ANGIOTONIN-ACTIVATOR, RENIN- AND ANGIOTONIN-INHIBITOR, AND THE MECHANISM OF ANGIOTONIN TACHYPHYLAXIS IN NORMAL, HYPERTENSIVE, AND NEPHRECTOMIZED ANIMALS*

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Renin when purified has been shown by Helmer and Page (1939) to be devoid of vasoconstrictor properties. Addition of a protein-like substance separated from blood and tentatively called renin-activator by Kohlstaedt, Helmer, and Page (1938) restores this quality. Page and Helmer (1939–1940) then found that if renin and renin-activator are allowed to interact, a third substance with strong pressor and vasoconstrictor properties was produced, which was called angiotonin. This they showed to be a heat-stable, diffusible substance from which crystalline derivatives, notably picrates and oxalates, could be prepared. It acts directly on peripheral blood vessels to produce constriction. It does not appear to be the end-product of the reaction between renin and renin-activator, for it is itself destroyed by further contact with renin. Unless blood or a 70 per cent acetone-soluble fraction of blood is added to it, loss of vasoconstrictor properties occurs when it is perfused through an isolated organ. The occurrence of an angiotonin-activator is thus indicated. We employ the name, as in the case of renin-activator, to connote merely that neither renin nor angiotonin exerts a pressor action in the absence of their respective activators.

When renin is repeatedly injected intravenously into animals within short periods, the pressor response ceases to be elicited. Such loss of response is called tachyphylaxis. This is due, as Page (1939) has shown, (*a*) to exhaustion of renin-activator in the blood and (*b*) to appearance of an inhibitor. Tachyphylaxis can also be produced in isolated organs such as the rabbit's ear perfused with blood which is recirculated. Here it is due chiefly to exhaustion of renin-activator, for addition of activator restores the vasoconstrictor properties of renin. This contrasts with the circumstances in intact animals made tachyphylactic to renin, where injection of renin-activator with renin does not restore responsiveness. Nor does addition of renin-activator restore vasoconstrictor properties to renin when a mixture of renin and plasma from tachyphylactic animals is injected into blood from a tachyphylactic animal used as the perfusing fluid in a rabbit's ear.

Some evidence suggests that the elevated blood pressure which follows constriction of the renal artery (Goldblatt, Lynch, Hanzal, and Summerville, 1934) or the renal parenchyma (Page, 1939) is due to renin. Indeed

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Kohlstaedt and Page (1940) have been able to make isolated perfused kidneys excrete renin by reducing the pulse pressure in the perfusing system. It is our opinion that angiotonin is the effector substance, originating as it does from interaction of the non-pressor substances, renin and renin-activator. The importance of investigating further the physiological properties of angiotonin is evident. During the course of this study, more evidence than that given by Page (1939) for the occurrence of an inhibitor to angiotonin was accumulated and therefore is included in this communication.

Methods

Dogs and cats were used in all experiments and usually under pentobarbital sodium anesthesia. Some of the animals were pithed under ether and then the ether blown off before the experiment was begun. Blood pressure was measured by a mercury manometer from a cannula inserted into the femoral or carotid artery. Injections were made into a femoral vein.

Renin prepared by the method of Helmer and Page (1939) or angiotonin prepared by that of Page and Helmer (1940) were given, usually by slow infusion from a flask with a calibrated side arm to measure the rate of inflow of the solution. Transfusions were made by collecting the arterial blood from the donor in a vacuum flask containing an adequate amount of sodium citrate solution to prevent coagulation. This blood was injected into the recipient within one-half hour after being drawn. When all the blood had been injected, the renin or angiotonin infusion was started 10 minutes later. The usual routine to make the animal tachyphylactic was to infuse renin or angiotonin solution diluted 1 to 10 with saline at a rate of 0.8 cc. per minute for 60 minutes, then double the rate for the next 15 minutes. Finally 1 cc. of concentrated renin was injected and this was followed by 1 cc. of angiotonin.

Test animals were prepared by performing a bilateral nephrectomy 1 to 4 days before the experiment. These animals are much more sensitive to renin than normal ones as most investigators have found.

Tests for the presence and amount of renin, renin-activator, angiotonin-activator, and inhibitor were made by injecting blood samples with appropriate additions of renin, renin-activator, or angiotonin into Ringer's solution perfused by pulsatile pressure through an isolated rabbit's ear.

Chronic hypertension was produced in the dogs by the perinephritis method of Page (1939).

RESULTS

Tachyphylaxis to Angiotonin.—Tachyphylaxis to angiotonin develops slowly in sharp contrast to renin. For example, angiotonin solution was injected repeatedly into a pithed cat, each injection following on the other as soon as the blood pressure had returned to the initial level with the following rises in arterial pressure: 38, 32, 34, 38, 38, 34, 38, 32, 26, 22, 20, 16, 14, 12, 14, 12, renin 2, angiotonin 6, 4, adrenalin 60 mm. Hg.

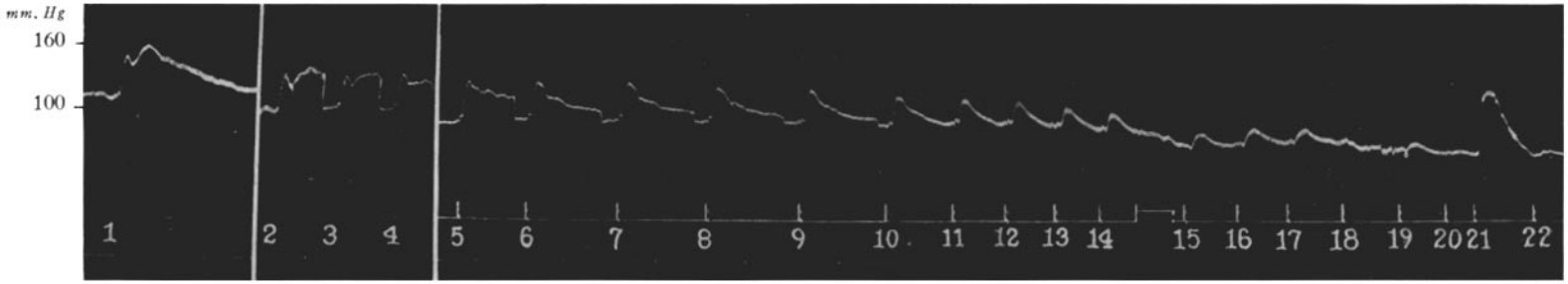

FIG. 1. Effect of repeated injections of angiotonin on arterial pressure of a cat anesthetized with ethyl urethane. Angiotonin was given in single doses (0.1 cc.) from 1 to 18 as indicated by the signal marker. At No. 19 renin 0.3 cc. was given. At No. 20 angiotonin, No. 21 adrenalin (0.5 cc. 1:100,000), and No. 22 angiotonin. Time marker 6 seconds.

