THE EFFECTS OF PRESSOR DRUGS AND OF SALINE KIDNEY EXTRACTS ON BLOOD PRESSURE AND SKIN TEMPERATURE¹

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Many pressor substances, including hormones, drugs, and variously prepared extracts of kidney tissue, have been studied intensively to determine their effects on blood pressure and their possible rôle in the pathogenesis of human hypertension. Many of these experiments were performed on anesthetized animals and observations usually dealt merely with changes of systemic blood pressure. According to recent studies, however, one of the remarkable features of human hypertension is the association of abnormally high arterial pressure with normal peripheral blood flow. When vasoconstrictor tone in the extremities is diminished by warming the body, blood flow in the forearm increases equally in hypertensive and normal subjects (1, 2). It seemed highly desirable therefore to determine whether the action of any of the known pressor substances simulated human hypertension to the extent of producing, in an experimental animal, a rise of blood pressure without reducing peripheral blood flow simultaneously.

In order to study the action of pressor substances on peripheral blood vessels it was found necessary to develop a method which (1) did not require anesthesia, (2) excluded variations arising from spontaneous changes in vasoconstrictor tone of nervous origin, (3) avoided trauma of the vessels studied, and (4) permitted intravenous injection at constant rates over long periods in order to reveal minor changes. The usual oscillometric technique was therefore modified to permit photographic recording of systolic blood pressure and pulse amplitude in the central artery of the rabbit's ear while vasoconstrictor tone of nervous origin was abolished by warming

the body of the rabbit. Changes of peripheral blood flow were detected by measuring skin temperature. The procedure required no anesthesia and observations could, if desired, be made on the same animal repeatedly on successive days.

This paper compares the effects of epinephrine, tyramine, pituitrin, pitressin, ergot derivatives, guanidine and saline extracts of normal rabbit kidney on blood pressure, amplitude of arterial pulsation, and skin temperature of the rabbit's ear. Of the substances tested, a specially treated saline kidney extract was the only one which elevated peripheral blood pressure without simultaneously reducing skin temperature in the ear of the warmed rabbit. This property of raising blood pressure without measurably decreasing peripheral blood flow appears to depend upon a protein-like substance which remains in solution during heating to 56° C., but is precipitated or destroyed by heating to 65° C.

METHODS

Unanesthetized male rabbits, weighing usually between 2.0 and 2.7 kilos, were used throughout. The animals were fed either purina rabbit chow with miscellaneous greens or a composite food known to be adequate (3). Hair was removed from both ears with a barium sulphide mixture at least twenty-four hours before the animals were to be used. Under ordinary conditions the vessels of the rabbit's ear dilate and constrict intermittently in response to excitement and the need for radiation or conservation of heat. To abolish such variations of vasoconstrictor tone the animals were placed in a metal box with a double wall through which warm water could be pumped continuously from a reservoir. The temperature of the circulating water was controlled by a thermostat, so that the warmth of the box could be adjusted to raise the rabbit's rectal temperature rapidly or slowly as desired. Ordinarily, warming the air within the box to between 43 and 45° C. elevated rectal temperature from the normal 38.5° C. to 40.5° C. in about forty-five minutes. The usual intermittent dilatation and constriction of the auricular vessels gave place to complete

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and persistent vasodilatation when rectal temperature was 40.0° C. or more (4). Emaciated or diseased animals did not respond normally to body warming and were discarded.

The head of the rabbit projected above the top of the heated box and was held in position by an adjustable support back of the occiput, a grooved board below the mandible, and a gauze bandage tied tightly over the nose. In this holder neither the auricular vessels nor the carotid sinuses could be pressed upon even though the animal struggled vigorously. The importance of avoiding pressure on the carotid sinuses cannot be overemphasized. In general, the animals were entirely quiet, or struggled only once or twice during an observation lasting one to two hours. In the warm animal, struggling usually in-

FIG. 1. DIAGRAM OF OSCILLOMETER FOR MEASURING THE PERIPHERAL BLOOD PRESSURE AND ARTERIAL PULSATION IN RABBIT'S EAR

For description see text.

creased both blood pressure and heart rate slightly for one-half to one minute, but did not produce the vasoconstriction which follows struggling of rabbits with normal body temperature. Very occasionally it was necessary to discard an animal that struggled too frequently. Sedatives were not used because even small doses of nembutal and morphine made the reactions of the auricular vessels unpredictably variable.

Rectal temperature was measured by a thermocouple which was enclosed in a soft rubber catheter and inserted into the rectum about two and one-half inches. A second thermocouple, held in contact with the mesial surface of the left ear by one thickness of adhesive plaster, measured changes of skin temperature (T, Figure 1). All injections were made intravenously through

a minute T-tube (C, Figure 1) consisting of a 20-gauge, short-bevelled needle and two side tubes of like caliber. The needle was inserted into the lateral vein of the left ear and sewed in position with a single silk stitch before observations were begun. The two side tubes at the base of the needle communicated with fine rubber tubing, so that one limb could be closed by a clamp (Figure 1) while the other limb was connected to a 20 cc. syringe. By opening the clamp, the solution about to be injected could be washed through the tubing and brought to the base of the needle, thus practically eliminating dead space in the injection system. All solutions were warmed to 41° C.; the dose was always 20 cc. and was injected at a rate of 1.6 to 1.7 cc. per minute by means of a motor-driven syringe. The injection of each dose required between twelve and thirteen minutes, which allowed ample time for detecting small changes in skin temperature.

