of nitrogen) filtration[2] through membranes of various density (30 to 400 minutes). The high pressure apparatus offered the great advantage that 10 cc. or more of ultrafiltrate could be obtained in ½ hour, the time depending on the porosity of the filter membrane.

Ultrafiltrates were prepared from plasma at normal pH and from plasma which had been made acid (pH 4.5) by the addition of acetic acid. Neutralization of the acid dialysates (to phenolphthalein) was performed immediately before injecting into the animal. Ordinarily the ultrafiltrate was not concentrated. When concentration was desired it was effected below 20°C. with the aid of a vacuum. The ultrafiltrates were clear and protein-free as estimated with the sulfosalicylic acid reagent. 52 ultrafiltrates have been tested.

Partition.—Active extracts prepared by the alcohol method were extracted with chloroform in a separatory funnel. The solvent was removed under vacuum after the addition of water. Such aqueous extracts were vaso-active. Many experiments have shown that the pressor substance is extracted from aqueous solution by this strong organic solvent, often almost completely. Reversing the extraction,

[1] The preparation of these ultrafiltrates was undertaken with the help of Dr. K. J. Anselmino of Düsseldorf.

[2] Pfaltz and Bauer apparatus.

that is extracting the substance from chloroform after acidification, was not so successful, only about 20 per cent being so recoverable. Ethyl acetate also removes some of the pressor substance from the water phase but does not appear as efficient as chloroform. Plasma which has been allowed to stand at room temperature yields an extract which is powerfully depressor and partition of this extract with chloroform does not leave the depressor substances in the water phase.

Heat Stability.—Plasma extract may be heated to boiling for about 1 minute with but partial loss of pressor activity (nine experiments). Longer heating even at lower temperatures either liberates depressor substances in such quantity that they overshadow the pressor principle or else the pressor substance is destroyed leaving the depressor substances unantagonized. The experimental evidence does not allow us to decide which reaction had occurred.

Is the Pressor Substance Lipid in Nature?—The gross fats may be frozen out of the extracts without substantial loss of pressor activity. These fats in aqueous emulsion when injected were not vaso-active. Further removal of phosphatide was achieved by precipitation with a large excess of acetone and allowing the precipitate to agglomerate at a temperature just above freezing. The supernatant liquor after the addition of water and the removal of the acetone under vacuum remained vaso-active. It thus seems unlikely that any of the ordinary lipids are involved in the activity of these extracts.

Vascular Responses of the Test Animals

Method.—Cats weighing 3 to 4 kilos which had been without food for 18 hours were usually employed as test animals. Lactating or pregnant animals responded to vascular stimuli in a peculiarly irregular manner, hence were not used in these experiments. The animal was anesthetized and a tracheal cannula inserted. Both vagi were then cut and the blood pressure recorded, by a mercury manometer, either from the right carotid artery, cannulated well below the cricoid cartilage, or from the femoral artery. Interference with the circulation of the thyroid gland was avoided. The femoral artery was employed for blood pressure measurement when injury to the carotid sinus and aortic nerves was to be avoided. Sodium citrate, 20 per cent, was employed as anticoagulant in the manometer tubing because it has been found that should some of the solution flow into the vascular system its action can be quickly nullified by the intravenous injection of 5 per cent calcium lactate. The injections of warmed extract, usually of 5 cc. volume, were made slowly and evenly into a cannulated femoral vein. The ani-

mals were kept warm and the temperature noted from time to time by means of a rectal thermometer.

Anesthetics.—A variety of anesthetics have been studied with regard to their suitability for pressor extract assay. Ether proved satisfactory when administered in gentle puffs of warm air from a windshield wiper type of artificial respirator but with the tracheal cannula open to the outside air. It was found essential to keep the respiration normal and unimpeded by the respirator.

Ethyl urethane given subcutaneously 3 to 4 hours before the experiment in doses of 7 to 10 cc. of a 25 per cent solution was found equally satisfactory and was employed in many of our experiments.

Amytal (iso-amyl ethyl barbituric acid) administered in doses of 60 mg. per kilo produced a smooth anesthesia without marked fluctuations in the level of the blood pressure but the response to extract was not as great as that when ether or ethyl urethane was employed. This lack of sensitivity is surmised to be due to the fact that this barbituric acid derivative stabilizes the autonomic nervous system in such a manner that vasomotor responses are buffered.

Pento-barbital (1-methyl-butyl ethyl barbituric acid) administered intraperitoneally in doses of 40 mg. per kilo gave an excellent anesthesia and the responses were similar to those following amytal narcosis, with the exception that early in the course of the experiment, depressor responses were likely to be elicited by extracts of known pressor activity.

Chloretone (0.4 gm. per kilo given by stomach tube) depressed the responsiveness to pressor extract to a marked degree.

Course of the Vascular Responses to Pressor Substance.—It was observed that the same extract at one time produced a marked rise in the animals' blood pressure, whereas, later in the course of the experiment either the response was small or none was elicited. That the extract had deteriorated in so short a time did not seem probable. Extracts which had assayed both active and inactive tested on the same animal were reassayed the next and subsequent days on other animals. Again the same variability was noted. The conclusion seemed justified that the variability lay in the response of the animal and not in the extracts.

On analyzing the data derived from the blood pressure records of a large number of cats it became evident that under the conditions of our experiments, especially when ether was employed as anesthetic, fairly definite periods could be recognized during which the magnitude of the response was reasonably constant. Since the responsiveness during the different periods varied greatly it became evident that to obtain comparable results the assay would have to be performed dur-

ing the same period. This observation was established on such a large number of animals, because the truth or falsity of this observation lies at the crux of the important problem of the quantitation of the pressor substance.

During Period 1 (Fig. 1) the blood pressure is high and tends to fall rather rapidly and progressively. The animal is usually but slightly responsive to plasma extract during this period, indeed pressor extracts may produce depressor reactions. Period 2 is characterized by a moderately low (about 108 to 120 mm. Hg) steady blood pressure and may last for 1 to 3 hours. It is during this interval that the animal is most sensitive to pressor substances. As the third period ap-

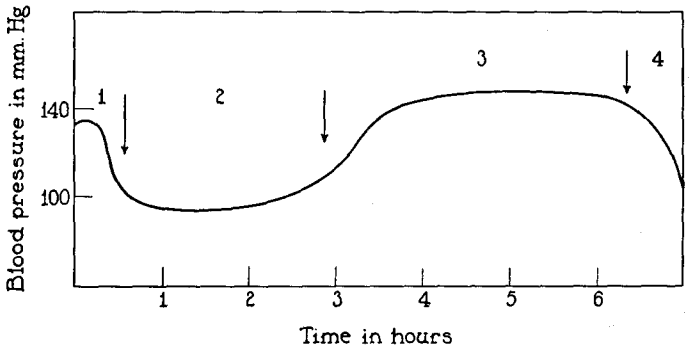

FIG. 1. Curve showing variations in blood pressure exhibited by a cat while anesthetized with ether.

proaches the pressure very gradually rises to a level usually greater than the initial pressure. At this level it remains almost fixed and will carry on for hours without significant alteration. The responses are exceedingly sluggish. It is during this period that an extract which had previously proven active may seem entirely devoid of pressor activity. Period 4 sets in when the animal is fast failing. During this period the responses are usually very irregular and are often confused by asphyxial blood pressure waves. Experiments performed to determine whether this course of events is the normal one, under prolonged anesthesia indicate that it is (Fig. 2).