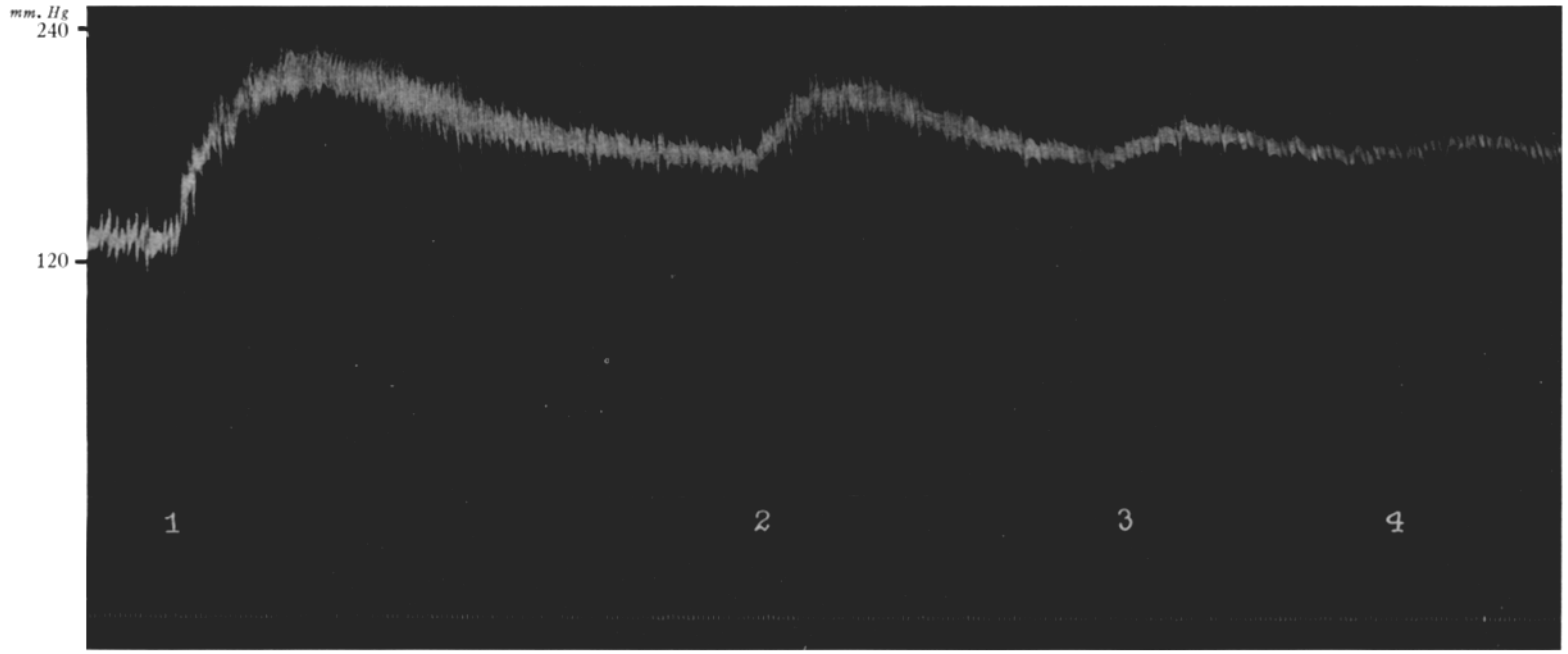

FIG. 2. Effect of repeated injections of renin on arterial pressure of a cat anesthetized with ethyl urethane. The same dose of renin (0.2 cc.) was given 4 times. Time marker 6 seconds.

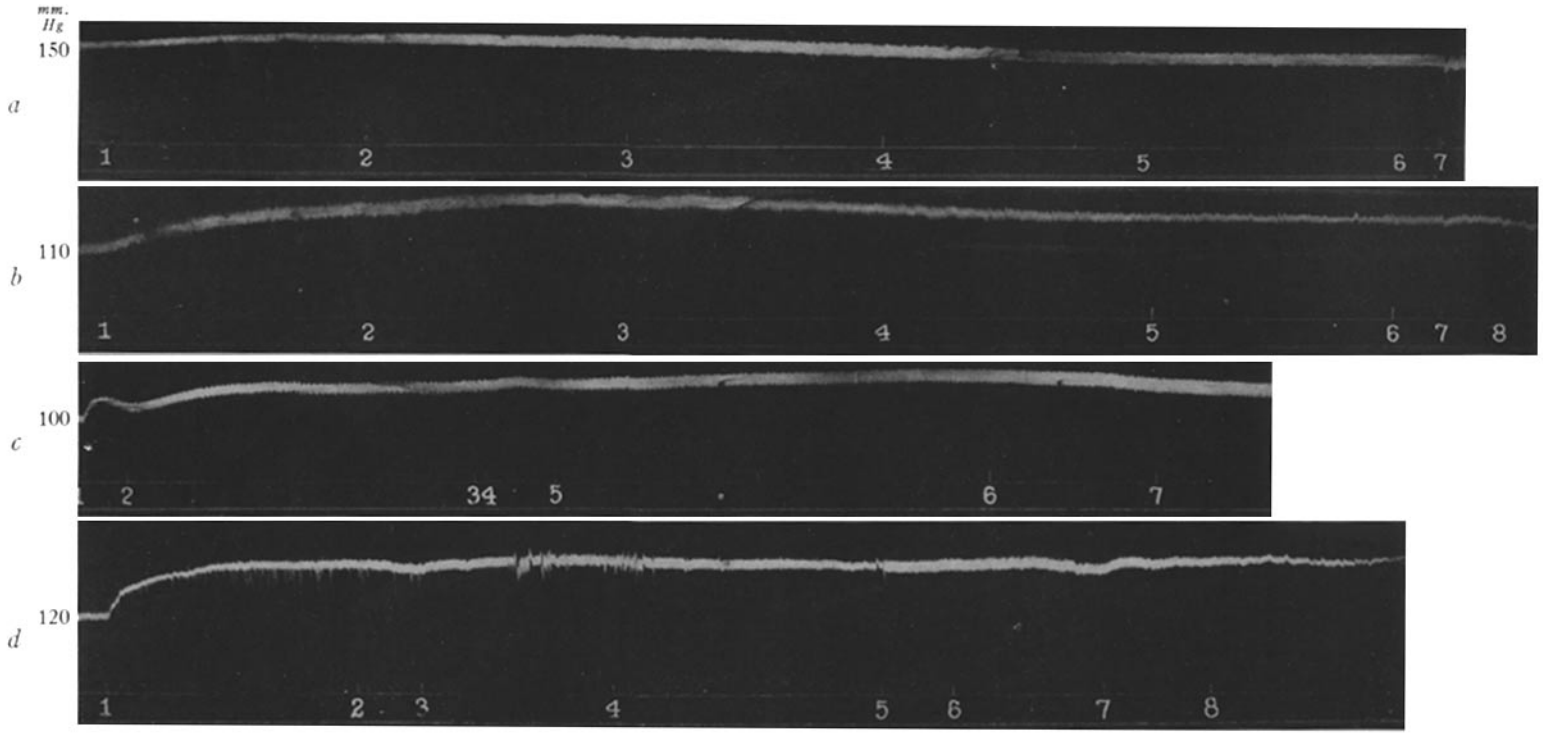

FIG. 3

(*a*) *Normal Dog.*—(*1*) Renin infusion. (*2*) 15 minutes. (*3*) 30 minutes. (*4*) 45 minutes. (*5*) 60 minutes. (*6*) 75 minutes. (*7*) Renin 1 cc.

(*b*) *Nephrectomized Dog.*—(*1*) Renin infusion. (*2*) 15 minutes. (*3*) 30 minutes. (*4*) 45 minutes. (*5*) 60 minutes. (*6*) 75 minutes. (*7*) Renin 1 cc. (*8*) Angiotonin 1 cc.

(*c*) *Nephrectomized Dog.*—(*1*) Angiotonin 0.6 cc. (*2*) Transfusion of renin-tachyphylactic blood (250 cc.). (*3*) After 25 minutes. (*4*) Angiotonin 0.6 cc. (*5*) Renin infusion started 0.8 cc. per minute. (*6*) 30 minutes. (*7*) 45 minutes. Renin and angiotonin tachyphylaxis in a nephrectomized dog following transfusion of renin-tachyphylactic blood.

(*d*) *Nephrectomized Dog.*—(*1*) Angiotonin infusion 0.8 cc. per minute. (*2*) 15 minutes. (*3*) Increased to 0.9 cc. per minute. (*4*) 30 minutes. (*5*) 45 minutes. (*6*) 1.0 cc. per minute. (*7*) 1.5 cc. per minute. (*8*) 65 minutes.