Peripheral blood pressure and the amplitude of peripheral arterial pulsation were measured in the right ear by means of a capsule (5, 6) and sensitive recording oscillogram. The concave surface of the ear rested on a hollow metal and glass support (S, Figure 1) warmed by circulating water to between 42 and 43° C. The central artery and the adjacent tissues were illuminated by light coming through the ear from a 6-volt bulb situated below a glass window in the center of the ear holder. An oval celluloid capsule, notched at both ends and covered with dental rubber dam, was mounted on a sheet of thicker celluloid (detailed drawing, Figure 1). By means of thumb screws the rubber membrane and capsule were brought down carefully upon the ear so that the central artery lay free in the notches while the sides of the capsule held the ear in place. The illumination from beneath made it possible to adjust the ear between the underlying support and the capsule so that blood flow through the central artery was unobstructed except when pressure was increased within the capsule.

The interior of the capsule communicated through pressure tubing with the oscillogram which was arranged for photographic recording to increase sensitivity (Figure 1). A heavy rubber manometer and mirror (M1, Figure 1) indicated the external pressure exerted upon the central artery of the right ear. This manometer was calibrated against a mercury column before and after each experiment (Figure 2). A second manometer (M2, Figure 1), closed by condom rubber and situated inside a glass chamber, recorded pulsation in the segment of artery enclosed by the capsule. Pressure within the oscillogram system and upon the auricular artery was changed automatically by a motor-driven valve from 0 to 180 mm. Hg and then back to 0, with four complete cycles per minute. The capillary resistances (CR1, CR2, Figure 1) and the capillary leak (CR3, Figure 1) were adjusted so that mean pressure changed at approximately the same rate everywhere in the system. For recording rapid changes in pressure the camera was allowed to run continuously, producing four readings of systolic pressure and pulse amplitude during each minute. In the experiments now described, however, interest centered upon

FIG. 2. PORTIONS OF RECORDS SHOWING CONTRASTING EFFECTS OF 5 PER CENT "HEATED (55 TO 56° C.) KIDNEY EXTRACT" AND TYRAMINE

The portions chosen illustrate control observations (9th and 10th minutes), injection (16th and 22d minutes, depressed base line) and recovery (32d and 33d minutes). Injection of 20 cc. dose was started at the end of the 10th minute and continued to the end of the 22d minute.

Above: Showing that 5 per cent heated kidney extract elevates blood pressure without changing either skin temperature or the amplitude of oscillation.

Below: Showing that 1:1,000 tyramine elevates blood pressure but reduces both skin temperature and amplitude of oscillation.

slow changes so the camera was arranged to run only nine seconds of each minute. The photographic record then showed (Figure 2) once every minute the change in pulsation produced by increasing the external pressure on the auricular artery from 0 to 180 mm. Hg. Sections of two typical experiments are reproduced in Figure 2 with the calibration of the heavy manometer to the left.

The sigmoid line (Figure 2), rising obliquely toward the right, indicates the increasing pressure exerted by the capsule upon the central artery, while the horizontal tracing shows simultaneous changes in amplitude of pulsation. As pressure rises from 0, the oscillations become at first greater, then disappear more or less suddenly. The pressure at which the last definite oscillation occurs is recorded as systolic pressure in the auricular

artery. Since all studies were comparative, this rough criterion was satisfactory, as may be seen from the control experiment shown in Figure 3.

Changes in heart rate were estimated by measuring the linear distance occupied by ten complete pulsations. The maximum amplitude of pulsation in each oscillometric tracing was measured to the nearest half millimeter in order to estimate gross changes in the volume of the pulse in the auricular artery. Lessening of the maximum amplitude of oscillation cannot be regarded by itself as a measure of increasing vasoconstrictor tone, since oscillation may be reduced also by increased heart rate or diminished blood pressure. When interpreted in conjunction with changes in skin temperature, blood pressure, and pulse rate, large changes in the amplitude of

oscillation can supply, it is believed, significant information concerning arterial tone. High grades of vasoconstriction make pressure readings difficult, and complete constriction makes them impossible. Hence, in studying drugs which produced conspicuous constriction of the auricular artery, numerous concentrations were used so that conclusions might be based primarily on those doses which elevated blood pressure significantly without diminishing the amplitude of pulsation by more than twenty or thirty per cent.

The dilute solutions of drugs or hormones were prepared from standard powder or ampules not more than thirty minutes before injection. Each solution was heated to 41° C. in the syringe and was kept warm during injection.

In preparing kidney extracts, normal white male rabbits, weighing between 2.0 and 2.7 kilos, were killed by concussion. The blood vessels of the neck were immediately severed, and the animal was suspended head down till bleeding ceased. Both kidneys, weighing together between 10 and 15 grams, were excised, their capsules stripped off, and the hilus tissues cut away. The kidneys were weighed and then cut into small fragments, including both cortex and medulla. Enough 0.9 per cent NaCl solution was added to make a 5 per cent extract based on wet kidney weight. The material was ground for fifteen minutes in a colloid mill cooled by a current of cold tap water so that during grinding the temperature of the extract did not exceed 40° C. The milky suspension was centrifuged and the supernatant cloudy fluid filtered under suction through Numbers 2 and 50 Whatman paper. The filtrate, termed "unheated kidney extract" was still cloudy or milky in appearance, though practically free of granules by microscopic examination.

Part of the original "unheated kidney extract" was kept for later injection and comparison with the corresponding "heated kidney extract." The remainder was immersed in a thermostatically controlled water bath, warmed gradually to the temperature of the bath and then kept for twenty minutes at the desired maximum temperature (50 to 51°, 55 to 56°, or 65 to 66° C.). As the temperature of the extract reached 51° C. a heavy flocculent precipitate formed. Filtration through Whatman Numbers 2 or 50 paper in a funnel at or near the temperature of the bath produced, after the first 10 or 20 cc., a water-clear, slightly yellowish fluid, termed in this paper "heated kidney extract."

