THE LIBERATION OF RENIN BY PERFUSION OF KIDNEYS FOLLOWING REDUCTION OF PULSE PRESSURE*

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Despite the fact that much evidence indicates the participation of renin in the genesis of experimental hypertension it has not been shown to the satisfaction of most investigators that renin is liberated from the kidneys of hypertensive animals into the blood of the renal vein. The experiments which have been tried have either failed completely to demonstrate any increased pressor action of renal venous blood or the conditions of the experiment were highly artificial and did not allow of a single interpretation. Even if it could be shown that renal venous blood exhibited greater pressor properties than arterial blood, this would not necessarily indicate that renin itself was being liberated.

It was our belief that to study this problem the conditions of the experiment should be made as simple and controllable as possible. Isolated kidneys perfused with blood were therefore employed allowing strict control of extra-renal hemodynamic changes. Increase in the amount of renin liberated was ascertained by addition of renin-activator (Kohlstaedt, Page, and Helmer, 1938, 1940) to samples of renal vein blood and perfusion of the mixture through an isolated rabbit's ear (Page, 1939, *b*). If renin is present it will react with the renin-activator to produce the pressor substance angiotonin (Page and Helmer, 1939, 1940). Since renin itself is not a pressor substance (Helmer and Page, 1939) it is understandable that attempts to show increased pressor activity of renal vein blood except under artificial conditions are foredoomed to failure.

In short, we believe that it is possible to demonstrate increased liberation of renin from normal kidneys by altering the extra-renal hemodynamics and employing what appears to be a specific activating system to demonstrate the increase.

Method

A dog weighing 10 to 12 kg. was given 1/50 grain of atropine sulfate subcutaneously and anesthetized with ether. The lower portion of the abdominal aorta and femoral

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vein were cannulated. Blood was collected from the aorta and simultaneously 200 cc. of Ringer-Locke's solution containing 30 gm. of acacia was infused into the femoral vein. Artificial respiration was begun as soon as spontaneous breathing ceased, to prevent development of pulmonary edema. The blood was defibrinated by stirring with a rubber brush and filtered through washed cotton gauze. After no more blood was obtained from the aorta, 100 cc. of Ringer-Locke's solution containing 12 mg. of heparin was infused to prevent clotting in the lungs and kidneys.

The right kidney was quickly removed together with several centimeters of its ureter and a part of the aorta adjacent to the renal artery. The right kidney was used because in the perfusion apparatus the vein comes to lie above the artery. The renal artery was cannulated through the attached portion of the aorta, so insuring perfusion of all its branches. A small glass cannula was inserted into the ureter.

During preparation of the kidney an assistant opened the thoracic cavity. A glass cannula was inserted through an incision in the right ventricle into the pulmonary artery and tied in place by a ligature which extended around the arch of the aorta and the pulmonary artery near its junction with the ventricle. Another cannula was inserted into the left auricle by cutting off the distal tip of the auricle and tying a ligature around the auricle. The lungs, trachea, and heart were freed, removed from the body, and suspended in a heated container by attaching the tracheal cannula to a ring stand. Artificial respiration with a mixture of 5 per cent CO_2 and 95 per cent O_2 was continued after the lungs were removed from the body. The cannula in the pulmonary artery was connected with one of the outflow tubes (*E*) of a double Dale-Schuster (1928) perfusion pump (Fig. 1) fitted with Hemingway valve chambers, and the cannula in the left auricle was connected with the arterial reservoir (*D*) of the opposite pump by a glass tube (*G*), thus completing a pulmonary circulation.

The cannula in the renal artery was connected to the outflow of the other perfusion pump and the blood from the renal vein was collected in the venous reservoir (*V*) which supplied blood to the pulmonary circulation.

The pumps were located at the bottom of a copper tank (*B*) which was filled with water maintained at 38°C. by an electric heating unit. A Kelly flask located directly above each pump made an excellent venous reservoir because the outlet of the flask was at the bottom and therefore it could be used with either large or small quantities of blood.

As the blood left the chamber of the pump, it passed through a heated glass coil (*F*) to insure the proper temperature. Beyond, on each outlet tube was a side arm which was connected to an adjustable flow resistance (*C*). This mechanism was constructed by enclosing a soft rubber tube 4 cm. in length within a glass tube which was closed tightly at both ends around the rubber tube. Constant pressure was maintained in the glass tube by compressing a rubber bulb (*A*) which was connected through a small opening in the side of the glass tube. One end of the rubber tube was connected to the side arm of the outlet of the pump and the other end of the rubber tube was connected to a glass tube which extended across the water tank to the reservoir from which the blood had been removed by the pump. Blood flowed through the rubber tube only when the pressure produced by the pump exceeded the pressure in the glass tube which surrounded the rubber tubing. This device protected the lungs and kidneys from the increased pressure incident to vasoconstriction.

Blood pressure in the tube between the pump and kidney was recorded on the kymograph by a mercury manometer (*S*).