Definite loss of responsiveness develops, although slowly. Large amounts of angiotonin are required. If large doses of renin are administered quickly, the response to angiotonin is abolished. Contrariwise, the response to renin is much weakened after large doses of angiotonin. When the pressor action of angiotonin is lost, that of renin also disappears. If renin-activator is exhausted, renin may fail to cause vasoconstriction, but angiotonin continues to do so.

In short, tachyphylaxis for renin develops much more quickly than for angiotonin, and under most circumstances establishment of renin tachyphylaxis abolishes the response to angiotonin.

The Occurrence of Angiotonin-Activator

Angiotonin was injected into Ringer's solution perfusing a rabbit's ear (Table I). The effect was to cause immediate constriction of the vessels with reduction of drop rate. The next injection, however, caused no vasoconstriction nor did a third. Injection of normal heparinized dog's blood alone caused no constriction, but if blood and angiotonin were mixed and injected together, marked constriction again occurred. This phenomenon could be repeated many times. Renin-activator had no activating effect on angiotonin.

These results suggested that some substance was contained in blood which was necessary for angiotonin to exert repeatedly its effect. It has been observed that the initial dose of angiotonin into the rabbits' ears always produced a response. Even long continued perfusion with Ringer's solution did not prevent it. Enough of the activator may be present in the cells of the blood vessels to allow the angiotonin to act initially, but it is apparently quickly exhausted and only restored by addition of blood or of a 70 per cent acetone extract of blood. If, instead of perfusing the rabbit's ear with Ringer-Locke's solution, normal blood is substituted and the blood washed out of the ear with fresh blood, it is doubtful whether tachyphylaxis develops.

When the blood is recirculated, tachyphylaxis quickly occurs and addition of fresh blood restores the lost activity of angiotonin. That it is the recirculated blood which has lost its ability to activate angiotonin and not inability of the blood vessels of the ear to respond is demonstrated by perfusing the blood through a second ear. Addition of angiotonin causes no significant constriction until more normal blood is added. Angiotonin plus samples of tachyphylactic recirculated blood added to Ringer's solution perfusing a rabbit's ear fail to cause vasoconstriction, whereas angiotonin added to normal blood does so.

TABLE I

Development of Angiotonin Tachyphylaxis in Perfused Rabbits' Ears

Source of perfusing fluid	Substance injected	Initial drop Rate per min.	Reduction of flow Time in min.	Per cent reduction of flow
	First Ear			
Normal dog's blood diluted 1:1 with Ringer's solution	0.5 cc. angiotonin	40	5	50
	0.5 cc. angiotonin	38	5	46
	0.5 cc. angiotonin	35	5½	49
	1.0 cc. angiotonin	32	9	56
	0.5 cc. angiotonin	34	5	50
	0.5 cc. angiotonin	32	5½	47
	0.5 cc. angiotonin	29	6	51
	Blood recirculated			
	0.5 cc. angiotonin	31	1	26
	0.5 cc. angiotonin	30	½	12
	0.5 cc. angiotonin	28	0	0
	0.5 cc. angiotonin	26	0	0
	0.5 cc. angiotonin + 5 cc. normal blood	28	5	57
	0.5 cc. angiotonin	28	0	0
	Second Ear			
Blood from first ear after reperfusion	0.5 cc. angiotonin	43	1	10
	0.5 cc. angiotonin	40	0	0
	0.5 cc. angiotonin + 2 cc. normal blood	42	2	39
Ringer-Locke's solution	0.5 cc. angiotonin	99	1	51
	0.5 cc. angiotonin	96	¼	11
	0.5 cc. angiotonin	94	0	0
	0.5 cc. angiotonin + 0.5 cc. reperfused blood	97	½	10
	0.5 cc. angiotonin + 0.5 cc. normal blood	97	5	60
	Third Ear			
Ringer-Locke's solution	1.0 cc. angiotonin	75	2	46
	1.0 cc. angiotonin	76	1	40
	1.0 cc. angiotonin	74	0	0
	0.1 cc. normal blood	72	0	0
	0.1 cc. normal blood + 1.0 cc. angiotonin	71	6	59
	0.2 cc. activator	70	0	0
	0.2 cc. activator + 1.0 cc. angiotonin	69	1½	27
	1.0 cc. angiotonin	70	½	11
	1.0 cc. angiotonin	70	0	0

Preparation of Angiotonin-Activator

Angiotonin-activator was prepared from ox serum by precipitating with potassium phosphate at pH 6.5. The greatest concentration of the activator is found in the

fraction precipitated by a concentration of phosphate up to 1.5 molar. The precipitate is freed of phosphate and other salts by dialysis against physiological saline. This fraction can be further purified by adding acetone to a concentration of 70 per cent and, after filtering, the precipitate is discarded. The filtrate is concentrated under reduced pressure to about one-fourth its original volume and the angiotonin-activator fraction precipitated by addition of 10 volumes of acetone. The mixture is allowed to stand in the refrigerator until the precipitate has settled, the supernatant fluid is decanted, the precipitate collected on a filter and washed with acetone. It is then dissolved in physiological saline.

Angiotonin-activator was prepared from laked ox red blood cells by the same technique, but without preliminary precipitation with phosphate. It has also been prepared from laked red blood cells by a method similar to that employed by Warburg and Christian (1935) for co-ferment.

Development of Angiotonin Tachyphylaxis in Cats and Its Demonstration on the Vessels of Rabbits' Ears

Angiotonin was infused into the femoral vein of a cat anesthetized with pentobarbital until, after a prolonged rise, the blood pressure began to fall. Blood samples from this animal were tested in rabbits' ears perfused with Ringer's solution (Table II).

Small amounts (0.1 cc.) of normal blood added to angiotonin produced marked vasoconstriction, but after the animal had received an infusion of angiotonin the same amount of blood did not restore its vasoconstrictor properties. In short, as angiotonin tachyphylaxis develops, the ability of blood to restore the pressor action of angiotonin in perfused organs is markedly reduced. It should be noted that even though the blood pressure in the animal receiving the infusion on angiotonin was falling, response was still elicited when concentrated angiotonin was injected. Evidently the fall in blood pressure after long continued infusion is not alone due to development of angiotonin tachyphylaxis.

Increased Sensitivity to Angiotonin and Renin after Bilateral Nephrectomy

The following example demonstrates the markedly increased pressor response to angiotonin and renin induced by bilateral nephrectomy.

A cat was anesthetized with pentobarbital and two test doses of angiotonin given. The responses were both 36 mm. Hg rise of pressure and that for renin was 52 mm. Hg. Both kidneys were removed and 24 hours later the animal was retested. The response to angiotonin at this time was 48 mm. Hg and to renin 80 mm. Hg. The blood pressure remains elevated roughly 4 to 10 times as long as when the kidneys were present.

Dogs behaved similarly though the hypertension tends to persist even longer.

TABLE II

Development of Angiotonin Tachyphylaxis by Infusion of Angiotonin into a Cat

	B.P.	Blood sample	Rabbit's ear perfused with Ringer's solution			
			Material injected	Initial drop Rate per min.	Reduction of flow Time in min.	Per cent reduction of flow
			First Ear			
	mm. Hg					
Initial level	154	No. 1	0.5 cc. angiotonin	119	2	67
			0.5 cc. angiotonin	121	0	0
			0.5 cc. angiotonin	118	0	0
Angiotonin* infused 1 cc. per min. for 10 min.	172		0.1 cc. blood No. 1	119	0	0
			0.5 cc. angiotonin	120	0	0
After 15 min. infusion	170		0.1 cc. blood No. 1 + 0.5 cc. angiotonin	118	3	79
After 30 min. infusion	168		0.1 cc. blood No. 1	119	0	0
			0.1 cc. blood No. 2	120	0	0
After 45 min. infusion	166		0.1 cc. blood No. 2 + 0.5 cc. angiotonin	122	1	30
After 60 min. infusion	148		0.1 cc. blood No. 2 + 0.5 cc. angiotonin	120	½	26
After 75 min. 2 cc. per min.	148	No. 2	0.1 cc. blood No. 1 + 0.5 cc. angiotonin		12	74
0.6 cc. undiluted angiotonin	118–130		0.5 cc. angiotonin	120	0	0
0.3 cc. renin	152–152	No. 3				
			Second Ear			
			0.5 cc. angiotonin	130	2	48
			0.5 cc. angiotonin	129	½	20
			0.5 cc. angiotonin	128	0	0
			0.5 cc. angiotonin + 0.1 cc. blood No. 1	130	5	89
			0.5 cc. angiotonin + 0.1 cc. blood No. 2	133	1	20
			0.5 cc. angiotonin + 0.1 cc. blood No. 3	134	½	12
			0.5 cc. angiotonin + 0.1 cc. blood No. 2	135	½	26
			0.5 cc. angiotonin + 0.1 cc. blood No. 3	136	0	0
			0.5 cc. angiotonin + 0.1 cc. blood No. 2	138	½	20
			0.5 cc. angiotonin + 0.1 cc. blood No. 1	140	4	71
			0.5 cc. angiotonin	141	0	0

* Angiotonin 1:10 dilution.