Each extract was analyzed for nonprotein nitrogen and total protein by a standard Kjeldahl procedure and Nesslerization.⁴ Ultrafiltrates were prepared by filtration through Number 600 cellophane at pressures between 100 and 115 atmospheres. Some of the heated extracts were salted out by saturation with ammonium sulphate at room temperature. After one to three hours the precipitate was filtered off, redissolved in 0.9 per cent NaCl solution, dialyzed, and the volume made up to equal that of the original heated extract. With the exception of the am-

monium sulphate precipitate, all extracts were used on the day of preparation. A fresh animal was used for each injection to avoid possible variations in response as a result of prior injection of protein to which the animal might conceivably be, or become, sensitive.

OBSERVATIONS

Control studies

The accuracy of the oscillometric method for measuring systolic blood pressure in the auricular artery was tested (*a*) roughly, by inspecting the artery to determine at what pressure the flow was entirely obstructed and (*b*) more accurately, by comparing the oscillometric records from the right auricular artery with direct determinations from the left auricular artery, using for the latter a gauge 20 needle and the manometer described by Hamilton *et al.* (7). In the resting animal oscillometric determinations of systolic blood pressure were equal to, or slightly lower than, the figures provided by the Hamilton technique (Table I). Elevations of blood pressure produced by abdominal compression, epinephrine and asphyxia were promptly detected by oscillometry, the peak figures being, however, slightly lower than those obtained by direct cannulation (Table I).

TABLE I

Comparison of simultaneous measurements of systolic blood pressure in the central artery of the rabbit's ear by oscillometric and direct (Hamilton) methods

	Systolic blood pressure	
	Right ear (oscillometric method)	Left ear (Hamilton method)
	<i>mm. Hg</i>	<i>mm. Hg</i>
Resting values	86	84
Rectal temperature 40.6 to 41.5°C.	95	98
	80	80
	68	70
	84	90
Abdominal compression by pneumatic cuff at 200 mm. Hg. Peak values only.	123	119
	113	112
	118	120
	110	112
	113	112
Epinephrine 1 : 100,000 in- jected at rate of 1.7 cc. per minute. Peak values only.	132	136
	124	138
	140	140
	122+*	125
	124	140

⁴ We are indebted to Miss E. H. Shiels for these analyses.

* Auricular artery much constricted. Oscillometric measurement uncertain.

FIG. 3. CONTROL EXPERIMENT SHOWING SYSTOLIC BLOOD PRESSURE, AMPLITUDE OF OSCILLATION (AURICULAR ARTERY), SKIN TEMPERATURE OF EAR, AND HEART RATE DURING INJECTION OF 0.9 PER CENT NaCl AND 1:200,000 EPINEPHRINE

Rectal temperature ranged from 39.4 to 43.0° C.

The systolic blood pressures in the auricular artery, recorded either indirectly by oscillometry or directly by needle, were as much as 20 mm. less than those obtained by direct puncture of the femoral artery both in the resting animal and during the action of epinephrine (Table II). From these comparisons it appears that a rise of

systolic pressure measured oscillometrically from the auricular artery indicates an equal, or slightly greater, rise in femoral systolic pressure. The oscillometric method was used because it presented certain advantages for comparative studies, *viz.*: (1) observations could be made over periods of hours, or on successive days, with relatively

TABLE II

Comparison of simultaneous measurements of systolic blood pressure in the central artery of the rabbit's ear (oscillometric) and in the femoral artery (Hamilton method)

	Systolic blood pressure	
	Right ear (oscillometric method)	Femoral artery (Hamilton method)
	<i>mm. Hg</i>	<i>mm. Hg</i>
Resting values	72	88
Rectal temperature 40.6 to 41.5°C.	86 77 92 83	102 92 110 95
Epinephrine 1 : 100,000 injected at rate of 1.7 cc. per minute.	138 132 140	160 155 160

little injury of the artery, (2) the animal was in a normal relaxed position and could be warmed easily, whereas direct femoral determinations required that the animal be stretched tightly on an animal board so that warming was difficult and struggling more frequent, (3) the amplitude of oscillation provided, within certain limits, additional objective evidence of changes in vascular tone which might be compared to changes in skin temperature.

A control experiment is shown in detail in Figure 3. At body temperatures below 40.5° C. the

ear vessels constricted and dilated intermittently, as shown by wide fluctuations of pulse amplitude and of skin temperature throughout the first twenty minutes. During constriction the readings of heart rate and systolic blood pressure were often questionable. After rectal temperature exceeded 40.5° C. the readings of systolic blood pressure became uniform, within the error of the method. Skin temperature and the amplitude of oscillation did not change significantly during four injections of 0.9 per cent NaCl. Rectal temperature was allowed to rise well beyond the usual level in order to demonstrate that, even at 43° C., the injection of epinephrine elevated blood pressure, slowed the heart rate, decreased slightly the amplitude of pulsation, and reduced skin temperature sharply. This experiment represents an extreme test with respect to the range of body temperature and the total amount of fluid injected. Ordinarily, rectal temperature was kept between 40.5 and 41.8° C., with occasional experiments between 41.8 and 42.5° C. In studying the effects of drugs, not more than four injections were made in any one animal. In studying the effect of kidney extracts the animals were discarded after a single injection of 20 cc. had been completed.