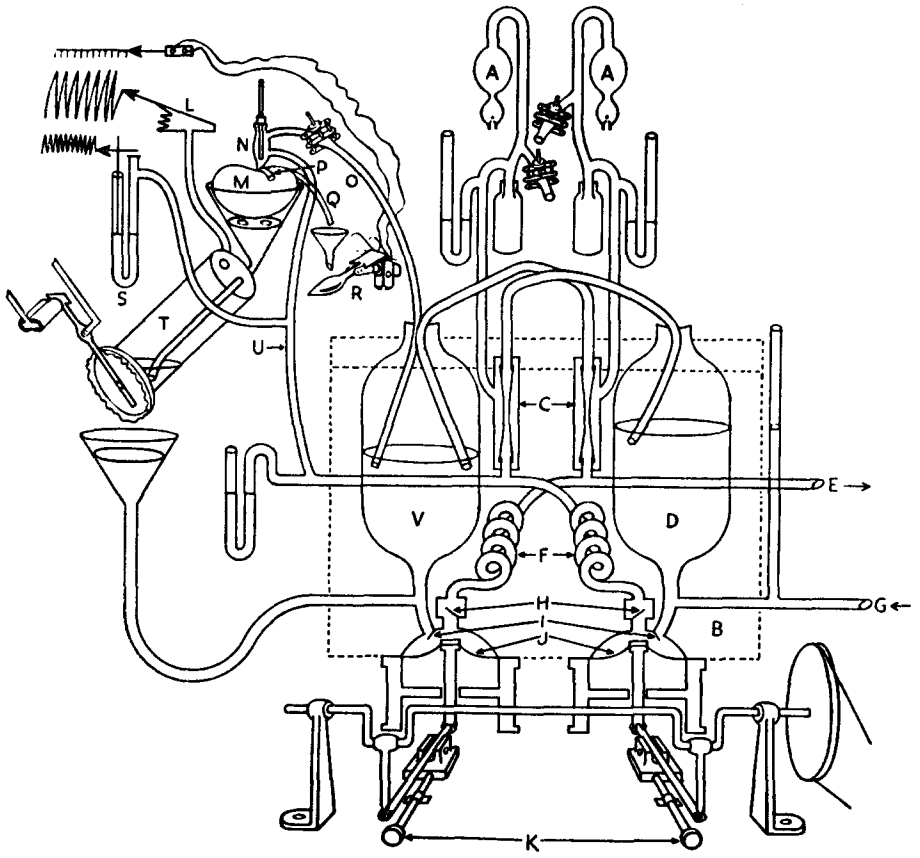

FIG. 1. Apparatus for perfusion of isolated dog's kidney with blood. *A*, rubber bag for maintaining peripheral resistance; *B*, constant temperature water bath; *C*, device for producing peripheral resistance; *D*, reservoir for arterial blood; *E*, outflow to pulmonary artery; *F*, warming coils; *G*, tube returned from the lungs; *H* and *I*, valves in pumps; *J*, rubber diaphragms in pumps; *K*, screws for altering stroke of pumps; *L*, Brodie bellows; *M*, dog's kidney; *N*, glass cannula in renal artery; *O*, tube for shunting blood around kidney; *P*, renal vein; *Q*, ureteral catheter; *R*, balanced spoon for collecting urine; *S*, mercury manometer; *T*, Gaddum recorder; *U*, site of constriction of renal artery; *V*, venous reservoir.

The cannula in the renal artery was constructed with a small side arm which was connected by a glass tube (*O*) to the pulmonary venous reservoir. This arrangement enabled blood to be shunted around the kidney and returned to the pulmonary circuit. It was used during the first part of the experiment, usually for about 30 minutes when the kidney vessels were constricted and blood flow was slow due, as Cushny showed, to

a vasoconstrictor substance present in defibrinated blood which can be removed by repeated circulation through the lungs.

The kidney was placed in a small evaporating dish which was supported by a glass funnel. Blood from the renal vein filled the dish and ran over into the funnel and was collected in a Gaddum recorder (*T*). From the recorder the blood flowed into the reservoir from whence it was pumped into the pulmonary circulation.

Urine flow was measured by collection in a balanced spoon (*R*) which recorded each spoonful on the kymograph.

After the kidney and lungs had been connected to their respective perfusion pumps, perfusion was begun with a peripheral resistance of 160 mm. Hg on the renal circuit and 80 mm. Hg on the pulmonary circuit. Pulse rate was 96 during the entire experiment.

As soon as a steady level of urine and blood flow was reached, urea clearance was measured. Blood samples were taken for plasma protein determination and for comparative study of vasoconstrictor properties of the blood.

When the urea clearance determination was completed, a screw clamp was placed on the rubber tubing near the cannula in the renal artery and just proximal to the mercury manometer (*U*, Fig. 1). The clamp was adjusted to reduce pulse pressure at least 50 per cent. The mean pressure was maintained as nearly constant as possible by increasing the stroke of the pump and raising peripheral resistance on the renal circuit. In most experiments by careful adjustment of all three factors, *i.e.*, amount of constriction produced by the clamp, increase in output of the pump, and amount of peripheral resistance added, it was possible to reduce pulse pressure with minimal change in the mean pressure.