Two dogs responded to injection of renin with rises of 18 and 19 mm. Hg respectively. Bilateral nephrectomy was performed; 3 hours later the responses were 20 and 32 mm. Hg and 48 hours later 36 and 40 mm. Hg. These along with several other such experiments (Table VI) indicate that the maximum increase in response is observed within 24 to 36 hours.

TABLE III

Increased Activating Effect of Blood from Nephrectomized Dog on Angiotonin

Ringer's solution perfused through ear			
Material injected	Initial drop Rate per min.	Reduction of flow Time in min.	Per cent reduction of flow
First Ear			
0.3 cc. angiotonin	149	3	56
0.3 cc. angiotonin	141	2	29
0.3 cc. angiotonin	140	0	0
0.3 cc. angiotonin + 0.3 cc. normal blood	137	4	51
0.3 cc. angiotonin + 0.3 cc. blood from nephrectomized dog	140	8	86
0.3 cc. angiotonin + 0.3 cc. blood from nephrectomized dog transfused with normal blood	136	2	39
Second Ear			
0.3 cc. angiotonin	129	3	42
0.3 cc. angiotonin	121	1	29
0.3 cc. angiotonin	120	½	18
0.3 cc. angiotonin	120	0	0
0.1 cc. angiotonin + 0.2 cc. normal blood	117	2	46
0.1 cc. angiotonin + 0.2 cc. blood from nephrectomized dog	119	5	70
0.1 cc. angiotonin + 0.3 cc. normal blood	120	3	49
0.1 cc. angiotonin + 0.4 cc. normal blood	120	4	56
0.1 cc. angiotonin + 0.2 cc. blood from nephrectomized dog	121	5	62
0.1 cc. angiotonin + 0.5 cc. normal blood	119	5	57

Infusion of angiotonin into nephrectomized dogs causes great elevation of blood pressure, which persists for several hours (Fig. 3*d*).

For example, the initial pressure of a dog was 118 mm. Hg. 15 minutes after the angiotonin infusion was started, the blood pressure had risen to 198 mm., after 30 minutes 205 mm., 60 minutes 200 mm., 80 minutes 201 mm., and 115 minutes 182 mm. Hg. After an hour or more, increasing the rate of infusion produced little further rise of pressure. When the infusion was stopped the arterial pressure fell quickly to 155 mm. Hg.

To ascertain whether angiotonin-activator was still present in the blood and hence

TABLE IV

Development of Angiotonin Tachyphylaxis in Dog and Transfusion of the Tachyphylactic Blood into a Bilaterally Nephrectomized Dog

Source of perfusing fluid	Substance injected	Initial drop Rate per min.	Reduction of flow Time in min.	Per cent reduction of flow	Blood pressure of dog (1) receiving angiotonin	Time after infusion begun
		Perfused rabbit's ear				
						min.
Blood from normal dog after infusion of angiotonin	0.5 cc. angiotonin	47	½	20	142	0
	0.5 cc. angiotonin	42	0	0	156	10
	0.5 cc. angiotonin + 0.5 cc. normal blood	41	0	0	152	15
	0.5 cc. renin	40	0	0	156	30
	0.5 cc. renin activator	41	0	0	160	40
	0.5 cc. renin + renin activator	42	1½	27	146	50
					142	60
					135	70
Blood from nephrectomized dog	0.5 cc. angiotonin	36	23	91		
		Second rabbit's ear perfused with same blood				
Same blood	0.4 cc. angiotonin	25	40	100		
Blood from nephrectomized dog after transfusion of tachyphylactic blood	0.5 cc. angiotonin	39	6	61		
	0.5 cc. angiotonin	32	7	56		
	0.5 cc. angiotonin	30	6	53		
					(2) Receiving transfusion	
Blood from nephrectomized dog after transfusion with 250 cc. tachyphylactic blood and infusion of angiotonin	0.4 cc. angiotonin	21	½	22	118	0
	0.4 cc. angiotonin	23	0	0	138	10
	0.4 cc. angiotonin + 0.4 cc. normal dog's blood	23	0	0	142	20
					(3) Receiving angiotonin	
	0.5 cc. renin	24	1	28	170	5
	0.5 cc. renin + 0.5 cc. activator	22	½	21	172	15
					167	30
	0.5 cc. angiotonin	21	½	17	170	45
					172	60
	0.5 cc. angiotonin	22	0	0		

TABLE IV—*Concluded*

Source of perfusing fluid	Substance injected	Initial drop Rate per min.	Reduction of flow Time in min.	Per cent reduction of flow	Blood pressure of dog (3) receiving angiotonin	Time after infusion begun
		Fresh ear				
						min.
Blood from nephrectomized dog after angiotonin infusion	0.5 cc. angiotonin	32	3	48		
	0.5 cc. angiotonin	29	½	20		
	0.5 cc. angiotonin	26	0	0		
	0.5 cc. angiotonin + 0.2 cc. blood from nephrectomized dog	27	0	0		
	0.5 cc. angiotonin + 0.4 cc. blood from nephrectomized dog	29	1	31		
	0.5 cc. angiotonin + 0.6 cc. blood from nephrectomized dogs	30	3	40		
	0.5 cc. angiotonin	27	0	0		
	0.5 cc. angiotonin + 0.6 cc. normal blood	29	0	0		
	0.5 cc. angiotonin + 0.8 cc. blood from nephrectomized dog	30	6	42		
	0.5 cc. angiotonin + 0.6 cc. blood from nephrectomized dog drawn 1 hr. later	36	10	94		

could still cause vasoconstriction in conjunction with angiotonin, a sample of blood was removed from the animal after 60 minutes of angiotonin infusion. Tachyphylaxis to angiotonin was established in a rabbit's ear perfused with Ringer-Locke's solution and angiotonin plus blood then injected into the perfusing fluid. Angiotonin (0.5 cc.) caused no constriction, while angiotonin (0.3 cc.) plus normal dog's blood (0.3 cc.) reduced the flow 62 per cent for 3 minutes. Blood from the nephrectomized dog receiving angiotonin infusion, however, reduced the flow 94 per cent for 25 minutes. A second example is given in Table III.

It is evident that far more than the usual ability to activate angiotonin is possessed by the blood of nephrectomized animals, despite the infusion of angiotonin and the preternaturally high arterial pressure.

Blood removed from the animal after 115 minutes of infusion and after the blood pressure had begun to fall exhibited roughly 25 per cent of the original activating ability for angiotonin.

After the angiotonin infusion was stopped and the blood pressure had fallen from 188 to 155 mm. Hg, an infusion of renin was started. Within 5 minutes the blood pressure

rose to 177 mm., within 15 minutes to 194, and after 60 minutes was still 194 mm. Hg. Blood removed at this point for assay in the rabbit's ear showed that ability to activate angiotonin was still present though reduced to 50 per cent of the initial value. A second infusion of angiotonin reduced it again to less than 15 per cent of the original level.