TABLE III

Effects of epinephrine, tyramine, pitressin, and pituitrin S on circulation of the rabbit's ear (20 cc. at rate of 1.6 cc. per minute)

Drug	Concentration	Number of observations	Body temperature at time of injection	Average change in			
				Skin temperature	Blood pressure	Heart rate	Pulse amplitude
			° C.	° C.	<i>mm. Hg</i>	<i>beats per minute</i>	<i>per cent</i>
Epinephrine hydrochloride	1 : 1,000,000	4	40.8-41.8	0	0	0	0
	1 : 500,000	2	41.1-41.9	-0.5	+5	0	0
	1 : 250,000	4	41.0-42.0	-1.9	+12	-60	-15
	1 : 100,000	5	40.7-41.9	-3.6	+54	-135	-30
Tyramine hydrochloride	1 : 100,000	5	40.2-41.7	+0.1	0	0	0
	1 : 10,000	6	40.6-42.3	-1.6	+5	-35	0
	1 : 5,000	5	41.6-42.5	-1.5	+8	-14	0
	1 : 1,000	5	40.8-42.6	-5.4	+29	-80	-75
Pitressin	1 : 10,000	3	40.3-41.3	-0.5	0	-20	0
	1 : 2,000	3	41.4-41.7	-0.9	0	-35	0
	1 : 1,000	3	40.6-41.8	-4.5	+6	-50	-6
	1 : 100	1	41.3	-8.3	+13	-80	-10
Pituitrin S	1 : 100,000	1	41.3	0	0	-40	0
	1 : 10,000	4	41.0-41.5	-0.2	0	-45	0
	1 : 1,000	3	41.1-41.5	-2.3	+16	-55	-15
	1 : 500	2	41.7-41.8	-3.4	+22	-70	-40

The effects of epinephrine, tyramine, pitressin, pituitrin S, guanidine, ergotoxine and ergotamine

The effects of epinephrine on auricular circulation and skin temperature are illustrated in Figure 4. The injection of 20 cc. of 1:500,000 epi-

changes were produced in greater degree by 1:100,000 epinephrine. Table III summarizes a series of fifteen observations on solutions ranging in concentration between 1:1,000,000 and 1:100,000.

During injection of epinephrine solutions, skin

FIG. 4. SHOWING THE EFFECTS OF EPINEPHRINE ON PERIPHERAL CIRCULATION AND SKIN TEMPERATURE OF THE RABBIT'S EAR

nephrine had no detectable effect, but 20 cc. of 1:250,000 epinephrine elevated blood pressure slightly, depressed skin temperature significantly, diminished amplitude of oscillation, and produced a definite slowing of the heart rate. These same

temperature always diminished when blood pressure rose; occasionally skin temperature diminished without a significant rise of blood pressure (Figure 4, last injection). The amplitude of pulsation diminished as blood pressure rose and

with the higher doses pulsation of the auricular artery was almost obliterated. The effects were always temporary; at the end of each injection peripheral circulation rapidly returned toward the normal as blood pressure decreased. With the more dilute solutions recovery began even before the injection was complete (second injection, Figure 4).

A reduced amplitude of oscillation, when associated with rising blood pressure, slower heart rate, and falling skin temperature, can be due only to increased arterial tone. Since peripheral blood flow diminished, it may be concluded that periph-

eral resistance and blood pressure did not increase proportionately, the increase in the former being relatively greater.

The effects of tyramine hydrochloride (Table III) resembled those of epinephrine qualitatively but began more slowly. Blood pressure rose only slightly and occasionally with 1:10,000 solution, but whenever pressor action appeared skin temperature also fell. More uniform and significant changes in blood pressure were obtained with higher concentrations and it can be concluded that tyramine, when given in amounts which elevate blood pressure, also constricts the auricular ar-

FIG. 5. SHOWING THE EFFECTS OF PITUITRIN S ON PERIPHERAL CIRCULATION AND SKIN TEMPERATURE OF THE RABBIT'S EAR

TABLE IV

Effects of guanidine, methylguanidine, dimethylguanidine and ergot derivatives on circulation of the rabbit's ear (20 cc. at rate of 1.6 cc. per minute)

Drug	Concentration	Number of observations	Body temperature at time of injection	Average change in			
				Skin temperature	Blood pressure	Heart rate	Pulse amplitude
			° C.	° C.	mm. Hg	beats per minute	per cent
Guanidine hydrochloride	1 : 10,000	1	40.9	-0.4	0	-35	0
	1 : 1,000	3	41.3-41.8	-0.1	0	-5	0
	1 : 100	3	41.2-42.0	-2.7	+18	-45	-55
Methylguanidine hydrochloride	1 : 1,000	3	41.2-42.4	-0.1	0	0	0
	1 : 100	3	41.4-42.2	-5.9	+13	-110	-70
Dimethylguanidine hydrochloride	1 : 1,000	4	41.4-42.4	-2.0	+2	-60	-10
	1 : 500	2	41.6-42.5	-3.1	+2	-55	-25
	1 : 100	2	42.2-42.4	-6.0	+32	-55	-50
Ergotoxine ethane sulphonate	1 : 10,000,000	1	41.3	-0.3	0	0	0
	1 : 1,000,000	2	40.2-41.4	-1.3	0	0	0
	1 : 100,000	1	41.6	-1.8	+12	-15	-80
Ergotamine tartrate	1 : 5,000,000	1	40.8	-0.2	0	0	0
	1 : 500,000	1	41.0	-0.3	+5	0	0
	1 : 50,000	1	41.3	-2.0	+12	0	-50
	1 : 25,000	1	42.0	-3.5	+7*	-60	-80

* Almost complete vasoconstriction prevented accurate pressure readings.

tery, diminishes skin temperature significantly, and reduces peripheral blood flow. As with epinephrine, very high concentrations constricted the auricular artery conspicuously and under these circumstances readings of pressure were difficult, as shown by portions of the record reproduced in Figure 2. The higher concentrations reduced skin temperature much more rapidly than the smaller doses (Table III and Figure 2). Recovery after the injection of tyramine was not quite so prompt as that following the injection of epinephrine.