In a group of control experiments the kidney was perfused for an equal length of time with defibrinated blood but without constricting the tube between pump and kidney or without altering the output of the pump.

The hind leg of a dog was perfused with defibrinated blood in a similar manner. The cannula was placed in the femoral artery and blood flowing from the femoral vein was collected in a Gaddum recorder. The rubber tube connecting the femoral artery and pump was constricted by a clamp and blood samples were taken before and after its application.

The renal venous blood samples were studied for vasoconstrictor properties by perfusion through a rabbit's ear with pulsatile pressure. In one group of experiments the ear was perfused with Ringer-Locke's solution and mixtures of blood and renin-activator or renin were injected. In a second group the ear was perfused with the defibrinated blood which had been perfused through the kidney and small amounts of renin or renin-activator were injected through a side arm of the perfusion apparatus into the artery of the rabbit's ear.

RESULTS

Perfusion of the Isolated Kidney without Constriction of the Renal Artery

Renal vascular relaxation with increase in pulse pressure and renal blood flow occurred as the constrictor substance normally present in blood directly after defibrination was removed by the lungs. When relaxation appeared complete, the average pulse pressure was 49 mm. Hg, mean pressure 112

mm. Hg, and blood flow 4.5 cc. per gm. of kidney per minute in 7 experiments (Table I). After 4 hours of perfusion the average pulse pressure was 47 mm. Hg, mean pressure 114 mm. Hg, and blood flow 3.9 cc.

Urine secretion which began as soon as an adequate renal blood flow was obtained, continued throughout the experiment. Urea clearance measured in 3 experiments (Nos. 2, 6, and 7) was found to be 0.15 cc., 0.16 cc., and

TABLE I

Perfusion of the Isolated Kidney without Constriction of the Renal Artery

Experiment No.		Kidney weight	Time perfused	Plasma protein	Blood pressure	Mean pressure	Pulse pressure	Change mean pressure	Change pulse pressure	Blood flow	Change blood flow	Urine flow
		gm.	*min.*	*gm./ 100 cc.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*	*per cent*	*cc./ min.*	*per cent*	*cc./ min.*
1	Maximum blood flow	28		1.74	150/114	132	36			120		1.8
	End of experiment		255		146/122	134	24	+1	−33⅓	88	−26	1.1
2	Maximum blood flow	38		4.39	132/92	102	40			180		0.3
	End of experiment		240		136/86	111	50	+9	+10	180	0	0.2
3	Maximum blood flow	60			140/80	110	60			260		0.1
	End of experiment		270		144/96	120	48	+9	−20	208	−20	0.2
4	Maximum blood flow	44.2		4.44	126/68	94	58			230		0.6
	End of experiment		240		126/66	96	60	+1	+1	220	−1	0.9
5	Maximum blood flow	33.3		2.9	138/84	111	54			130		4.2
	End of experiment		224		134/84	109	50	−1	−7	70	−42	0.9
6	Maximum blood flow	31			148/96	122	52			120		0.3
	End of experiment		226		140/90	115	50	−5	−3	140	+16	0.4
7	Maximum blood flow	32		3.95	142/90	116	48			168		0.7
	End of experiment		240		142/88	115	54	−1	+11	148	−12	0.4

0.27 cc. per gm. of kidney per minute. These values were lower than the urea clearance of 0.4 cc. per gm. of kidney per minute reported for intact dogs by Van Slyke, Rhoads, Hiller, and Alving (1934). In the third experiment (No. 7) the urea clearance repeated after 4 hours of perfusion was 0.18 cc. per gm. of kidney per minute.

Two hours of perfusion in 2 experiments (Table III) caused the rate of oxygen consumption to vary insignificantly.

Samples of renal venous blood collected at the beginning and the end of

TABLE II

Perfusion of the Isolated Kidney before and after Constriction of the Renal Artery