The ability of blood to supply activator for renin was also determined. The angiotonin infusion caused no significant change in the amount of renin-activator, but the infusion of renin reduced it below the limits of accuracy of the method of assay.

An excess of renin was present in the blood since addition of purified renin-activator caused roughly four times as much vasoconstriction as was caused by blood taken at the start of the experiment.

Development of Angiotonin Tachyphylaxis in Normal Dogs and Transfusion of the Tachyphylactic Blood into Bilaterally Nephrectomized Dogs

Angiotonin was infused into normal dogs until, after a prolonged rise of arterial pressure, a decrease occurred. A sample of this blood was then used for perfusing a rabbit's ear (Table IV). Injection of angiotonin into the blood caused little or no vasoconstriction, demonstrating that tachyphylaxis was not lost when the blood was transferred from the intact animal to the isolated, perfused organ. Addition of 0.5 cc. of normal dog's blood to the angiotonin caused no constriction, but if 0.2 cc. of blood from a nephrectomized dog was added, constriction occurred (42 per cent reduction of flow for 3 minutes). Renin plus renin-activator also caused no significant constriction. If for the tachyphylactic blood, blood from a bilaterally nephrectomized dog was substituted, then angiotonin caused intense constriction, far greater than that associated with perfusion of blood from normal animals.

Transfusion of 250 cc. of citrated blood from the normal animal made tachyphylactic with angiotonin into a nephrectomized animal appeared to reduce, but not to abolish, the pressor response to infusion of angiotonin.

Thus, in one nephrectomized animal the initial pressure after transfusion from the tachyphylactic normal dog was 151 mm. Hg. Infusion of angiotonin elevated the arterial pressure to 160 mm. after 5 minutes but it had returned to 151 mm. after 15 minutes and 126 mm. after 30 minutes. Under the same conditions a nephrectomized animal without having had a transfusion of tachyphylactic blood exhibited the following rise of blood pressure: initial pressure 104 mm., after 15 minutes 166 mm. and after 30 minutes 146 mm. Hg. In another experiment blood from a bilaterally nephrectomized dog was taken 10 minutes after an infusion of angiotonin was begun. The arterial pressure was high, showing that the animal was responding actively to angiotonin. Transfusion of 300 cc. of blood from this dog into a normal dog did not alter the response of the recipient to single injections of angiotonin.

Blood removed after the transfusion for perfusion in the rabbit's ear exhibited markedly reduced vasoconstrictor properties when angiotonin was added to it—roughly 20 per cent of the response expected from blood of a nephrectomized animal not having received a transfusion.

After infusion of angiotonin the blood produced no pressor response when either angiotonin or renin plus renin-activator was injected into it. Addition of the same amount of blood (0.2 cc.), which had partially restored activity when injected with angiotonin into normal blood made tachyphylactic with angiotonin, no longer did so. 3 times as much (0.6 cc.) was required to produce the same degree of vasoconstriction while the same amount of normal blood had no effect. One hour after the blood sample was drawn the response in the rabbit's ear after addition of blood from a nephrectomized animal to angiotonin was more than twice as great.

Estimation of the Amount of Angiotonin and Renin-Activator in Blood of Normal and Nephrectomized Dogs before and after Transfusion

The amount of angiotonin or renin-activator present in the blood was determined by mixing a known amount of angiotonin with varying amounts of blood and injecting the solution into the Ringer-Locke's solution perfusing a rabbit's ear. Renin-activator was determined by mixing blood with renin in the proportion of 17 to 1—the proportion found by Page (1939) to yield satisfactory pressor response—and allowing the solution to incubate for 10 minutes. Since this method has proved of great usefulness, a sample protocol is given in its entirety (Table V).

In this experiment transfusion of blood from the normal animal appeared to reduce the response of the nephrectomized dog to renin.

The titration illustrated in Table V shows (*a*) the great increase in ability of blood from nephrectomized animals to activate angiotonin and renin over normal blood, (*b*) that injection of small amounts of renin, while markedly elevating blood pressure, does not greatly reduce the ability of blood to activate angiotonin: activation of renin is depressed, (*c*) that transfusion of normal blood reduces angiotonin activation, (*d*) that further renin injections cause no change in the already reduced angiotonin and renin activation, and (*e*) that transfusion of renin-tachyphylactic blood from a nephrectomized to a normal dog abolishes the power of the blood of the recipient to activate renin.

Transference of Inhibitor from Normal and Renin-Tachyphylactic Dogs to Nephrectomized Dogs by Transfusion

Dogs were anesthetized with pentobarbital (30 mg. per kg. body weight) and the arterial pressure recorded from a femoral artery. A standard dose of renin was given. 7 hours later one of two procedures was followed. After anesthetization (15 mg. per

TABLE V

Estimation of the Amount of Angiotonin and Renin-Activator in Blood of Normal and Nephrectomized Dogs before and after Transfusion

Material injected	Blood sample No.	Initial drop Rate per min.	Reduction of flow Time in min.	Per cent reduction of flow
0.5 cc. angiotonin		142	3	61
0.5 cc. angiotonin		139	1	40
0.5 cc. angiotonin		140	0	0
0.1 cc. angiotonin + 0.1 cc. normal blood	6	142	1	28
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	1	140	3	42
0.1 cc. angiotonin + 0.2 cc. nephrectomized blood	2	143	5	46
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	3	139	4	43
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	3	134	4	41
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	4	132	1	26
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	1	130	5	50
0.1 cc. angiotonin + 0.3 cc. normal blood	6	129	3	47
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	1	130	4	41
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	2	127	4	42
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	5	129	1	36
0.1 cc. angiotonin + 0.3 cc. nephrectomized blood	4	130	3	39
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	1	128	5	53
Second Rabbit's Ear				
Renin + 0.5 cc. normal blood	6	129	2	38
Renin + 0.5 cc. nephrectomized blood	1	127	4	46
Renin + 0.5 cc. nephrectomized blood	2	124	½	20
Renin + 0.5 cc. nephrectomized blood	3	127	1	26
Renin + 0.5 cc. nephrectomized blood	4	130	1½	21
Renin + 0.5 cc. nephrectomized blood	5	132	2	29
Renin + 0.5 cc. normal blood	7	134	0	0
Renin + 0.5 cc. nephrectomized blood	1	132	4	51
Renin + 0.5 cc. normal blood	6	127	2	42
Third Rabbit's Ear				
0.5 cc. angiotonin		139	4	48
0.5 cc. angiotonin		131	1	30
0.5 cc. angiotonin		136	0	0
0.1 cc. angiotonin + 0.1 cc. normal blood	6	132	3	47
0.1 cc. angiotonin + 0.1 cc. normal blood	7	131	1	31
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	4	135	4	40
0.1 cc. angiotonin + 0.1 cc. nephrectomized blood	1	135	10	86

Conditions under Which Blood Samples Were Removed for Testing

No. 1, from nephrectomized dog at start of the experiment.

No. 2, after injection of 0.1 cc. of renin. Blood pressure had risen 62 mm. Hg.

No. 3, after 150 cc. of normal blood had been transfused into the nephrectomized dog.

No. 4, after total of 300 cc. of normal blood had been transfused.

No. 5, after 2 more injections of 0.1 cc. each of renin. The blood pressure rose 18 mm. after the first and 8 mm. Hg after the second injection.

No. 6, from normal dog before start of the experiment.

No. 7, after normal dog had received a transfusion (300 cc.) from the nephrectomized dog made tachyphylactic to renin. Injection of 0.1 cc. of renin elevated blood pressure only 14 mm. while the same amount elevated it 62 mm. Hg in the nephrectomized animal.

kg. body weight of pentobarbital), either a second dose of renin was administered or a transfusion of fresh citrated dog's blood (275 to 500 cc.) was given, followed within 5 to 10 minutes by the test dose of renin.