Pitressin (Table III) and Pituitrin S (Figure 5 and Table III) reduced skin temperature conspicuously and increased blood pressure very little. The smaller doses of these two substances merely blanched the ear, constricting the minute vessels visibly without affecting pulsation of the central artery; under these conditions skin temperature occasionally fell even though blood pressure was not elevated measurably (pitressin 1:2,000; Table III). The larger doses constricted the minute vessels conspicuously and, to a lesser degree, the central artery as well; skin temperature then decreased by several degrees. The auricular circulation returned to normal very slowly (Figure 5).

Guanidine and its derivatives had no clearly detectable effect on blood pressure in concentrations less than 1 per cent (1:100, Table IV). The larger doses regularly produced vasoconstriction and reduced skin temperature but the effects on blood pressure were variable. Dimethylguanidine reduced skin temperature even in 1:1,000 solution but did not raise blood pressure at that concentration. Toxic effects were frequently observed; one animal was killed by 15 cc. of 1:100 methylguanidine and two animals had convulsions after the larger dose of guanidine was injected. Peripheral vasoconstriction, when induced by guanidine or its derivatives, persisted in some instances for several hours. Skin temperature continued to fall after the injection ceased, or fell further after a short, but incomplete, period of recovery. There was nothing to suggest that guanidine or its derivatives could produce a significant rise of blood pressure without persistent peripheral vasoconstriction and reduced peripheral blood flow.

Two derivatives of ergot (Table IV) were included in order to test by the same method the action of substances which are generally known to exert a powerful constrictor action on the cutaneous vessels particularly. Ergotoxine ethane sul-

phonate in 1:1,000,000 dilution reduced peripheral blood flow without affecting blood pressure significantly. Higher concentrations of both ergotoxine and ergotamine reduced amplitude of oscillation, elevated blood pressure slightly, and diminished skin temperature conspicuously. Vasoconstriction persisted long after injection ceased. It appears that these substances could not produce a significant rise in blood pressure without reducing peripheral blood flow.

Unheated 5 per cent saline kidney extract

Filtered 5 per cent saline kidney extracts, prepared as described under "Methods," were cloudy even after careful centrifugation and filtration. They contained between 0.24 and 0.51 per cent protein (Table V, left). These extracts were relatively unstable, since precipitates formed whenever they were kept for more than a few hours after preparation. When injected intra-

venously, their action was highly variable, except for their effect on skin temperature. To the left in Table V are summarized representative experiments which include most of the variations observed in over thirty injections of 5 per cent unheated extract of normal kidney tissue. Systolic blood pressure occasionally rose during injection but more frequently fell. An initial rise of systolic blood pressure might be followed by a fall, or *vice versa*. The temperature of the ear always diminished by amounts ranging between 1.5 and 8.0° C. Heart rate usually remained the same or increased; but when blood pressure rose, definite slowing occurred. The amplitude of pulsation was always diminished significantly, and frequently to such degree that accurate pressure readings were impossible.

The unheated kidney extracts were often toxic. In some instances, after the first 5 or 10 cc. were injected, the ear blanched, respiration became slower, heart rate increased, and the animal died

TABLE V

Effects of unheated and heated 5 per cent saline extracts of normal rabbit kidney (20 cc. at rate of 1.6 cc. per minute)

Unheated kidney extract					Heated kidney extract					
Change in				Total protein	Extract heated for 20 minutes to	Change in				Total protein
Systolic blood pressure	Skin temperature	Heart rate	Pulse amplitude			Systolic blood pressure	Skin temperature	Heart rate	Pulse Amplitude	
mm. Hg	° C.	beats per minute	per cent	grams per cent	° C.	mm. Hg	° C.	beats per minute	per cent	grams per cent
-10	-2.6	0	-75	0.38	50-51 ‡	+20 +16	-0.7 -0.4	-85 -65	-25 -15	0.13
-30 ?	-8.0	+30	-90	0.50	50-51 ‡	+13 +12	-0.4 +0.4	-15 -10	-15 -10	0.15
+5	-3.4	0	-85	0.39	50-51 ‡	+30 +35	+0.4 +0.2	-20 -70	0 +10	0.15
+10 ?	-3.2	+30	-100	0.51	55-56	+27	+0.2	-10	0	0.13
-? *	-6.6 *	+? *	-100	0.45	55-56	+35	0	-60	0	0.12
+35	-1.5	0	-60	0.31	55-56	+25	+0.1	0	0	0.13
+35, -7 †	-1.6	-95, +25	-30	0.24	55-56	+30	+0.4	-70	0	0.09
- ?	-6.7	+70	-100	0.46	65-66	-8	+0.1	+10	0	0.06
-20	-2.4	+30	-90	0.47	65-66	-10	+0.5	+20	0	0.06
+30	-2.2	-55	-70	0.35	65-66	-5	+0.2	+10	0	0.06

* Animal killed by injection.

† Blood pressure rose then fell during injection.