Experiment No.	Condition at time of observation	Kidney weight	Time perfused	Plasma protein	Blood urea nitrogen	Urine urea nitrogen	Urea clearance	Blood pressure	Mean pressure	Pulse pressure	Change mean pressure	Change pulse pressure	Blood flow	Change blood flow	Urine flow
		gm.	*min.*	*gm./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*cc./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*	*per cent*	*cc./min.*	*per cent*	*cc./min.*
8	Before clamping	31	135					138/84	111	54			108		0.5
	Just after clamping							120/94	107	26	−3	−51	90	−10	0.1
	End of experiment		115					114/94	104	20	−3	−25	58	−22	0
9	Before clamping	46	160				11.1	130/84	97	46			130		7.2
	Just after clamping							118/104	111	14	+14	−70	120	−8	5.3
	End of experiment		100					138/120	129	18	+18	+10	86	−38	1.0
10	Before clamping	52	150		10.05	78.1	6.0	140/88	114	52			180		0.7
	Just after clamping							98/80	89	18	−22	−65	156	−14	2.9
	End of experiment		205		9.3	47.0	3.5	114/90	102	24	+12	+33⅓	156	0	0.7
11	Before clamping	25	145		10	17.7	2.11	140/100	120	40			70		1.8
	Just after clamping							124/118	121	6	+0.9	−85	66	−5	0.3
	End of experiment		130					138/128	133	4	+10	−33⅓	50	−28	0.11
12	Before clamping	38	120		7.9	41.1	6.0	144/112	128	32			130		1.3
	Just after clamping							132/118	125	14	−2	−56	120	−8	1.6
	End of experiment		120					138/130	134	8	+15	−40	100	−30	0.15
13	Before clamping	33	108	5.8	17	73	2.1	136/98	117	38			108		0.45
	Just after clamping							126/118	122	8	+4	−78	87	−19	0.6
	End of experiment		80		15	56.5	1.14	130/120	125	10	+3	+20	40	−68	0.3
14	Before clamping	40	120	2.57	9.25	106	7.03	118/64	91	54			240		0.7
	Just after clamping							90/78	84	12	−7	−77	228	−5	0.6
	End of experiment		165					126/118	122	8	+38	−33⅓	188	−12	0.05
15	Before clamping	43	103		9.25	49	5.16	138/88	113	50			168		1.1
	Just after clamping							120/100	110	20	−2	−60	168	0	0.8
	End of experiment		165					168/164	162	4	+52	−80	112	−58	0.05
16	Before clamping	40	100	4.39	7.55	43.8	10.2	124/78	101	46			220		1.5
	Just after clamping							104/82	93	22	−8	−52	191	−10	1.4
	End of experiment		140					164/136	150	28	+49	+2	170	−12	0.5
17	Before clamping	23.4	150	3.33	13.3			150/92	121	58			124		0.8
	Just after clamping							108/90	99	18	−20	−69	108	−15	0.1
	End of experiment		120					200/170	185	30	+64	+40	92	−15	0
18	Before clamping	40	135	4.26	9.8	88.5	3.6	140/80	107	56			112		0.4
	Just after clamping							128/112	120	16	−13	−71	95	−13	0.4
	End of experiment		150					178/156	171	26	+47	+6	32	−68	0.15
19	Before clamping	34	80	3.23	9.8	65.6	18	128/82	105	46			208		2.0
	Just after clamping							92/76	84	16	−20	−65	160	−23	0
	End of experiment		240					184/158	170	26	+83	+10	68	−77	0.3

TABLE II—*Concluded*

Experiment No.	Condition at time of observation	Kidney weight	Time perfused	Plasma protein	Blood urea nitrogen	Urine urea nitrogen	Urea clearance	Blood pressure	Mean pressure	Pulse pressure	Change mean pressure	Change pulse pressure	Blood flow	Change blood flow	Urine flow
		gm.	*min.*	*gm./ 100 cc.*	*mg./ 100 cc.*	*mg./ 100 cc.*	*cc./ min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*	*per cent*	*cc./ min.*	*per cent*	*cc./ min.*
20	Before clamping	31.4	92	4.0	13.7	134	3.0	156/98	127	58			180		0.3
	Just after clamping							126/100	113	26	−14	−55	160	−11	0.1
	End of experiment		160					150/136	143	14	+30	−49	80	−50	0.4
21	Before clamping	25	100					150/110	130	40			144		0.7
	Just after clamping							130/114	122	16	−6	−60	128	−11	0.4
	End of experiment		110					142/122	132	20	+10	−20	78	−38	0.1
22	Before clamping	34	130	4.35	12.8	173	6.5	146/96	121	50			160		0.5
	Just after clamping							120/102	111	18	−9	−64	132	−17	0.2
	End of experiment		140					140/120	130	20	+19	+11	105	−20	0.05
23	Before clamping	26.5	108	3.92	17.2	191	2.8	146/96	121	50			136		0.2
	Just after clamping							116/98	107	18	−13	−64	140	+2	0.2
	End of experiment		204		14.4	131	2.3	98/76	87	22	−20	+22	140	0	0.2
24	Before clamping	33	120	4.05	7.1	167	6.1	154/98	126	56			160		0.26
	Just after clamping							138/110	124	28	−1	−50	180	+12	0.3
	End of experiment		210		7.1	107	1.4	144/118	131	26	+7	−8	168	−7	0.1
25	Before clamping	34	124	4.11	7.15	70.8		146/106	121	40			100		0.8
	Just after clamping							124/112	118	12	−2	−70	96		0.6
	End of experiment		156		5.7	25.5		136/124	126	12	+1	0	86		0.3

each experiment showed no difference in vasoconstrictor properties when tested with renin and renin-activator on the isolated rabbit's ear.