A gradual increase in sensitivity to renin was observed for 24 to 30 hours after bilateral nephrectomy. As no further increase was observed the experiments were begun after 24 hours.

TABLE VI

The Effect of Transfusion of Normal and Tachyphylactic Blood on the Pressor Response of Nephrectomized Dogs to Injection of Renin

No.	Day after nephrectomy	Response to 0.1 cc. renin	Transfusion 275 cc. blood	Rise of B.P. during transfusion	Time after initial renin injection	Response to 0.1 cc. renin
		mm. Hg		*mm. Hg*	*hrs.*	*mm. Hg*
6-87	2	57	—	—	7	42
6-89	2	36	Normal	14	7	18
7-13	1	70	Normal (500 cc.)	0	7	34
7-14	1	48	Normal (500 cc.)	36	7	20
	3	36	Normal	6	7	32
7-04	2	62	—	—	7	52
7-04	3	54	Normal	68	7	28
7-05	1	52	Normal	−3	6	24
7-07	1	62	—	—	6	30
	2	76	Normal	34	6	20
	3	84	—		7	68
7-10	1	46	Normal	20	7	16
7-11	1	44	—		7	34
6-98	1	38	Tachyphylactic*	40	6	20
6-98	2	62	Normal	4	6	42
6-94	1	58	Tachyphylactic	66	6	0
6-95	1	56	Normal	10	6	74
6-95	3	52	—			
6-97	1	50	—		6	30
6-97	3	58	Tachyphylactic†	24	6	24

* Made tachyphylactic with 3 single injections of 0.1 cc. of renin.

† Partially tachyphylactic, 2 single injections of 0.1 cc. of renin.

As a control it was necessary to show that transfusion of blood by itself caused no change in response to renin. In eight experiments cross transfusions were carried out between two bilaterally nephrectomized dogs for periods up to 4 days after the operation. The response to renin was not significantly altered by the transfusion of blood from a nephrectomized dog into a nephrectomized recipient.

The results show that transfusion of normal blood into nephrectomized animals markedly reduces their responsiveness to renin, especially when large amounts (500 cc.) of blood are transfused and the experiment is performed during the first day or two after nephrectomy. In about 30 per

cent of the experiments in which transfusion is given on the 3rd day, no significant reduction in response to renin is observed.

Transfusion of blood from animals made tachyphylactic to renin usually abolishes completely the response to injections of renin into the recipient. This is probably due not only to transfusion of inhibitor but to the occurrence in the transfused blood of excess of renin. This can be easily demonstrated by adding renin-activator to the plasma and testing on a perfused rabbit's ear.

Transfusion of renin-tachyphylactic blood also reduces the response of the recipient to angiotonin. For example in one nephrectomized dog, angiotonin elevated arterial pressure 26 mm. Hg. After transfusion the same amount caused a rise of only 4 mm. Hg.

The Effect of Renin and Angiotonin on the Vasoconstrictor Action of Blood from Nephrectomized and Hypertensive Dogs before and after Transfusion with Blood from Normal Dogs and Dogs Made Tachyphylactic with Renin

The effect of transfusion of normal dogs' blood on the ability of blood of the nephrectomized recipient to cause vasoconstriction when renin was added to it and the mixture perfused through a rabbit's ear was studied in six experiments. After the initial test dose of renin, the response was roughly halved. 7 hours later, and before the transfusion was given, the response increased but usually not to its original level. Transfusion again reduced the response and the second test dose of renin reduced it still further. To illustrate a typical experiment:

0.1 cc. of plasma at the beginning of the experiment when renin was added and incubated 10 minutes caused constriction of 72 per cent lasting 3.5 minutes. The test dose of renin elevated blood pressure 70 mm. Hg and 10 minutes after its administration the plasma treated as described caused 36 per cent constriction lasting 1.5 minutes. 7 hours later the plasma caused 91 per cent constriction lasting 3.5 minutes. After transfusion of 500 cc. of normal blood, the constriction was reduced to 40 per cent lasting 1.5 minutes. Following the second test dose of renin, which elevated blood pressure 34 mm. Hg, the constriction caused by plasma-renin was 21 per cent lasting one minute.

After transfusion with blood (250 cc.) from a normal animal made tachyphylactic by infusing renin, the response of the blood sample plus renin is negligible, though the response to angiotonin may be retained. In other experiments the response to angiotonin is also lost.

After infusion of renin into the recipient, the blood sample removed from the dog causes no vasoconstriction when either renin or angiotonin is added to it.

TABLE VII

Transfusion of Renin-Tachyphylactic Blood into Nephrectomized and Hypertensive Dogs

	B.P.	Amount transfused	Blood sample	Rabbit's ear perfused with Ringer's solution			
				Material injected	Initial drop Rate per min.	Reduction of flow Time in min.	Per cent reduction of flow
Nephrectomized Dog (bilateral nephrectomy 2 days before)							
	mm. Hg	*cc.*					
Initial level before transfusion	146	150	No. 1	0.5 cc. blood No. 1	92	0	0
				0.5 cc. blood No. 1 + 0.1 cc. renin	90	3	76
Initial level after transfusion	171		No. 2	0.5 cc. blood No. 2 + 0.1 cc. renin	91	½	14
				0.1 cc. blood No. 1 + 0.3 cc. angiotonin	91	3	84
5 min. after renin infusion	+9			0.1 cc. blood No. 2 + 0.3 cc. angiotonin	90	2½	90
				0.3 cc. angiotonin	87	0	0
15 min. after renin infusion	+17		No. 3	0.5 cc. blood No. 3 + 0.1 cc. renin	91	0	0
				0.2 cc. blood No. 3 + 0.3 cc. angiotonin	93	0	0
				0.2 cc. blood No. 1 + 0.3 cc. angiotonin	92	6	87
				0.2 cc. blood No. 3 + 0.3 cc. angiotonin	95	0	0
Hypertensive Dog (cellophane perinephritis, unanesthetized, B.P. = 216 mm. Hg)							
Initial level before transfusion	140	300	No. 1	0.5 cc. blood No. 1	130	0	0
				0.5 cc. blood No. 2 + 0.1 cc. renin	129	0	0
				0.5 cc. blood No. 2 + 0.1 cc. renin	130	0	0
Initial level after transfusion	210		No. 2	0.5 cc. blood No. 1 + 0.1 cc. renin	121	3	63
				0.1 cc. blood No. 2 + 0.2 cc. angiotonin	118	3	47
				0.1 cc. blood No. 1 + 0.2 cc. angiotonin	119	3	79
5 min. after renin infusion	−10			0.5 cc. blood No. 3 + 0.1 cc. renin	118	0	0
				0.5 cc. blood No. 1 + 0.1 cc. renin	120	4	76
15 min. after renin infusion	−24			0.1 cc. blood No. 3 + 0.2 cc. angiotonin	120	½	11
				0.2 cc. angiotonin	120	0	0
30 min. after renin infusion	−50						
45 min. after renin infusion	−56						
60 min. after renin infusion	−58						
75 min. after infusion stopped	−74						
Angiotonin 2 cc.	0						
Renin 1 cc.	0		No. 3				

Transfusion of larger amounts (300 cc.) of renin-tachyphylactic blood into a dog made hypertensive (216 mm. Hg mean pressure) by cellophane perinephritis abolishes the vasoconstrictor response anticipated when renin is added to the blood sample. Angiotonin still produces vasoconstriction. But if renin is infused—and in this case marked fall of arterial pressure rather than rise occurred—the response to angiotonin is lost as well.