‡ In this group (50 to 51° C.) the same heated extract was injected into 2 animals to illustrate range of variation.

after a few convulsive movements. Many of the animals, though surviving the injection itself, died within twenty-four hours after the injection of a single dose of 20 cc. of 5 per cent extract. Graded injections of 1 per cent, then 3 per cent, and finally 5 per cent extract reduced the immediate toxicity, but did not clearly increase ultimate survival of the animal. Extracts prepared in the usual way from kidneys of animals that had been starved for one to five days seemed to be less toxic with respect to survival, but the immediate vascular effects were not essentially different from those obtained with extracts of kidneys from normally fed animals. The results were not modified by various diets, including purina plus miscellaneous greens, oats only, lettuce only, and the composite diet described by Friedman (3). The incidence of pressor activity did not have any clear relation to diet.

From these experiments it appears that crude saline extracts of normal rabbit kidney tissue, while producing occasional pressor effects, do not do so with sufficient regularity to justify any conclusions. Even in those experiments in which pressor activity was demonstrated clearly, the simultaneous fall of skin temperature indicated that peripheral blood flow was reduced so that the effects of unheated kidney extracts did not differ from those of the other pressor substances which had been tested.

Heated 5 per cent saline kidney extracts

It was found, by accident, that heating the extracts of normal kidneys decreased toxicity and rendered pressor responses much more uniform. A series of extracts were therefore exposed to graded heating as described under "Methods." At temperatures above 50° C. a heavy flocculent precipitate formed; filtration of the warm solution in a heated funnel produced a clear, slightly yellowish filtrate which contained less protein and produced effects entirely different from those described for the unheated extracts. Table V (right) presents the results of a series of observations in which the effects of heated extracts (50 to 51°, 55 to 56°, and 65 to 66° C.) are compared with those of the unheated 5 per cent extracts from which they were prepared (Table V, left). A fresh animal was used for each in-

jection, with duplicate animals in the 50 to 51° C. series.

Extracts heated to between 50 and 51° C. for twenty minutes and filtered while warm contained from 0.13 to 0.15 per cent protein compared to 0.38 and 0.50 per cent in the original unheated extracts. While the unheated extracts diminished blood pressure (two instances) or produced a questionable rise (one instance), the slightly heated extracts raised blood pressure by amounts ranging from 12 to 35 mm. Hg (Table V, upper right). Nevertheless, skin temperature and pulse amplitude were diminished significantly in at least half the injections, so that it must be concluded that blood flow was decreased slightly.

Those extracts which were heated to between 55 and 56° C. and filtered at or near that temperature, contained from 0.09 to 0.13 per cent protein, compared to 0.24 and 0.51 per cent in the original unheated extracts. On injection, these heated extracts elevated systolic blood pressure by 25 to 35 mm. Hg, without reducing the amplitude of oscillation (Table V, middle right). Heart rate diminished during the pressor response, as is to be expected in intact animals. The extracts were non-toxic, and the animals remained in good condition until sacrificed. The temperature of the ear never fell significantly; on the contrary it remained constant or rose slightly, by amounts which might well result from gradual increase of rectal temperature during the experiment. It seems safe to conclude that suitably heated kidney extracts produce a pressor response without diminishing blood flow in the ear of the heated rabbit. A slight increase in blood flow may occasionally occur, especially with 10 per cent extracts, but the conditions of the present experiments permit no definite conclusions on that point.

Extracts heated to between 65 and 66° C. contained only 0.06 per cent protein and had lost their pressor activity entirely (Table V, lower right). Injection of these extracts reduced blood pressure by 5 or 10 mm. Hg without affecting pulse amplitude. Skin temperature and pulse rate increased by amounts which were to be expected from the gradual elevation of body temperature in the course of each experiment.

Representative experiments are illustrated in Figure 6, which compares the effects of three

FIG. 6. COMPARISON OF THE EFFECTS OF 5 PER CENT SALINE KIDNEY EXTRACTS BEFORE AND AFTER HEATING

Heated extracts (below) were prepared from corresponding unheated extracts (above).

unheated extracts (above) with the effects of the same extracts after heating and filtration at the three temperatures chosen (below). Figure 2 reproduces a typical record from an experiment in which the injection of 20 cc. of heated (55 to 56° C.) 5 per cent kidney extract increased blood pressure by 40 mm. Hg, without affecting pulse amplitude or skin temperature significantly. The contrast between the effects of heated kidney extract (Figure 2, upper record) and those of tyramine (Figure 2, lower record) is striking.

From these experiments, it may be concluded that heating the original kidney extract to between 55 and 56° C. for twenty minutes, with filtration at or near that temperature, (1) reduced the protein content of the extract, (2) reduced toxicity, (3) rendered pressor activity much more constant, and (4) revealed in extracts of normal rabbit kidney the presence of a substance which elevated blood pressure but did not reduce peripheral blood flow as measured by skin temperature.

Heating the original extracts to between 50 and 51° C. produced solutions with properties midway between the unheated material and the 55 to 56° C. extract. Heating to 65° C. reduced the protein content still further and destroyed all pressor activity. Simple dilution of unheated extracts reduced their protein content and diminished the intensity of their action, but did not produce the qualitative changes of action which were observed when the protein content was reduced by heating. Suitable heating apparently removed certain substances responsible for depressor and toxic reactions and left in solution

other substances which elevated blood pressure without diminishing peripheral blood flow.

The protein of heated (55 to 56° C.) kidney extract and its effects on blood pressure and skin temperature

The substance responsible for the pressor activity of kidney extracts or pressed juice does not pass an ultrafilter, and is precipitated by ammonium sulphate (8, 9, 10).