It must be recognized that this division of the vascular reactivity into periods is arbitrary. The periods are not so distinct when the

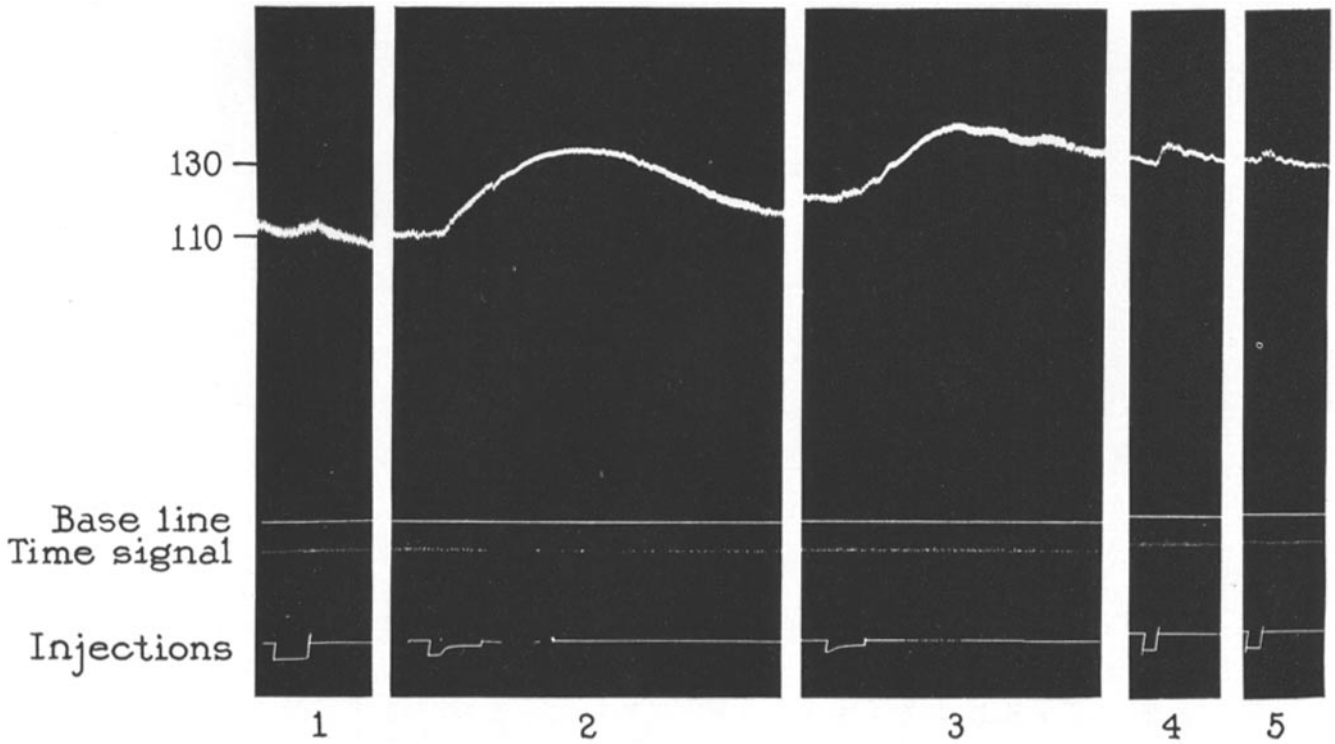

FIG. 2. Comparison of the pressor effects of plasma extract during the reactive and inactive period of testing. Cat under ether anesthesia. (1) Control injection of 5 cc. saline. (2) Extract of 14 cc. of plasma from a moderately severe essential hypertensive patient (B.P. = 202/116). (3) Extract of 14 cc. plasma from severe hypertensive patient (B.P. = 290/130). (4) The ether was cut off ½ hour before this injection, but the animal showed no signs of recovery. The same extract as (1). (5) Same extract as (2). Initial pressure = 116 mm. Hg. Time signal = 10 seconds.

fixed hypnotics are employed as anesthetics. Some hypnotics have the serious disadvantage of damping the vascular responses to pressor extracts to a degree that makes the interpretation of the records difficult. Furthermore, the non-reactive phases (Phase 3) are more difficult to detect.

If extracts are not tested during periods of approximately the same reactivity it is apparent that false results will be obtained. Our problem, namely whether the pressor substance is increased in amount in the plasma of patients suffering from hypertension, could not be answered until this fact was understood. It is not sufficient to test the animal at the beginning of the experiment with extracts of the plasma of normal individuals, and if no response is elicited, assume that any other extracts which produced a rise in pressure are "active." The extracts to be compared must be tested within as short a time interval as is feasible, and in alternating order.

Methods for Estimating the Reactivity of Test Animals to Pressor Extracts

Vascular Response to Peripherally Acting Substances.—The hope that the character of the response of animals to a pressor extract might be predicted by preliminary study of the responses to such powerful pressor and depressor drugs as adrenalin, pitressin, choline, adenylic acid, and histamine, proved illusory. Systematic studies, on a large number of animals, showed conclusively that no parallel or reciprocal relationship existed. These drugs which act primarily on the peripheral blood vessels produced excellent responses when pressor extracts no longer influenced the blood pressure level. Indeed, as will be evident later, the recognition of this fact proved an important key to the understanding of the pharmacology of the pressor substance.

Reactivity as Measured by Carbon Dioxide Inhalation.—The reactivity of the "vasomotor center" was tested from time to time during the course of the experiment by the administration of carbon dioxide-air mixtures.

10 per cent carbon dioxide air mixtures were prepared in a gasometer and 800 cc. portions delivered into a rubber balloon. The gas mixture was administered by means of a No. 10 French catheter, inserted through the rubber tube which delivered the ether, into the tracheal cannula. The tip of the catheter reached

to the bifurcation of the trachea in order to insure delivery of the carbon dioxide to the lungs. The tracheal cannula remained partially open in order not to interfere with spontaneous breathing. The test dose of carbon dioxide-air mixture required 100 seconds for delivery. An alternative method consisted in the insertion of a flutter valve between the gas balloon and the catheter and a second one on the tracheal outlet.[3] It was necessary to stop the administration of the anesthetic when this latter system was employed.

During the administration of the test dose of gas the blood pressure began to rise and remained at an elevated level for 2 to 3 minutes, then fell to the original

TABLE I

Comparison in Etherized Cat of the Vascular Response to Carbon Dioxide-Air Mixture and Pressor Extracts

Test substance	Amount	Time	Blood pressure	Magnitude of change
	cc.	*p.m.*		*mm. Hg*
NaCl	5	1.10	140	0
Ascitic extract	5	1.30	122	+ 6
CO_2-air	800	1.40	116	+ 8
CO_2-air	800	2.00	114	+12
Ascitic extract	5	2.15	100	+16
CO_2-air	800	2.40	98	+12
Ascitic extract	5	3.00	88	+14
CO_2-air	800	3.30	74	+14
Ascitic extract	5	3.45	82	+36
CO_2-air	800	4.00	112	+32
CO_2-air	800	4.20	114	+16
Ascitic extract	5	4.40	116	+20
CO_2-air	800	5.10	126	+ 4
Ascitic extract	5	5.30	146	+ 6
CO_2-air	800	5.40	148	0
Pure CO_2	600	6.20	154	−37

level. In most of our experiments the reactivity of the vascular system was first tested with CO_2-air, then the test dose of plasma extract given.