Perfusion of the Isolated Kidney before and after Constriction of the Renal Artery

When maximum renal vascular relaxation had occurred, the average pulse pressure was 45 mm. Hg, mean pressure 114 mm. Hg, and blood flow 4.2 cc. per gm. of kidney per minute in 18 experiments before the renal artery was constricted. Immediately after applying the clamp to the renal artery and increasing the output of the perfusion pump, the average pulse pressure was 17 mm. Hg, mean pressure 109 mm. Hg, and blood flow 3.9 cc. (Table II).

Changes in mean pressure which occurred in some experiments after constriction of the renal artery, were not accompanied by similar variations in renal blood flow. For example, renal blood flow decreased in 4 experiments (Nos. 9, 11, 13, and 18) immediately after clamping although mean pressure was higher and, contrariwise, blood flow increased after applica-

tion of the clamp in 2 experiments (Nos. 23 and 24) although the mean pressure was lower.

Renal function as measured by the rate of urine secretion and urea clearance was impaired by constricting the renal artery. Urea clearance was reduced 41, 40, 20, and 70 per cent, 2 hours after application of a clamp in 4 experiments.

The average rate of oxygen consumption before clamping was 0.092 cc. per gm. of kidney per minute and after constricting the renal artery 0.078 cc. (4 experiments, Table III). These values were similar to the average of

TABLE III

Oxygen Consumption of Isolated Dog's Kidney during Perfusion with Defibrinated Blood

Experiment No.	Blood sample collected when maximum flow occurred						Sample collected at end of experiment						
	Blood flow per gm. of kidney	Urine flow	Blood oxygen content Volumes per 100 cc.		A-V difference Volumes per 100 cc.	Oxygen consumption per gm. of kidney	Time perfused	Blood flow per gm. of kidney	Urine flow	Blood oxygen content Volumes per 100 cc.		A-V difference Volumes per 100 cc.	Oxygen consumption per gm. of kidney
			Arterial	Venous						Arterial	Venous		
	cc./min.	*cc./min.*				*cc./min.*	*min.*	*cc./min.*	*cc./min.*				*cc./min.*
6	3.9	0.35	18.03	16.7	1.33	0.0519	120	4.5	0.3	17.77	16.7	1.07	0.0482
7	4.2	0.5	16.53	15.0	1.53	0.0643	130	5.0	0.4	14.78	13.21	1.57	0.0785
	Before constriction of renal artery						After constriction of renal artery						
19	6.1	2.0	18.94	17.01	1.93	0.1127	40	4.7	1.0	18.8	16.9	1.90	0.0893
20	5.7	0.3	18.52	16.72	1.8	0.1025	120	5.0	0.1	16.22	13.82	2.4	0.120
21	4.4	0.4	16.8	15.04	1.76	0.0775	130	1.9	0	11.0	8.47	2.53	0.0481
23	5.1	0.2	16.18	14.6	1.58	0.0806	120	5.2	0.2	17.21	16.15	1.06	0.0551

0.08 cc. of oxygen consumed per gm. of kidney per minute by intact dogs (Van Slyke, Rhoads, Hiller, and Alving, 1934).

Perfusion for at least 80 minutes and at most 240 minutes after constricting the renal artery resulted in a rise in mean renal arterial pressure distal to the constriction (average before clamping was 114 mm. Hg and at the termination of 18 experiments was 135 mm. Hg). Renal blood flow was reduced from an average of 3.9 cc. to 2.9 cc. after constricting the renal artery. Pulse pressure was not altered (average immediately after clamping was 17 mm. Hg and at end of experiment was 18 mm. Hg). Changes in renal blood flow and mean pressure did not occur until the renal artery had been constricted at least 40 minutes.

Renal venous blood (0.2 cc.) collected before application of a clamp to the

renal artery even after several hours of perfusion caused no vasoconstriction when perfused with Ringer-Locke's solution through an isolated rabbit's ear. Addition of renin-activator caused modest constriction but this was not increased by prolonged perfusion (Experiments 6 and 7, Table IV).

When the renal artery was constricted 100 minutes or more (11 experiments) renal venous blood caused far greater vasoconstriction on addition of renin-activator than blood removed before clamping. For example in

TABLE IV

Effect of Injection of a Mixture of 0.2 Cc. of Renin-Activator and 0.2 Cc. of Defibrinated Blood into Ringer-Locke's Solution Perfusing a Rabbit Ear with Pulsatile Pressure

Experiment No.	Renal vein blood collected as soon as maximum renal vascular relaxation had occurred		Renal vein blood collected at end of experiment		
	Duration of reduction of flow	Reduction of flow	Duration of reduction of flow	Reduction of flow	Length renal perfusion
	min.	*per cent*	*min.*	*per cent*	*min.*
6	2	46	2	42	320
7	0.6	30	1	31	296
			Renal vein blood collected after renal artery constricted more than 120 min.		
8	1	32	4	86	115
9	2	51	8	81	100
10	4	39	10	94	205
11	2.5	59	9	81	130
19	0.5	12	1	21	240
20	2	30	5	87	160
21	2.5	47	7.5	85	110
22	1.5	30	5	75	140
23	1	31	4	61	204
24	1.8	50	4	87	210
25	1	59	9	91	180