The ultrafiltrates of 5 and 10 per cent heated (55 to 56° C.) kidney extracts were practically inert (Table VI and Figure 7). The original heated extracts elevated blood pressure by 23 to 45 mm. Hg, but their protein-free ultrafiltrates had only a very slight depressor action. Neither the original extracts nor the ultrafiltrates reduced skin temperature or the amplitude of pulsation. The protein-containing extracts produced the cardiac slowing which is associated with rise in blood pressure in the intact animal; the ultrafiltrates had no appreciable effect on heart rate.

A number of 10 per cent heated (55 to 56° C.) extracts were saturated with ammonium sulphate at room temperature as described under "Methods." After dialysis, the volume of the protein solution was made equal to that of the extract originally used for salting out. Some protein was lost in the process so that the protein content of the redissolved precipitate was generally less than that of the original 10 per cent extract. This protein, or associated substance, elevated blood pressure but did not decrease pulse amplitude or reduce skin temperature (Figure 7 and Table VII). The pressor activity of this protein

TABLE VI

Comparison of heated (55 to 56° C.) kidney extract and its ultrafiltrate (20 cc. injected at rate of 1.6 cc. per minute)

Material used	Protein content	Change in			
		Systolic blood pressure	Skin temperature	Pulse amplitude	Heart rate
	<i>grams per cent</i>	<i>mm. Hg</i>	<i>° C.</i>	<i>per cent</i>	<i>beats per minute</i>
5 per cent extract	0.12	+23	+0.6	0	-25
Ultrafiltrate	0.00	0	+0.3	0	0
10 per cent extract	0.17	+45	+1.1	0	-40
Ultrafiltrate	0.00	-10	+0.6	0	+10
10 per cent extract	0.19	+42	+0.4	+10	-70
Ultrafiltrate	0.00	-5	+0.1	0	0

FIG. 7. COMPARISON OF THE EFFECTS OF 10 PER CENT HEATED (55 TO 56° C.) EXTRACTS WITH THOSE OF THE ULTRAFILTRATE, AND THE AMMONIUM SULPHATE PRECIPITATE

TABLE VII

Comparison of heated (55 to 56° C.) kidney extract and ammonium sulphate precipitate in same volume of 0.9 per cent NaCl solution (20 cc. injected at rate of 1.6 cc. per minute)

Material used	Protein content	Change in			
		Systolic blood pressure	Skin temperature	Pulse amplitude	Heart rate
	<i>grams per cent</i>	<i>mm. Hg</i>	<i>° C.</i>	<i>per cent</i>	<i>beats per minute</i>
10 per cent extract	0.16	+30	+0.2	0	-10
Redissolved precipitate	0.15	+22	+1.1	0	-50
10 per cent extract	0.18	+45	-0.2	0	-65
Redissolved precipitate	0.14	+35	+0.6	0	-10
10 per cent extract	0.15	+30	+0.2	0	-20
Redissolved precipitate	0.11	+30	+0.7	0	-80

fraction, like that of the original extract, was destroyed by heating to 65° C. On standing, these solutions of kidney protein again became toxic and resembled unheated kidney extract in their effects on the peripheral circulation. If such solutions were heated to 55 or 56° C. for twenty minutes a faint precipitate appeared; filtration at or near 55° C. produced a clear solution which once more was non-toxic and raised blood pressure without reducing skin temperature. Some protein was lost in the reheating and the intensity of pressor activity was correspondingly reduced. Further studies are being carried out with this protein fraction, which has been dried to a white powder by the lyophile process of Florsdorf and Mudd (11). This treatment offers greater opportunity for storage and repeated use of standard material.

DISCUSSION

In general, injections of fresh saline extracts of normal kidney tissue have produced mixed depressor and pressor effects, the latter predominating according to Tigerstedt and Bergman (8), Bingel and Strauss (9), Prinzmetal and Friedman (12) and Harrison *et al.* (13). Others found that depressor effects were more prominent (10, 14, 15, 16, 17). To some extent the variable results with crude kidney extracts may have been a result of (1) injecting extracts from one species into another species, demonstrated by Pearce (18) and Miller and Miller (19), (2) variation in the thoroughness with which kidney tissue was ground, and (3) the presence in varying amounts of unidentified toxic,

depressor, and pressor substances in the original extract itself, or arising as a result of treatment.

In our experiments only one variety of rabbit, the New Zealand white, was used so that heterologous injections might be avoided. The duration of grinding and the degree of subdivision were also constant. Nevertheless, the centrifuged and filtered unheated saline extracts were often toxic, and produced such variable pressor and depressor effects that no conclusion seemed warranted. On the other hand, the extracts which were heated to between 55 and 56° C., and filtered at or near that temperature, elevated the blood pressure of unanesthetized heated rabbits uniformly and conspicuously. Except for the absence of a preliminary depressor effect, the results agreed with certain successful experiments described by others (8, 9, 12, 13).

Tigerstedt and Bergman (8) gave the name "renin" to a pressor material extracted from the normal rabbit's kidney and showed that the active substance withstood heating to 56° C., was destroyed by boiling, and did not dialyze. Bingel and Strauss (9) found that the addition of ammonium sulphate (between $\frac{1}{3}$ and $\frac{7}{12}$ saturation) to pressed juice of rabbit's kidneys precipitated a protein-like substance which elevated blood pressure, was coagulated and inactivated by heating to 58° C., and was retained by an ultrafilter. Heated kidney extracts, and the protein-like substance salted out from such extracts, resembled "renin" in all these respects. In addition, it has been shown that by appropriate heating, pressor activity can be made more consistent and that

peripheral blood flow is not reduced during the period when blood pressure is elevated.