The results show that when the animal exhibits the greatest response to CO_2-air mixture the response to pressor extract is also marked. Exact parallelism is not always seen. After some time has elapsed (1 to 3 hours) the action of both may become weaker. Finally,

[3] The valves can be made conveniently from the rubber of surgical gloves, the edges of the valve being glued together by grippit (a form of rubber cement).

they elicit no further response. Even high concentrations of carbon dioxide are ineffective (Table I). This is the period designated as 4 (Fig. 1).

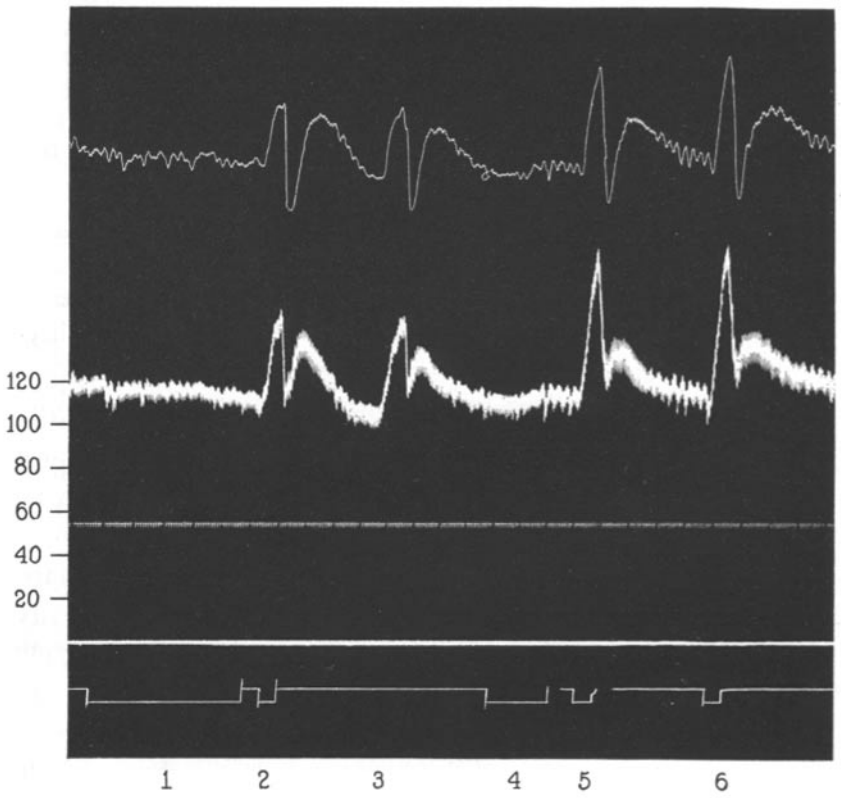

FIG. 3. Increase in response to carotid sinus stimulation following extract injection, without marked change in blood pressure level. Dog anesthetized with 0.10 gm. chloralose per kilo body weight. Upper curve records pressure from peripheral end of the femoral artery. Lower curve is record of the pressure in the central end of the femoral artery. (1) 15 cc. ascitic extract. (2) Sinus stimulation. (3) Sinus stimulation. (4) 15 cc. ascitic extract. (5) and (6) Sinus stimulation. Initial blood pressure = 118 mm. Hg.

Reactivity as Measured by the Response to Carotid Sinus Stimulation.— Occlusion of both common carotid arteries after bilateral vagotomy normally produces a marked rise in systemic blood pressure, due to

stimulation of the carotid sinuses. The nervous connection with the vasomotor center is through the glossopharyngeal nerve. This reflex has been utilized as a means of determining the reactivity of the animal to pressor extract. It has been found that there is a general parallelism between the rise in blood pressure produced by occlusion of the carotid arteries and the responsiveness of the animal to extracts. The parallelism is by no means exact, but is a useful means of determining non-reactive periods.

It has also been possible to demonstrate that the extract in some manner sensitizes the mechanism responsible for the sinus reflex[4] (Fig. 3).

The rise in blood pressure following sinus stimulation is considerably greater after the injection of extract than before. Extracts which, through some fault in preparation, were depressant to the systemic blood pressure level, invariably markedly reduced the reflex sensitivity. The substance which causes increased sensitivity is contained in the pressor extract alone because ultrafiltrates of plasma had no effect in altering the response to reflex stimulation.

It has not been ascertained whether the rise in blood pressure incident to injection of extract is responsible for the increased sensitivity to the reflex stimulation. Increase in sensitivity has often been observed in experiments where the rise of blood pressure level due to the injected extract was very small.

Other Methods of Control and Precautions Necessary to Observe in Assaying Pressor Extract.—

(*a*) Rise in blood pressure incident to the intravenous injection of indifferent fluids must always be estimated by the injection of 5 cc. of warm saline. Certain animals are sensitive to changes in blood volume and this, although rare, cautions against the acceptance of one or even two positive results as indicating the presence of pressor substance.

(*b*) Since in the preparation of plasma extracts the water-soluble elements of the blood become more concentrated than normal, for control purposes artificial extracts were prepared containing three times the concentration of sodium chloride and urea found in normal blood. No clear effect on the blood pressure level was observed from the injection of 5 cc. of this solution.

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[4] We were fortunate in having the direction of Professor Dautrebande of Liége, Belgium, during this part of the work. Dogs under chloralose anesthesia were employed in some of the experiments. These animals, according to Professor Dautrebande's experience, are most suitable for carotid sinus experimentation.

(*c*) Injections should not be made when the blood pressure is falling at a rapid rate. The pressure fall may be checked by the extract injection and the blood pressure assume a fixed level; it may continue to fall or it may suddenly climb rapidly. None of these responses give any clue as to the potency of the pressor substance in the extract.

(*d*) Injections must not be attempted when changes either in the rhythm or the amplitude of the pulse are noted on the kymographic record.

(*e*) The injection must be made slowly and with great evenness, otherwise sharp transient elevations in the pressure curve often occur. In spite of this precaution in some animals a temporary rise in pressure cannot be avoided even when small amounts of saline are injected.

(*f*) In general it has been found undesirable to test extracts in animals in which the blood pressure is exceptionally high or low. The usual level at which our tests were made was about 100 to 140 mm. of mercury.

(*g*) The respiration must be moderately slow and regular and in no way hindered by the respirator. Usually, during periods when the natural breathing had stopped, the vascular responses were at first very sluggish and if anoxemia supervened they became highly irregular.

(*h*) In order to compare the potency of two extracts, the injections should be given within as short a space of time as is feasible, to guard against changes in the sensitivity of the animal to the extract. It has been found best to administer the smallest doses of the extract that will produce a rise in blood pressure which is definite, and to follow this injection with the extract with which it is to be compared as soon as the pressure has returned to the original level. The first extract is then again injected to make sure that the animal is still reactive. While this method demands much time and relatively large amounts of extract it has seemed the only one which is reliable.

Pharmacology of Pressor Extracts

The typical pressor response consists in a relatively slow rise in the level of the blood pressure and the steady maintenance of the elevated level for some time (Fig. 4). No preliminary depression of the level of blood pressure occurs.