Experiment 9 (Table IV) injection of renin-activator plus renal venous blood collected at the beginning of the perfusion of the kidney reduced the flow in the rabbit's ear 51 per cent for 2 minutes, whereas blood collected 100 minutes after application of the clamp reduced the flow 81 per cent for 8 minutes. The increase in vasoconstricting power of mixtures of renal venous blood and renin-activator did not appear until 40 minutes after the renal artery was constricted. This change in vasoconstrictor properties was followed by a sharp reduction in renal blood flow and a rise in mean pressure in the kidney (Fig. 2).

If, instead of perfusing the rabbit's ear with Ringer-Locke's solution,

blood from the renal vein of a kidney perfused under normal pressure relationships was substituted, addition of renin-activator caused no vasoconstriction. Contrariwise, addition of renin produced constriction. Altering the hemodynamics by reducing the pulse pressure caused a change to occur in the renal vein blood such that the addition of renin-activator produced intense vasoconstriction, while renin itself caused none (Table V).

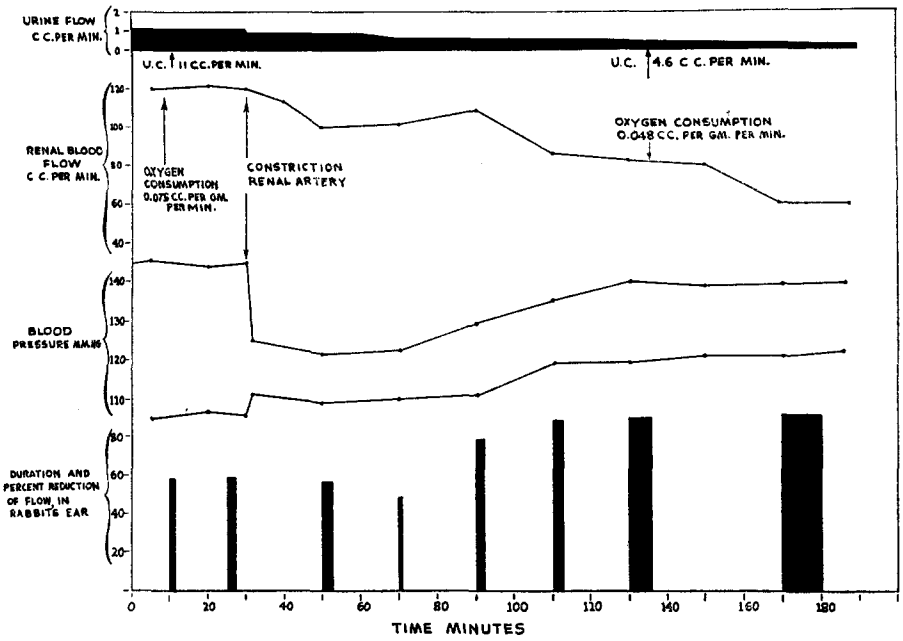

FIG. 2. Experiment 21. Renal hemodynamics and changes in vasoconstrictor properties of renal venous blood before and after reduction of pulse pressure. Kidney weight 26 gm. Vasoconstriction measured by injection of plasma samples and renin-activator into perfused rabbit's ear. Height of columns represents per cent reduction of flow in the ear and width represents the duration of reduced flow in minutes.

Perfusion of the Hind Leg of a Dog before and after Constriction of the Femoral Artery

Perfusion of the hind leg of dogs under conditions similar to that employed for the kidneys furnished suitable control experiments. For example, the blood pressure in the femoral artery was 144/80 mm. Hg and blood flow 165 cc. per minute. The pulse pressure was reduced from 65 to 22 mm. Hg and the mean pressure from 112 to 99 mm. Hg with a blood flow of 136 cc. per minute. Despite this marked alteration, perfusion for 4 hours caused no changes in blood pressure or blood flow. Nor did it alter the response of

the blood to renin and renin-activator. Renin caused marked vasoconstrictor properties to manifest themselves both before and after reducing the pulse pressure but there was no appreciable difference even after 4 hours of perfusion.