Renin-Tachyphylactic or Normal Dog's Blood Does Not Reduce Blood Pressure Elevated by a Single Injection of Renin into a Nephrectomized Dog

A normal dog was made tachyphylactic to renin by infusing renin for 75 minutes. A single injection (0.1 cc.) of renin was then given a nephrectomized dog and after the blood pressure had become elevated and showed no further tendency to change, blood (300 cc.) from the renin-tachyphylactic dog was transfused. The blood pressure of the recipient rose moderately (46 mm. Hg). After 2½ hours there was little tendency for it to fall from its elevated level, nor did it respond to further injections of renin. An attempt was made to reduce it with choline and histamine, but, except for the temporary sharp fall directly following the injection, within a short period it had resumed its former level.

Tests for activator in the rabbit's ear showed the usual intense vasoconstrictor effect of blood from the nephrectomized animal with renin and angiotonin. After injection of renin into the nephrectomized animal, the blood still caused marked vasoconstriction when renin or angiotonin was added to it. After the transfusion of tachyphylactic blood, addition of renin to the blood sample caused no constriction and the response with angiotonin was reduced to one-fourth its original intensity.

In another experiment the single injection of 0.05 cc. of renin elevated the blood pressure of a nephrectomized dog from 122 mm. to 190 mm. Hg. Transfusion of 300 cc. of blood from a normal dog caused no significant change in pressure. Further injections of renin caused only slight rise of blood pressure.

Sensitivity of Normal, Hypertensive, and Bilaterally Nephrectomized Dogs to Renin and Angiotonin

Renin was diluted 1 part to 10 of saline solution and infused into the femoral veins of normal dogs anesthetized with pentobarbital sodium at the rate of 0.8 cc. the first 60 minutes, 1.6 cc. the next 15 minutes. Tachyphylaxis was always established as demonstrated by the fact that injections of 1 cc. of concentrated renin or angiotonin produced no rise in blood pressure. After the renin or angiotonin infusion, blood was withdrawn, citrated, and transfused into the anesthetized recipient dog. Renin or angiotonin was infused into the recipient at the same rate as into the donor dog and the effect on the blood pressure again recorded.

Hypertensive dogs were found to respond with a greater and more prolonged rise in blood pressure after renin infusion, but were somewhat less sensitive than were bilaterally nephrectomized animals (Tables VI and VIII). After transfusion of tachyphylactic blood from normal animals

TABLE VIII

Pressor Response of Normotensive and Hypertensive Dogs to Infusion of Renin

Weight	Initial B.P.	Change of B.P. in mm. Hg at various intervals after infusion began						
		5 min.	15 min.	30 min.	45 min.	60 min.	75 min.	
Normal Dogs								
kg.	*mm. Hg*							
10.6	166	18	16	12	4	0	−4	
9.0	138	12	28	32	27	22	10	
12.0	116	4	8	11	7	4	4	
8.6	136	9	19	17	4	4	2	
10.8	150	10	12	8	−5	−10	−14	
9.4	172	12	17	11	−1	−18	−24	
	160	2	−4	−22	−20	−25	−28	
10.0	138	17	32	37	37	22	12	
11.8	160	16	13	22	14	0		
Hypertensive Dogs								
11.3	184	16	30	34	32	32	27	
13.1	154	24	40	58	50	44	34	
Bilaterally Nephrectomized Dogs								
								Nephrectomy
10.4	130	18	26	44	41	42	42	1 day before
10.7	114	38	48	58	56	51	46	3 days before

TABLE IX

Effect of Transfusion of Blood Tachyphylactic to Renin on Response of Bilaterally Nephrectomized and Hypertensive Recipients to Infusion of Renin

Dog	Initial B.P.	Amount blood transfused	Maximum rise of B.P. after transfusion	Initial B.P.	Blood pressure in mm. Hg at various intervals after renin infusion					
					5 min.	15 min.	30 min.	45 min.	60 min.	75 min.
	mm. Hg	*cc.*	*mm. Hg*	*mm. Hg*						
Nephrectomized 2 days before	146	150	23	171	9	17	6	3	—	—
Nephrectomized 4 days before	140	300	42	168	−4	−2	−7	−4	−8	—
Nephrectomized 1 day before	116	250	32	150	8	12	16	4	—	—
Hypertensive B.P. = 202 mm. Hg	182	450	16	118	−16	−36	−40	—	—	—
Hypertensive B.P. = 216 mm. Hg	140	300	72	210	−10	−24	−50	−56	−58	−74
Hypertensive with one normal kidney B.P. = 175 mm. Hg	166	300	47	111	15	34	9	4	—	—

to either hypertensive or nephrectomized animals, the response to renin infusion was markedly reduced (Table IX).

DISCUSSION

Tachyphylaxis to angiotonin has been shown to develop in animals when angiotonin is repeatedly injected, but much more slowly than does renin tachyphylaxis. When it occurs, the response to renin is also lost. Large doses of renin quickly abolish the pressor properties of angiotonin. Clearly, the development of tachyphylaxis to these two substances is interrelated. It is possible that the greater effectiveness of renin in establishing tachyphylaxis to both itself and to angiotonin is due to the prolonged exposure to angiotonin, which follows when angiotonin is formed from the interaction of renin with renin-activator rather than the brief exposure following single injections of angiotonin itself.

It is necessary to diverge from the main current of thought to point out that a fraction has been separated from both plasma and red blood cells which appears necessary for angiotonin to exert its pressor action in perfused isolated organs. The first injection of angiotonin into, for example, a rabbit's ear perfused with Ringer-Locke's solution causes vasoconstriction, but subsequent injections are ineffective. If normal blood or the chemically separated fraction of blood is added, the response is immediately restored. This co-substance, or as it has been called in this communication, "angiotonin-activator," is reduced or lacking when the animal is made tachyphylactic by infusing angiotonin or renin. It is not identical with renin-activator because addition of the latter does not cause return of the response to angiotonin when it is lost, nor does it exhibit similar chemical properties.

Tigerstedt and Bergmann (1898) showed that removal of both kidneys in an animal increased its sensitivity to injection of extracts of kidney. Subsequent work with purer renin has amply confirmed this observation (Merrill, Williams, and Harrison, 1938; Wakerlin and Chobot, 1939). We have shown that nephrectomy similarly increases sensitivity to angiotonin. It is of interest that either a single injection or infusion of angiotonin or renin causes elevation of arterial pressure in nephrectomized animals which shows little tendency to return to normal even after several hours. Angiotonin-activator is present in the blood, but if the infusion of angiotonin is persisted in, the amount of activator is gradually reduced. After several hours the blood has almost no activating power when assayed in the rabbit's ear with angiotonin. Nor does injection of more angiotonin into

the animal which has received the infusion cause further rise of blood pressure.

Infusion or single injections of renin causes the same prolonged rise of blood pressure as does angiotonin, but renin-activator is more quickly lost. It is apparent from these and the earlier results of Page (1939) that the amount of renin-activator is usually a limiting factor in that it can be exhausted in the blood with ease. Perhaps the increased amounts of it found in the blood of hypertensive animals by Kohlstaedt, Page, and Helmer (1940) is significant in this connection.

That the prolongation of elevation of arterial pressure may not be due to spasm is possibly indicated by the fact that administration of histamine or choline in amounts sufficient to cause profound fall of blood pressure does not prevent the pressure from rising to its initial level when administration is discontinued.

The increased sensitivity to angiotonin and renin following nephrectomy does not seem to be caused primarily by alteration in the blood vessels themselves. Rather it is a property of the blood which bathes them. This is demonstrated by the fact that blood from nephrectomized animals is abnormally potent in causing vasoconstriction when perfused with renin or angiotonin through a rabbit's ear.

Page (1939) showed that tachyphylaxis to renin in normal animals is due not only to exhaustion of renin-activator but also to development of an inhibitor. In rabbit's ears perfused with Ringer's solution tachyphylaxis is due chiefly to exhaustion of renin-activator. If the lack of response developed to renin and angiotonin in animals is due to liberation of an inhibitor as well as to exhaustion of activator, and if this inhibitor was liberated in part by the kidneys, then increased sensitivity should follow their removal. It should also be possible to reduce the response to these substances by transfusions of blood from normal animals and animals made tachyphylactic with angiotonin and renin.