The Action of Plasma and Ascitic Fluid Extracts on Cats Treated with Ergotoxine.—In order to determine whether the response to pressor extracts could be influenced by ergotoxine, cats under ethyl urethane anesthesia were first tested with 1 cc. of 1:100,000 adrenalin followed by 5 cc. of pressor extract as control. Now, 1 mg. of ergotoxine tartrate (gynergen, Sandoz) was injected intravenously and after 2 minutes the animal was retested with adrenalin and extract. It could be shown that although reversal of the adrenalin pressor effect had

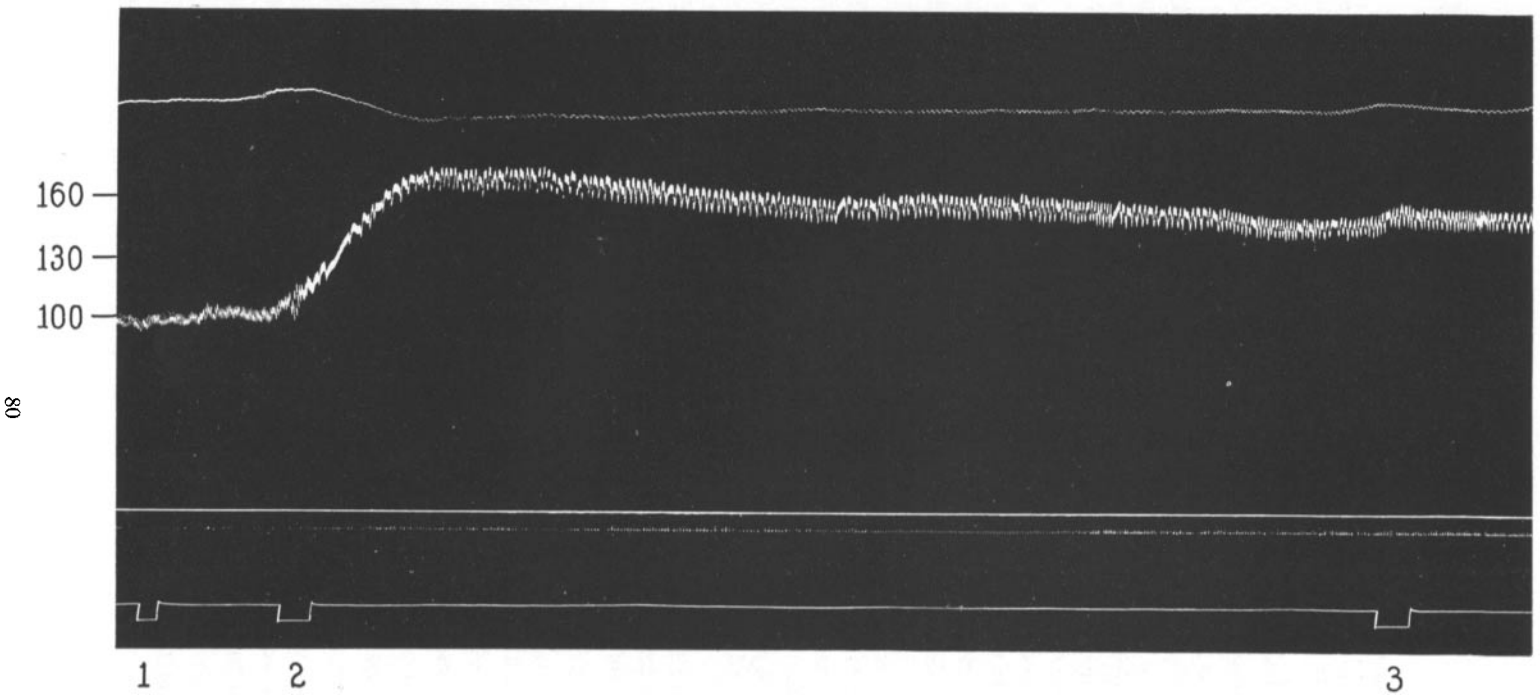

FIG. 4. Example of a very active response to plasma extract from a patient with toxemia of pregnancy and hypertension. (1) Saline 5 cc. (2) Extract of 10 cc. plasma from patient (B.P. = 140/104). (3) Same extract boiled 3 minutes. Upper tracing represents renal volume. Experiment 124 (3–5). Initial pressure = 100 mm. Hg. Time markings = 10 seconds.

been produced the rise in blood pressure resulting from plasma or ascitic fluid extract was not altogether abolished. After large doses of ergotoxine the central nervous system of the animal appeared depressed as shown by the fact that the responses to occlusion of the carotid arteries were very weak. When this occurred the pressor extracts were also almost ineffective.

Influence of Cocaine and Atropine on Response to Extracts.—It is well known that cocaine "sensitizes" the vascular response to adrenalin. Therefore it seemed desirable to ascertain whether this drug would influence the response to pressor extracts.

Cocaine (2 mg. per kilo) was injected intravenously into the cat under ethyl urethane anesthesia after the vascular response to adrenalin solution (1.5 cc. of 1:100,000) and to 5 cc. of pressor extract had been determined. The same amount of adrenalin was again injected to be certain that the magnitude of the response had increased and the pressor extract then injected. Adrenalin raised the pressure 10 mm. Hg before cocaine was given and 30 mm. Hg afterward. The extract raised the pressure level 18 mm. Hg and after cocaine 21 mm. It seems safe, therefore, to conclude that cocaine in the dosage we have employed does not affect the response to extract to any marked degree. No evident alteration in the responsiveness of the vascular system to carotid sinus stimulation was observed as the result of cocaine injection.

To ascertain the effect of atropine on the pressor response both cats and rabbits were given atropine by vein until the characteristic reversal of the action of choline from depressor to pressor had occurred. Active plasma extracts were found quite as active after atropinization as before.

Effect of Curare on the Pressor Response.—Animals under ether anesthesia were curarized by repeated intravenous injections of 3 mg. doses of curare until normal respiration was completely paralyzed. Artificial respiration was now begun.

The response to pressor extract was sluggish in such animals. As there seemed no advantages over the ordinary method of assay and there were definite disadvantages, the procedure was not further employed.

Response of the Kidneys to Pressor Extracts.—In order to ascertain what effect the pressor extracts exerted on the kidneys' volume, the

right kidney of the test animal was placed within a Livingston glass oncometer. The volume changes were registered by a small Brodie bellows.

Observation of many curves from such experiments convinces one of the extraordinary autonomy of the kidneys' circulation. Under apparently identical circumstances the kidneys may shrink or swell following the injection of certain substances. In general, the active plasma extracts tended to cause the renal volume to increase slightly though the opposite effect may be observed when the blood pressure rise is great. Without measurements of the blood flow through the kidney it is not possible to state whether vasodilatation or constriction had occurred. As has been pointed out by Richards and Plant (52) epinephrin may cause the renal volume to increase but simultaneously constrict the efferent arterioles, thus causing increased intraglomerular pressure.

As the result of pithing animals the renal volume curve follows accurately that of the carotid blood pressure, regardless of the agent employed to produce blood pressure changes (Fig. 5).