It is conceivable that heating kidney extract to 55° C. not only precipitates or destroys depressor substances, but also partially denatures a protein constituent which is naturally inactive. Opposed to this explanation is the occasional undoubted pressor activity of untreated extracts of normal kidney tissue. The pressor activity of unheated extracts of both normal and ischemic kidneys (12, 13) also suggests that pressor substances exist in the original extract, and that suitable heating merely reveals their presence. Preliminary experiments indicate that similarly heated extracts of liver, skin and muscle do not elevate the blood pressure of the heated rabbit. Extracts of other tissues and of kidneys from other species are being studied to determine the distribution of this pressor substance which can be separated by suitable heating.

All of the substances which have been studied in our experiments have at some time been considered with relation to their possible rôle in the pathogenesis of human hypertension. The literature covering the proposal and disposal of each substance is too vast to review here. However, the recent production in animals of a persisting experimental hypertension due to renal ischemia (20) has once more directed attention to the kidney as a source of pressor material, particularly since this one form of experimental hypertension is not affected by sympathetic denervation either partial (21, 22), or complete (23).

The qualitative difference between temporary hypertension produced by suitably heated kidney

extract and that produced by the better known pressor drugs is summarized in Table VIII. The average change in skin temperature associated with a 10 mm. rise of systolic blood pressure (in the heated rabbit) has been calculated for each substance. Thus pitressin reduced skin temperature conspicuously and raised blood pressure very little so that the change in skin temperature per unit rise of blood pressure is high (Table VIII). Below pitressin the substances are arranged in a decreasing series with epinephrine at the bottom.

The degree to which skin temperature of the heated animal is reduced with unit increase of blood pressure measures roughly the disproportion between local cutaneous vasoconstriction and the rise of systemic blood pressure. Because the auricular vessels are constricted excessively, blood flow in the ear is reduced in spite of the greater head of arterial pressure. It would appear that if human hypertension were due solely to the action of any of these substances, peripheral blood flow should be much decreased, with manifest peripheral vasoconstriction.

Suitably heated kidney extract, however, elevates blood pressure for thirty to forty-five minutes without reducing auricular blood flow measurably. The effects of heated kidney extract can be explained most easily by assuming a universal and uniform increase of vascular tone. At least in the ear of the heated rabbit, the constancy of blood flow indicates that peripheral resistance and blood pressure rise and fall together by proportionate increments at every stage throughout the pressor response. Information concerning changes of vascular tone in other tissues is lacking except for the statement by Hessel and Maier-Hüser (24) that the vessels of the extremities, intestines, and kidneys as well as excised arteries (9) are constricted by active kidney extracts.

Though suitably heated kidney extracts are unique in that they elevate blood pressure without reducing peripheral blood flow, this pressor response simulates the true hypertensive state only partially. The effects of heated extract are temporary, lasting only thirty to forty-five minutes. According to earlier studies on renin (8, 9) the pressor effect of active kidney extracts becomes less when repeated doses are injected in rapid succession into the same animal, though a full

TABLE VIII
Summary showing average relation between skin temperature and rise of systolic blood pressure

Substance	Change in skin temperature per 10 mm. rise of blood pressure
	°C
Pitressin	-7.0
Methylguanidine	-4.5
Tyramine	-2.3
Dimethylguanidine	-1.9
Ergotamine	-1.7
Ergotoxine	-1.5
Pituitrin S	-1.5
Guanidine	-1.5
Epinephrine	-1.1
Heated (55 to 56°C.) kidney extract	+0.1

response can be obtained again after the lapse of several hours. Further studies with standard extracts or precipitates will be required to determine whether or not the reactivity of vascular smooth muscle is changed by repeated exposure to pressor material obtained from kidney substance.

SUMMARY

An oscillometric method of studying the peripheral vascular responses of the unanesthetized, warmed rabbit is described. Oscillometric determinations of systolic blood pressure in the auricular artery were compared with simultaneous measurements by direct cannulation of the opposite auricular artery. In control experiments, the slow injection of 0.9 per cent NaCl solution produced no significant variations in peripheral circulation.

Epinephrine, tyramine, pituitrin, pitressin, guanidine, methylguanidine, dimethylguanidine, ergotamine and ergotamine elevated blood pressure but always decreased skin temperature with varying degrees of vasoconstriction in the ear of the heated rabbit. The temporary hypertension produced by these substances was associated with a disproportionate increase of peripheral resistance and a decrease of blood flow through the auricular vessels.

"Unheated kidney extracts" elevated blood pressure occasionally, reduced blood pressure more frequently, and always reduced skin temperature. Many of the "unheated kidney extracts" were toxic.

Heating the original saline kidney extracts to between 55 and 56° C. for twenty minutes precipitated considerable protein, and subsequent filtration at or near 55° C. produced a clear yellowish solution which elevated blood pressure without diminishing skin temperature and without reducing the amplitude of arterial pulsation. Peripheral blood flow was not measurably decreased at any time during the pressor response; a slight increase of peripheral blood flow could not be excluded by the methods used.

In agreement with earlier studies, the pressor activity of kidney extracts was destroyed by heating to 65° C. for twenty minutes; the pressor substance did not dialyze or pass through an ultrafilter. Saturation of heated (55 to 56° C.)

extracts with ammonium sulphate yielded a precipitate which, after solution and dialysis, also produced a conspicuous rise of blood pressure without diminishing peripheral blood flow. Certain properties of this material are compared with those of "renin" described by earlier investigators.

Suitably heated kidney extracts, and a protein-like material precipitated from such extracts, were the only pressor substances which elevated blood pressure without decreasing peripheral blood flow, a relationship which is characteristic of certain forms of human hypertension.

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