It has been found that transfusion of amounts of normal, citrated blood of the order of 300 to 500 cc. into small nephrectomized animals reduces the response to renin or angiotonin, demonstrating, we believe, that an inhibitor has been transferred from the donor to the recipient. Blood from nephrectomized or hypertensive dogs after transfusion exhibits much reduced power to activate renin and angiotonin. This suggests that transfusion exerts a specific effect on the mechanism responsible for hypertension following injection of renin and angiotonin.

Most of our experiments show that transfusion of tachyphylactic blood has a more pronounced inhibitor action than does normal blood. The

difference may in part be due to transference of an excess of renin employed in producing tachyphylaxis in the donor as well as to transference of inhibitor.

Single, small doses of angiotonin or renin injected into nephrectomized animals cause hypertension which even after several hours shows little tendency to disappear. For 30 minutes or more after the rise of blood pressure angiotonin and its activator can be demonstrated in the blood in large amounts. Injection of tachyphylactic blood reduces the angiotonin-activating power of the blood but it does not reduce the blood pressure. This suggests that inhibitor interferes with the reaction which initiates vasoconstriction rather than causing vasodilatation after it is established.

If angiotonin and renin are concerned in the genesis of experimental hypertension it is not unreasonable to suppose that like the nephrectomized animal, the animal with hypertension would be more sensitive to the pressor action of these substances. According to the experiments described, there would be less inhibitor in their blood as well as more activator. The rise of blood pressure during infusion of renin was, in fact, greater and more prolonged than in normal animals and more comparable to that observed in nephrectomized than normal dogs. Leiter and Eichelberger (1939) state in an abstract that they also found a greater and more prolonged rise in hypertensive animals. It thus appears that in experimental hypertension there is a lack of secretion by the kidneys of inhibitor to angiotonin and renin. The hypertensive animal stands roughly midway between normal and nephrectomized dogs as regards its ability to inhibit the response to angiotonin and renin.

It may aid the reader to summarize the evidence for the existence of an inhibitor to angiotonin and renin:

1. Blood from an *intact animal* made tachyphylactic to renin or angiotonin does not produce vasoconstriction when perfused along with renin or angiotonin and activator in an isolated organ. When tachyphylaxis is developed by repeated injections of renin or angiotonin into blood recirculated in an *isolated organ*, addition of renin plus activator or angiotonin plus activator causes vasoconstriction. This suggests that an inhibitor is added to the blood by the intact animal which does not occur in the isolated organ.

2. Nephrectomy greatly increases the sensitivity of animals both to angiotonin and renin. Induction of experimental hypertension by perinephritis also increases sensitivity but not so markedly. Addition of angiotonin or renin to blood from such animals causes preternaturally great constriction in perfused organs.

3. Extracts of kidneys and muscle reduce arterial pressure of animals with experimental renal hypertension. The evidence for this has been, in part, presented (Page, 1939). Similar results were reported for extracts of kidneys by Grollman, Harrison, and Williams.

4. Transfusion of normal and tachyphylactic blood into nephrectomized or hypertensive animals reduced the response to injections or infusion of renin.

Our present views regarding the nature of the renin-angiotonin vasopressor system are summarized in the following schema:

Reduced pulse pressure → efferent arteriolar constriction (due to renin formation?) → relative tubular ischemia → increased permeability of tubular cells → renin + renin-inhibitor

Renin + renin-activator → angiotonin

Renin-inhibitor + renin + renin-activator → inactive substance

Renin + angiotonin → inactive substance

Angiotonin + angiotonin-activator → vasopressor substance

Angiotonin + angiotonin-activator + angiotonin-inhibitor → inactive substance.

SUMMARY

1. Angiotonin does not exert its vasoconstrictor effect in the absence of a substance contained in red blood cells and serum which we have called "angiotonin-activator." A fraction has been separated from blood in which angiotonin-activator is concentrated. It contains little or no renin-activator.

2. Repeated intravenous injections of angiotonin into animals causes the pressor response gradually to lessen and finally to disappear (the phenomenon of tachyphylaxis), but much more slowly than when renin is injected. When the response to angiotonin is abolished, renin also fails to act. Large doses of renin reduce and finally abolish the responsiveness to angiotonin. Exhaustion of renin-activator in the blood abolishes the response to renin without abolishing the response to angiotonin.

3. Blood from animals made tachyphylactic by infusion of angiotonin contains greatly reduced amounts of angiotonin-activator. An inhibitor also appears in the blood.

4. Bilateral nephrectomy prolongs and greatly enhances the rise of arterial pressure following injection of angiotonin and renin. The enhancement reaches a maximum in from 24 to 30 hours after operation. Blood from these animals exhibits greatly increased ability to activate angiotonin and renin when tested in isolated perfused organs. Large amounts of angiotonin are required to reduce the amount of activator in their blood. Renin-activator is simultaneously but little affected.

5. Transfusion of blood from an animal made tachyphylactic to angiotonin into a nephrectomized dog reduces the response of the latter to angiotonin. Angiotonin when added to the blood of the recipient of the transfusion and perfused through a rabbit's ear also exhibits greatly reduced vasoconstrictor action.

6. Transfusion of normal blood in large amounts into nephrectomized or hypertensive dogs reduces the recipient's response to renin. If renin-tachyphylaxis is established in the donor, transfusion abolishes the response to renin in the recipient. The blood from such animals exhibits greatly reduced vasoconstrictor properties when perfused through an isolated organ with renin or angiotonin.

7. Renin-tachyphylactic or normal dog's blood does not reduce arterial pressure elevated by a single injection of renin into nephrectomized dogs.

8. Nephrectomized dogs exhibit the greatest pressor response to infusion of angiotonin and renin, normal animals the least, and hypertensive animals about midway between.

CONCLUSIONS

Angiotonin requires an activator differing from renin-activator to exert its vasoconstrictor action. It is contained in a fraction of blood and has been concentrated.

Tachyphylaxis occurs when angiotonin is repeatedly injected but much more slowly than with renin. The mechanisms for the production of tachyphylaxis to angiotonin and renin are interrelated. Tachyphylaxis appears to be due to exhaustion of their respective activators as well as to development of inhibitors.

Inhibitors to renin and angiotonin seem to originate in part in the kidneys and it is their loss which accounts in large measure for the increased sensitivity of animals to these substances after nephrectomy.

Hypertensive animals may not only have increased amounts of activators in their blood, but reduced amounts of inhibitors as well. This concatenation of circumstances, due apparently chiefly to the kidneys, adds credibility to the view that experimental renal hypertension is mediated by the renin-angiotonin vasopressor system.

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BIBLIOGRAPHY

Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.
Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 214.
Kohlstaedt, K. G., and Page, I. H., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 136.
Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 92.
Leiter, L., and Eichelberger, L., *J. Clin. Inv.*, 1939, **18**, 477.
Merrill, A., Williams, J. R., and Harrison, T. R., *Am. J. Med. Sc.*, 1938, **196**, 18.
Page, I. H., *Science*, 1939, **89**, 273.
Page, I. H., *J. Am. Med. Assn.*, 1939, **113**, 2046.
Page, I. H., *J. Exp. Med.*, 1939, **70**, 521.
Page, I. H., Newer aspects of hypertension, in Blood, heart and circulation, The American Association for the Advancement of Science, in press.
Page, I. H., and Helmer, O. M., *Proc. Centr. Soc. Clin. Inv.*, 1939, **12**, 17.
Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.
Tigerstedt, R., and Bergmann, P. G., *Skand. Arch. Physiol.*, 1898, **8**, 223.
Wakerlin, G. E., and Chobot, G. R., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 331.
Warburg, O., Christian, W., and Griese, A., *Biochem. Z.*, 1935, **282**, 157.