Changes in Peripheral Blood Supply as Measured Oncometrically.—Changes in peripheral blood supply of the hind leg were measured by an oncometer connected with a Brodie bellows. The results of measurements on sixteen animals show that ordinarily a moderate constriction in the leg volume occurs following the injection of plasma extracts. The constriction is initiated rather more slowly than the change in renal volume but it also lasts somewhat longer.

Response to Pressor Extract after Evisceration.—It could be shown by the oncometric method that, while both the renal and the peripheral blood vessels constricted as the result of the injection of the pressor extract, the action was not powerful. It seemed possible, therefore, that the splanchnic vessels constituted a not inconsiderable portion of the constricted area during the rise in systemic blood pressure.

After preliminary determination of the response to 5 cc. of plasma extract and the reactivity of the carotid sinus reflex, the anesthetized animal was eviscerated in the usual manner. The responses were again tested shortly after the blood pressure had steadied itself following the operation. An example may be given to illustrate the results of this procedure. Before evisceration the pressor response to the extract consisted in a rise in blood pressure of 22 mm. Hg, and

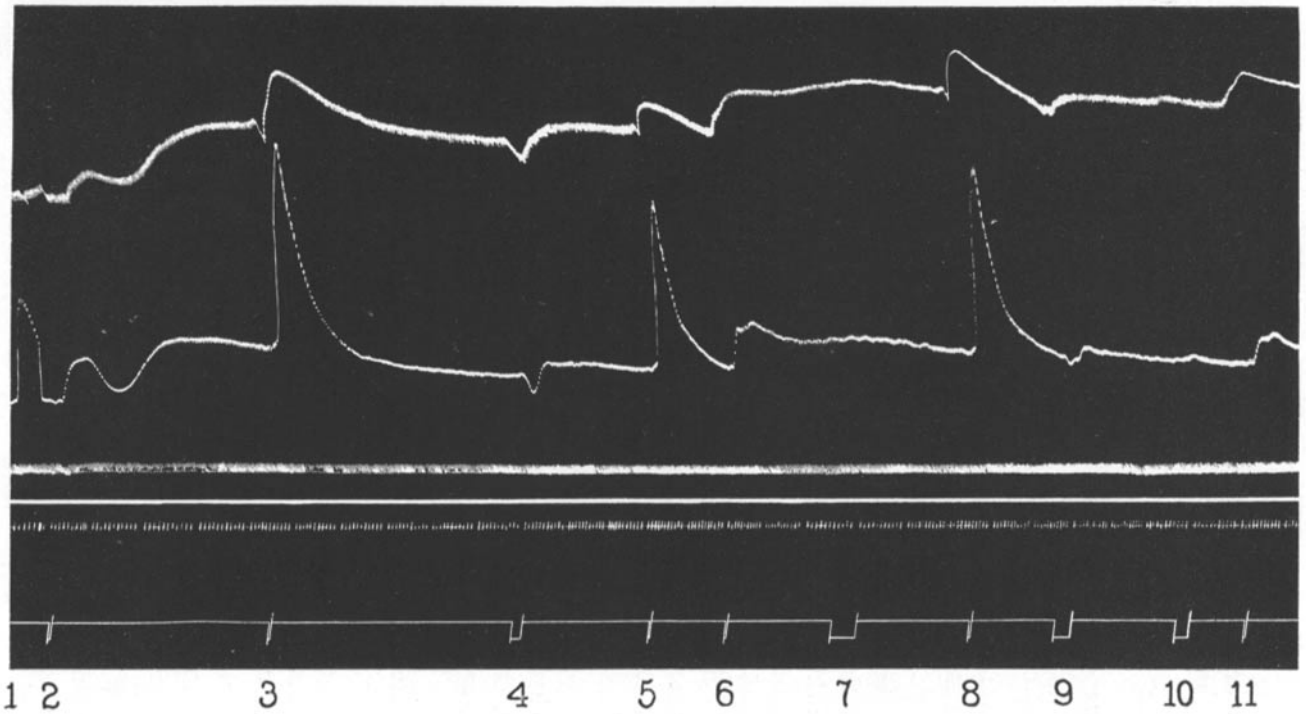

FIG. 5. Action of plasma from a nephritic patient on sensitivity of the pithed cat to epinephrin. The upper line represents renal volume; the second line, arterial blood pressure; and the third is the pneumographic record of respiration. No anesthetic. (1) Epinephrin 2 cc. (2) Pitressin 1.5 cc. of 1:25 dilution. (3) Epinephrin 2 cc. (4) 2 cc. plasma of severe nephritic patient with hypertension. (5) Epinephrin. (6) Pitressin. (7) Extract of 10 cc. plasma of same patient. (8) Epinephrin. (9) Nephritic plasma 5 cc. (No. 2). (10) NaCl 5 cc. (11) Pitressin. Experiment 116 (27–34). Blood pressure = 40 mm. Hg.

that due to carotid sinus stimulation was 16 mm. Hg. After evisceration the extract raised the blood pressure level 10 mm. Hg and the carotid sinus stimulation, 24 mm. Hg. The systemic blood pressure fell from 108 mm. Hg to 84 mm. Hg as the result of the operation.

The findings suggest that constriction of the vessels of the splanchnic area plays a large part in the elevation of blood pressure level resulting from the injection of pressor extract.

Effect of Adrenalectomy on the Effect of Pressor Extracts.—The possibility that the pressor action of plasma extracts might be due to stimulation of the adrenal gland causing the secretion of adrenalin was studied by bilateral removal of these glands. Recent experiments have shown that certain pharmacological agents do in fact owe at least part of their action to the increased liberation of epinephrin into the circulation.

After preliminary testing of the potency of an extract on a cat under ether anesthesia, the adrenal glands were either tied off or extirpated. The lumbar approach to the glands was employed. The extracts were again tested within 10 minutes and 1 hour after adrenalectomy.

The results from ten such experiments indicate that rather than a decreased response, the opposite was found. The increase, however, was relatively small, and in view of the spontaneous alterations in reactivity of the animal it does not seem justifiable to conclude that adrenalectomy causes sensitization to pressor extracts.

The Effect of Bilateral Vagotomy.—Extracts tested before and after bilateral vagotomy did not exhibit any characteristic difference. When the vagi were intact, spontaneous fluctuations in the level of the blood pressure were more apt to appear, consequently in most of our experiments this operation was performed.