TABLE V

Effect of Injection of 1.0 Cc. of Renin and 1.0 Cc. of Renin-Activator into Artery of Rabbit's Ear during Its Perfusion by Pulsatile Pressure with Oxygenated, Defibrinated Renal Vein Blood

Experiment No.	Blood collected when renal relaxation maximal			Blood sample collected at end of experiments (renal artery *not* constricted)				
	1.0 cc. renin injected		10 cc. renin-activator	Time perfused when sample collected	1.0 cc. renin injected		1.0 cc. renin-activator injected	
	Duration of reduction	Reduction of flow	Reduction of flow		Duration of reduction	Reduction of flow	Duration of reduction	Reduction of flow
	min.	*per cent*		*min.*	*min.*	*per cent*	*min.*	*per cent*
1	2	51	0	255	1.5	36	0	0
2	16	91	0	240	11	98	0	0
3	6	68	0	270	4	44	0	0
4	7	50	0	240	5	50	0	0
5	10	90	0	224	7	80	0	0
6	10	47	0	212	8	61	0	0
7	9	78	0	240	6	68	0	0
				Blood sample collected after renal artery constricted by clamp				
12	6	81	0	150	0	0	10	90
13	8	76	0	80	0	0	5	70
14	10	84	0	150	0	0	6	72
15	12	76	0	150	0	0	6	75
16	10	79	0	143	0	0	9	83
17	8	84	0	120	0	0	9	54
18	11	85	0	150	0	0	5	61
19	15	75	0	200	0	0	10	65

DISCUSSION

These experiments were designed to demonstrate the effect of altered hemodynamic states on the liberation of renin from isolated kidneys. The kidneys were therefore perfused under conditions which as nearly as possible approximated physiological states. The average blood flow was maintained at levels comparable to those observed by Van Slyke, Rhoads, Hiller, and Alving (1934) and Corcoran and Page (1939), respectively 4.0 and 4.7 cc. per minute per gm. of kidney. However, since their observations were made in uninephrectomized dogs whose remaining kidney had

been explanted, this rate of flow is probably higher than normal. The low urea clearances and relatively high rates of renal blood flow suggest, as has been observed by Shannon and Winton (1940), that there is a loss of tone of glomerular afferent arterioles in the perfused kidney. Renal blood flow, urine flow, and urea clearance could, in most experiments, be maintained for 4 or more hours.

From measurements of the renal clearances of phenol red and inulin, Corcoran and Page (1938) concluded that hypertension could be obtained in uninephrectomized dogs by constriction of the renal artery with Goldblatt's clamp in the absence of renal ischemia. It was therefore decided to observe the effect of constriction on the liberation of renin by the perfused kidney without altering renal blood flow. Hence, when the renal artery was clamped, efforts were made to keep mean arterial pressure distal to the clamp approximately the same as it had been before clamping and thus to maintain renal blood flow near its former level. This was done by increasing the stroke of the perfusion pump and the peripheral resistance after application of the clamp.

Constriction of the renal artery by the clamp reduced the pulse pressure in the renal artery by about one-half in 17 experiments. The mean pressure was not easily readjusted to its initial level since both pulse and mean pressures were raised by increasing the output of the pump. Probably as a result of loss of pulsatile pressure, blood flow was slightly reduced from its control level after restoration of mean pressure in most experiments. The blood flow increased in 2 experiments after clamping and adjustment of mean pressure, presumably because the clamp had been applied before maximum vasodilation had occurred. A critical point was found in each experiment beyond which tightening the clamp caused a precipitate decrease in mean pressure and blood flow.

The store of renin-activator in blood is small and rapidly exhausted by the addition of renin (Page, 1939, *b*). Consequently, if renin were liberated by the isolated kidney in more than minute amounts, its presence could not be demonstrated until renin-activator had been added to react with renin and liberate the vasoconstrictor, angiotonin (Page and Helmer, 1940). Renin-activator was therefore added to renal venous plasma and, after 10 minutes incubation, the renin content was assayed by perfusing the mixture through the isolated rabbit's ear perfused with Ringer-Locke acacia solution. The liberation of renin could alternatively be shown in the rabbit ear system perfused with blood obtained from the renal vein. If sufficient renin had been liberated into the blood to saturate its store of activator,

addition of more renin to the perfusion had no effect, while the addition of activator caused vasoconstriction.

Renin was present in no more than minute amounts in blood samples from the renal vein taken before the clamp was applied or after several hours of perfusion in control experiments. The renin content of the venous plasma was regularly increased several fold after about 90 minutes of perfusion at reduced pulse pressure, while blood flow and mean pressure were maintained at or near their former levels. This increased liberation of renin was also demonstrated in the two experiments in which renal blood flow increased after clamping. No liberation of renin was observed in a control experiment in which the hind leg of a dog was perfused with blood at reduced pulse pressure. Renin is therefore liberated by the isolated dog's kidney perfused at lowered pulse pressures.

Continued perfusion at low pulse pressures resulted in (*a*) gradual reduction of renal blood flow and (*b*) an increase in mean arterial pressure distal to the clamp, apparently the results of renal vasoconstriction. Similar constriction did not appear in 7 control experiments in which the kidneys were perfused for as long a time, but in which the clamp was not applied. Since, then, these changes are not the artefacts of prolonged perfusion, they probably result from accumulation of renin in the kidney. It is therefore not unlikely that they are due to constriction of glomerular efferent arterioles, caused by the liberation of angiotonin, which has been shown to result from infusion of renin or angiotonin into normal dogs with explanted kidneys (Corcoran and Page, 1940).