Does the Animal Develop Immunity to the Pressor Action of Plasma Extracts?—Since it is well known that repeated injection of extracts of the posterior lobe of the pituitary result in the gradual development of immunity in animals to further vascular stimulation, it seemed desirable to ascertain if this were true of plasma extracts. It was found that the second response is just as great and often greater than the first. Indeed, the pressure level can be built up step-wise by the repeated injection of extract. As the blood pressure reaches levels above 160 mm. Hg the response to additional extract is likely to be

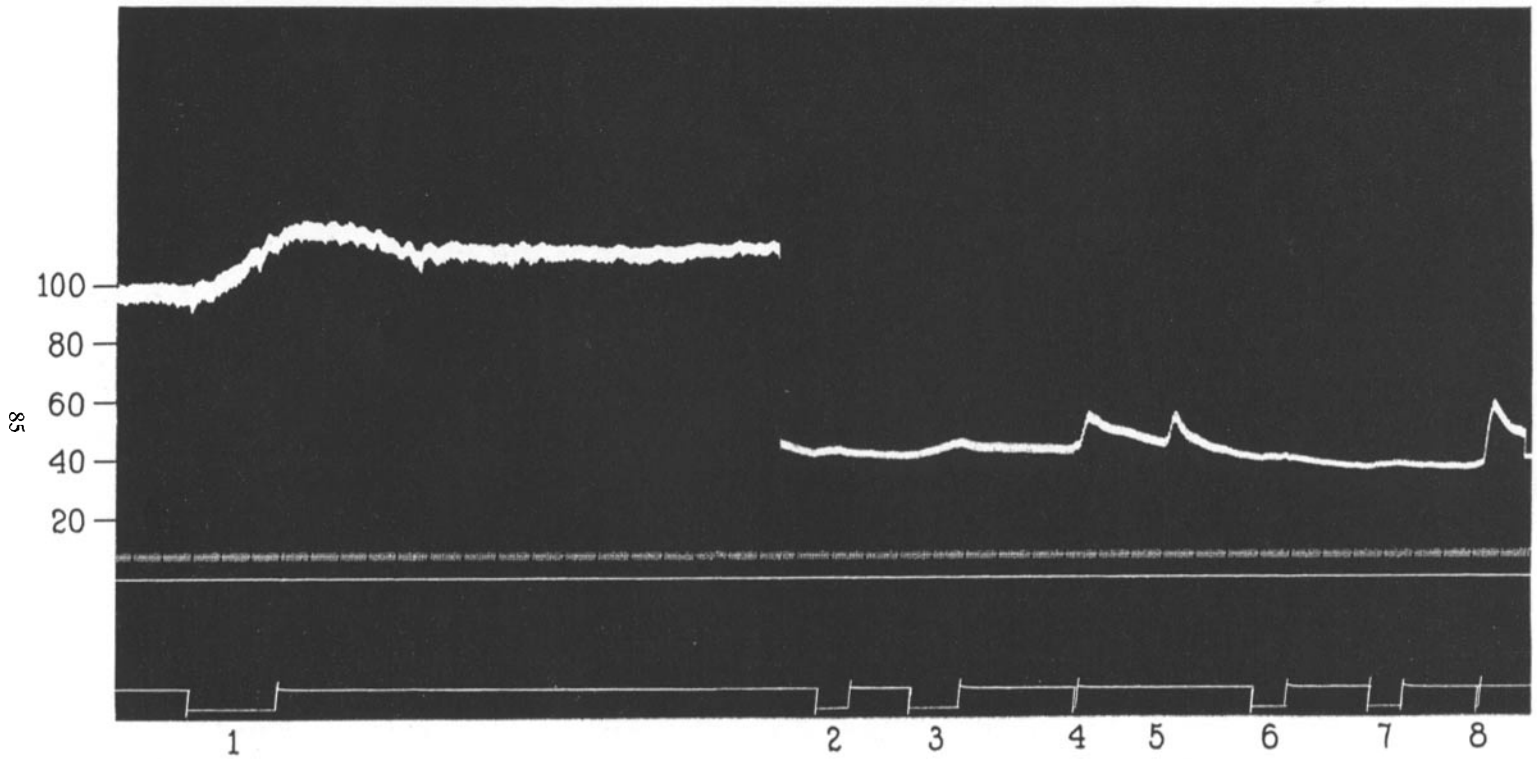

FIG. 6. Pressor response to 5 cc. of extract of 10 cc. ascitic fluid. Before and after pithing in cat under ethyl urethane anesthesia. (1) 5 cc. extract injected. (2) After pithing, 5 cc. extract injected. (3) 5 cc. normal saline. (4) and (5) Adrenalin 0.5 cc. 1:200,000. (6) 5 cc. extract. (7) Same. (8) Adrenalin 1 cc. 1:200,000. Initial pressure before pithing = 100 mm. Hg. After pithing pressure = 44 mm. Hg.

reduced. While "immunity" may develop in the sense that the animal no longer responds to extract this has seemed to be due to failure of the central nervous system because, coincident with the "immunity," the animal fails to respond to inspired CO_2-air mixture and usually to carotid sinus stimulation.

Effect of Progressive Destruction of the Central Nervous System.—The great variability of response noted in the test animal suggested that the substance or substances with which we were dealing might depend for their action on the cooperation of some portion of the central nervous system. It was assumed that during the initial period (Period 1), the lack of response was due to a temporary paralysis of the central nervous system, caused by anesthesia, when perforce an excess of ether was ordinarily employed. The sluggish reactions again, in Period 3, suggest that a more complete paralysis had occurred.

In order therefore to determine whether the central nervous system is an essential part of the mechanism of the pressor response, anesthetized cats were pithed to the second lumbar segment through a trephine hole in the skull, after tying off both carotid arteries. These animals responded markedly and regularly to adrenalin and pitressin. Such preparations are more sensitive to peripherally acting pressor substances than those in which the central nervous system is intact, provided the artificial respiration is properly regulated.

It could be shown that no response whatever was elicited by the injection of active pressor extracts (Fig. 6) into these pithed animals.

In order to localize more precisely the portion of the nervous system involved in the pressor response, the brain was removed at various levels in anesthetized, vagotomized cats, by means of the Sherrington guillotine. The action of pressor extract was not unusual until the cut passed through the brain somewhat above the bony tentorium. At and below this level, the responsiveness of the animal was slightly increased. However, section just below the tentorium abolished all effect of extracts.

Action on Unanesthetized Animals.—That the pharmacological action of many substances is markedly influenced by the anesthetic employed during the testing, is well known. It seemed important, therefore, to eliminate this factor as an influence in the action of the pressor extract.

For this purpose rabbits were found most satisfactory since they would lie quietly while the blood pressure was being taken. The method of Koch and Mies (53) was used to measure the blood pressure. This consists essentially in the preliminary operative transplantation of the carotid artery above the muscles of the neck of the anesthetized animal in such a way that a metal chamber containing a glass window can be clamped over the artery. One side of the chamber is covered by rubber membrane, and it is on this membrane that the artery is placed; that is, between the membrane and the glass window. Air pressure is applied against the membrane until the column of blood in the artery is just obliterated, and the pressure is then read from a mercury manometer. The transplantation of the artery was performed a day or two before the experiment. Often 0.5 cc. of 50 per cent ethyl urethane was given subcutaneously 15 minutes before the experiment in order to insure that the animal would remain quiet. By gentle handling we have had no difficulties. After applying the chamber it is well to wait 15 minutes before taking readings. A control period of 30 minutes was carried out, taking readings every 5 minutes to observe the range of spontaneous fluctuation in blood pressure. Our experience has been that most animals maintain under the conditions of the experiment a blood pressure of about 115 to 135 mm. of Hg without wide fluctuation.

Under the influence of ether, amytal, or ethyl urethane, rabbits have been found but moderately responsive to pressor extracts which were proven highly active in cats. The intravenous injection in non-anesthetized animals (ear vein) of 5 cc. portions of extract induced blood pressure elevation of 15 to 22 mm. of Hg. which appeared almost immediately after injection, and lasted 3 to 8 minutes. The same amount of normal saline produced practically no effect on the blood pressure.

This procedure has been controlled by the more usual method in which the carotid artery is cannulated and the pressure recorded graphically. The vessel was prepared, the cannula inserted under light ether anesthesia, and as soon as the operation was complete the cannula was stoppered. The animal was then allowed to recover from the anesthesia. 1 hour later blood pressure level was recorded. Records taken over 15 minute periods exhibited blood pressure of remarkable constancy. Pressor extracts ordinarily gave responses some 5 to 10 mm. of Hg greater than the same extracts tested on the same animal while anesthetized with ether.

Miscellaneous Observations Related to the Problem of Pressor Substances in Blood

Ultrafiltrates of Plasma Tested on Non-Anesthetized Animals.—In view of the observation of Anselmino and Hoffmann (15–17) that

ultrafiltrates of plasma or serum of blood from patients suffering from eclampsia, in whom the blood pressure was greater than 180 mm. of Hg, produced a rise in blood pressure of rabbits, it seemed desirable to test ultrafiltrates derived from the plasma of cases of essential and nephritic hypertension by the same method. The Koch-Mies method (53) has been used in the manner suggested by Anselmino and Hoffmann.[5]

After the control period, during which we convinced ourselves that the blood pressure of the animal was constant, 10 cc. portions of neutralized (to phenolphthalein) ultrafiltrate were injected subcutaneously into each flank of the animal and the injection site well massaged. Pressure readings were then taken every 5 minutes for a period of 30 minutes. Four plasma extracts from cases of essential and malignant hypertension (blood pressures ranging from 220 to 290 mm. systolic and 120 to 160 diastolic), and four cases of hemorrhagic Bright's disease with blood pressures from 195 to 250 systolic and 128 to 145 diastolic) were examined.

In no experiment was any effect on the blood pressure of the rabbit observed. The conclusion seems justified that while the conditions were excellent for a positive outcome of the experiment, the results were uniformly negative.

Ultrafiltrates of Plasma Tested on Anesthetized Animals.—Ultrafiltrates of plasma exhibited no marked action on the blood pressure level of anesthetized cats when injected intravenously in amounts up to 20 cc. While it is possible that a small quantity of pressor substance may pass the filter membrane, the fact that almost 90 per cent of the active material may be extracted by alcohol from the nonfilterable residue, clearly indicates that the amount which is dialyzable is very small. Alteration of the pH of the plasma before ultrafiltration did not change this result.

The extract of plasma prepared by the alcohol method as contrasted with native plasma was easily passed through the filter membrane without loss of activity.

Extracts of Blood Corpuscles.—Extracts of red blood cells prepared in the same manner as plasma extracts generally depress the blood pressure level. This is not always true as rare specimens have been found which produced slight pressor action and others have had none.

[5] We were fortunate in having the aid of Dr. Anselmino.

120 samples of red blood cells from patients suffering from essential hypertension, hemorrhagic Bright's disease, with and without hypertension, and red cells from normal individuals, have been compared. Analysis of the many graphs so obtained reveals no effect which is characteristic for any especial group of patients. It does not, therefore, seem necessary to present the detailed data.

Extracts of blood cells which have been hemolyzed by the addition of distilled water and then added to alcohol (20 cc. red cells + 20 cc. water in 380 cc. alcohol 95 per cent) and the extract prepared in the manner described for plasma also exhibited no characteristic difference from red cells of the same blood specimen in which hemolysis had been scrupulously avoided.

The amount of depressor substance in very fresh red blood cells (in alcohol within 5 minutes of venipuncture) is ordinarily not great. Extracts which correspond to 2 cc. of centrifuged cells may depress the blood pressure level of a 3.5 kilo cat 12 to 20 mm. of mercury. Allowing corpuscles to stand before addition to alcohol longer than 2 hours may cause to be liberated most powerful depressor substances. The injection of such an extract, corresponding to 2 cc. of corpuscles, may be quickly fatal to the animal. Atropine does not alter the depressor action of these extracts.

CLINICAL RESULTS

Since it could be shown that a pressor substance was extractable from the blood of man, it was of importance to ascertain whether the amount of this substance was altered in patients suffering from hypertension regardless of the pathogenesis of the symptom.

165 experiments have been performed, and from these data it seems almost certain that there is no significant correlation between the blood pressure response in the cat and the blood pressure of the patient from whom the plasma was obtained. Suffice it to say that the blood of 33 normal individuals, 8 cases of latent hemorrhagic Bright's disease, 12 cases of acute hemorrhagic Bright's disease, 11 cases of chronic active hemorrhagic Bright's disease, 19 cases of terminal nephritis, 8 cases of nephrosis or the nephrotic stage of Bright's disease, 38 cases of essential hypertension, 11 cases of toxemia of pregnancy, 1 case of cirrhosis of the liver without hypertension, and 2 cases of severe eclampsia have

been examined.[6] From many of these patients blood samples have been taken at intervals of a week for periods upwards of 16 months. During this time marked variations in the level of the patient's blood pressure had occurred (Fig. 7). Because of inherent difficulties in the method of assay, it was necessary to secure data which would be

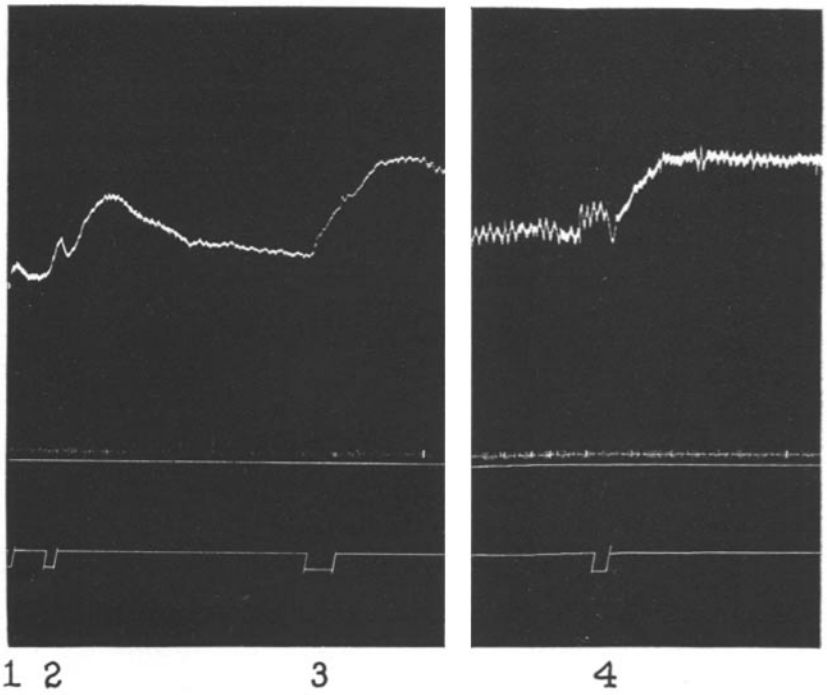

FIG. 7. Comparison of extract of nephritic plasma with ascitic fluid extract. (1) Saline 5 cc. injected. (2) Extract of 14 cc. plasma from severe nephritic patient with hypertension (B.P. = 200/122). (3) Extract of 14 cc. of ascitic fluid from patient with cirrhosis of liver without hypertension. Experiment 166 (8–9). (4) Extract of 10 cc. plasma from the patient with liver cirrhosis. Experiment 163 (No. 15). Initial pressure = 84 mm. Hg.

statistically significant. This goal, we believe, has been achieved, and it must be fairly stated that no correlation appears to exist between the amount of pressor substance and the height of the blood pressure.