If it be true that experimental renal hypertension is caused by liberation of renin from the kidneys, it appears from these experiments that adequate reduction of renal arterial pulse pressure is the necessary stimulus. Reduction of renal blood flow may follow, either as a result of the operation, or, gradually, as angiotonin exerts its effect on the efferent arterioles. As has been the experience of most investigators who have used Goldblatt's (1934) method of obtaining renal hypertension, best results are obtained at a critical level of arterial compression. If this level is not reached, hypertension is slight and transient, or does not appear, while if it is exceeded, and a severe reduction of renal blood flow occurs, *in vivo* ischemic atrophy and uremia result.

Within the parenchyma of the kidney, pulse pressure probably falls rapidly as the blood passes from small arteries to arterioles. Any additional obstruction to the flow of blood, as, for instance, pressure on the parenchyma, would greatly decrease pulse pressure thus supplying the stimulus

to renin liberation. This, we believe, offers a reasonable explanation for the ease with which hypertension is produced by pressure on or constriction of the renal parenchyma as in the cellophane method for producing hypertension (Page, 1939, *a*).

The mechanism by which reduction of pulse pressure leads to the liberation of renin from the kidneys is a matter of conjecture. Smoothing of the pulse wave must ultimately lead to replacement of pulsatile by continuous flow. The effects of continuous flow of blood have not been extensively studied in intact animals. However, it is well known that perfused organs rapidly develop morbid changes when continuous is substituted for pulsatile pressure. McMaster and Parsons (1938) have shown that edema occurs rapidly in tissues thus perfused because pulsatile pressure is one of the chief forces in draining lymph from the intercellular spaces. A similar mechanism probably obtains in the perfused kidney.

Evidence of renal anoxia in hypertension is conflicting. The oxygen consumption of cortical tissue is believed to be reduced in experimental hypertension (Gerbi, Rubenstein, and Goldblatt, 1940), although this view is contradicted by Mason, Blalock, and Robinson (1940). Arteriovenous oxygen differences do not differ significantly from the normal in experimental hypertension (Levy, Light, and Blalock, 1938). No unequivocal interpretation of these observations can be made. However, some degree of ischemia of the renal tubular mass is regularly present in hypertension in human beings (Smith, 1939). It is not unlikely that the renal tubular tissue in experimental hypertensive animals is also anoxic. Since renin is a protein-like substance and of high molecular weight, it would not be anticipated that it would normally diffuse with facility through the membranes of the cells of the tubules unless some change occurred which increased their permeability. Anoxia is one of the most important of such changes and may be a factor in the release of renin.

To summarize these speculations: reduction of pulse pressure leads to partial conversion of pulsatile to continuous blood flow in the kidneys with edema and anoxia of the cells of the tubules as the chief results. Increase in cellular membrane permeability follows and allows the liberation of the large renin molecule. Renin reacts with renin-activator to produce angiotonin which itself raises blood pressure and causes efferent glomerular arteriolar constriction and further tubular anoxia. A vicious circle may be thus set up which results in sustained arterial hypertension.

Renal venous blood from dogs made hypertensive either by silk perinephritis or clamping the renal artery also contains greater than normal amounts of renin as demonstrated by addition of renin-activator and per-

fusing the mixture through an isolated rabbit's ear (Page, 1940). The demonstration of increased liberation of renin from perfused kidneys with reduced pulse pressure and from kidneys of intact hypertensive animals adds weight to the belief that experimental hypertension is due to the action of renin. Stimulus to this process lies apparently in a reduction of pulse pressure within the kidney.

SUMMARY

1. Isolated dogs' kidneys have been perfused with defibrinated blood under hemodynamic conditions similar to those in the body. Under these circumstances blood flow, urine secretion, and oxygen consumption are well maintained, but urea clearance is low. Renal venous blood collected initially and at the end of 3 or more hours of perfusion exhibited no difference in vasoconstriction properties when perfused along with renin or renin-activator through an isolated rabbit's ear.

2. Reduction of pulse pressure by constricting the renal artery may be performed without reducing mean pressure significantly. Impairment of urea clearance and rate of urine secretion follow, and oxygen consumption is slightly reduced.

3. After an hour or more of perfusion with reduced pulse pressure, gradual rise in mean renal arterial pressure distal to the clamp and reduction of blood flow occur.

4. Renal venous blood collected after about one hour of perfusion with reduced pulse pressure differs from that collected before reduction of pulse pressure in that it causes intense vasoconstriction when perfused with renin-activator through an isolated rabbit's ear.

5. Perfusion of a dog's hind leg under similar circumstances does not cause this change in the venous blood to occur.

CONCLUSIONS

It is possible to demonstrate increased liberation of renin from the kidneys in the renal venous blood by addition of renin-activator and perfusion of the mixture through isolated organs. Kidneys perfused under what appear to be normal hemodynamic conditions liberate little of it. Reduction of pulse pressure is the stimulus eliciting the outpouring of renin. Reduction of blood flow follows but appears to be an effect rather than the cause of the increased liberation of renin.

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