[6] The blood samples from the cases of toxemia of pregnancy and eclampsia were furnished by Dr. E. Stander and Dr. L. Maclean of the Cornell Woman's clinic. We are grateful to Drs. Stander and Maclean for their courtesy.

It has been possible to examine blood from cases[7] of the recently described pituitary basophilism. Three of these cases had rather marked hypertension[8] and in the other two the pressure averaged 140 to 150 mm. Hg systolic and 90 to 94 diastolic. All the patients exhibited more or less typically the signs and symptoms of this disease as set forth by Cushing. No characteristic difference was observed in the ultrafiltrates or extracts of plasma from these patients as compared with extracts of normal blood.

As an incidental observation it seems worth recording that from a limited number (3) of patients suffering from convulsive uremia extracts of blood plasma seemed peculiarly toxic when injected into a cat. Instead of the usual rise in blood pressure, a precipitous fall occurred, causing the death of the animal. It should be emphasized that these patients were in the terminal stage of hemorrhagic Bright's disease and not examples of the so called pseudo-uremia.

Cerebrospinal fluids from nineteen assorted cases of nephritic and essential hypertension have also been examined. Pressor substance is present in at least as large amounts as in plasma. Injection of fresh spinal fluid from a variety of patients suffering from hypertension failed to show the presence of any appreciable amount of free pressor substance when tested either on anesthetized animals with the central nervous system intact, or on pithed cats. The possibility that unbound secretion of the posterior pituitary body is contained in the spinal fluid in amounts which might be considered significant in the pathogenesis of hypertension, has not been demonstrated. This statement must be qualified to a certain extent in that the ventricular fluid from one case of adrenal tumor contained a powerful pressor substance with properties somewhat different from those of the pressor extracts of plasma. Spinal fluid of one patient with a severe malignant hypertension also was powerfully vaso-active.

DISCUSSION

The pressor substance evidently is widely distributed since it has been found in human plasma, ascitic and cerebrospinal fluids. Corpuscles seem to contain little or none of it because alcohol extracts

[7] Through the kindness of Dr. Harvey Cushing and Dr. B. S. Oppenheimer.

[8] Dr. Henry Turner, Oklahoma City, very kindly sent samples of blood from one such case.

either do not influence or strongly depress the level of the blood pressure of cats. That it is not free in these fluids follows from the fact that ultrafiltration yields a protein-free filtrate which has no vasoactivity. It seems probable that the substance is bound to the plasma colloids but the union must be loose, to be split by such mild treatment as that with cold alcohol. The active substance is water-soluble and behaves not unlike an organic base.

The pharmacological properties of the pressor substance appear in some respects unique. The evidence, in the first place, gathered from comparison with drugs acting on peripheral arteries such as adrenalin, choline, histamine, and adenosine, does not lend support to the view that its action resembles that of these other substances. The effect of ergotoxine and cocaine on the response to pressor extract also demonstrates the dissimilarity in action.

The second group of data indicates that the functional intactness of the central nervous system is essential in order that pressor responses be obtained from the extract under investigation. The most cogent of these proofs consists in the demonstration that if the brain is removed close to the bony tentorium, the pressor response is undisturbed, conversely the response is completely abolished on pithing or cutting the cord below the medulla. Anesthesia, furthermore, prolonged to the point where inhalation of carbon dioxide-air mixtures or stimulation of the carotid sinus no longer elevate the blood pressure, also inhibits its action. The unanesthetized animal (rabbit) is, in fact, more responsive than the anesthetized. It is not improbable that some substance in the extract sensitizes the mechanism responsible for the carotid sinus reflex, for after injection of extract the reflex is considerably more active than before.

A third group of more miscellaneous data indicates that the splanchnic area is actively involved in the vascular constriction. The evidence consists largely in the demonstration that evisceration strongly depresses the response and oncometric measurement of the leg and renal volume shows relatively little constriction when marked elevation of the level of pressure has occurred. Stimulation of the adrenals to secrete more epinephrin apparently plays little or no part in the mechanism because bilateral adrenalectomy does not influence the result. An assay of the potency of the extract has been difficult

because the degree of response depends on the functional state of the nervous system. To some degree success has been attained in predicting the response of the animal to pressor extract by relying on the reflex from the carotid sinus and on that from stimulation by carbon dioxide. No evidence has been found indicating that there is an increase in the amount of pressor substance in hypertensive states of varied pathogenesis.

This investigation was undertaken to ascertain whether, by direct methods which involve as slight chemical manipulations as possible, a single substance or a group of substances is extractable from blood which causes or is associated with arterial hypertension in man. There are numerous possibilities. Such substances, present in normal blood, may be increased in the blood of hypertensive patients; they may be present only in hypertensive states; though constant in amount, the sensitivity of the vascular system may be so increased that its response is pathological; the amount bound to plasma colloid may remain constant though the amount liberated, due to the activity of the reacting cells (*Erfolgsorgan*), may be greater than normal; and, finally, they may be set free into the blood stream and either be quickly destroyed or inactivated. While it may be necessary to reckon with these manifold possibilities this investigation has been confined to the effort to demonstrate the presence of pressor substances in circulating blood and their quantitative aspects in relation to the hypertensive state in man.

CONCLUSIONS

1. Extracts of human blood plasma, ascitic and cerebrospinal fluids have been shown to contain a substance or substances which have a prolonged and powerful pressor action when injected into test animals.

2. The chemical properties of the substance suggest those of an organic base. It is extracted with alcohol, soluble in water and acetone, extracted from water by chloroform, and probably is but slightly heat-stable. The plasma colloids seem to hold the substance in a bound state since it does not appear in the ultrafiltrate and is liberated on coagulation of the colloids by alcohol. Coagulation alone of the blood does not cause the substance to be formed.

3. Its action suggests that its pressor effect is brought about by

mediation of the central nervous system. This inference was drawn from the following observations. (*a*) The functional intactness of the central nervous system is essential in order that pressor responses be obtained. Unanesthetized animals exhibit greater vascular responses than do anesthetized. (*b*) Pithing animals completely abolishes the response. Progressive ablation of the brain to the level of the hind brain does not alter the response, but below this level, injury abolishes the activity of the extract. (*c*) Some substance in the extract sensitizes the mechanism responsible for the carotid sinus reflex. (*d*) There is no parallelism between the response to peripherally acting drugs and pressor extracts. (*e*) Removal of the adrenal glands does not affect its character.

4. The rise in blood pressure appears to be due especially to constriction of the arteries in the splanchnic region.

5. Assay of the pressor extracts is made difficult because of the dependence of the vascular response on the functional state of the central nervous system. The carotid sinus reflex and stimulation with carbon dioxide-air mixtures have proved most useful means for the estimation of this functional state. It has been pointed out that the vascular responses to extract, stimulation of the carotid sinus, and inhalation of carbon dioxide-air vary greatly during the course of an experiment on anesthetized animals. This natural history of the vascular responses has been described.

6. No evidence has been produced by the method employed that the amount of this pressor substance is increased in the blood or spinal fluid of patients with hypertension of varied pathogenesis (nephritic hypertension, essential hypertension, malignant hypertension, eclampsia, and pituitary basophilism).

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