

## STUDIES ON EXPERIMENTAL HYPERTENSION

### I. THE PRODUCTION OF PERSISTENT ELEVATION OF SYSTOLIC BLOOD PRESSURE BY MEANS OF RENAL ISCHEMIA\*†

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PLATES 23 AND 24

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The production of elevated blood pressure in animals has been attempted (1-16) by various methods involving injury to the kidneys. In the experiments of long duration (7-16), for the purpose of producing persistent hypertension, the methods used were injection of nephrotoxic substances (7), irradiation of the kidneys by Roentgen rays (8), renal venous stasis (9-11) and excision of varying amounts of kidney tissue, with or without ligation of some branches of the renal arteries (12-16). The elevation of blood pressure which occurred as a result of some of these methods did not prove persistent. Cash (15), one of those who used the method mentioned last, drew attention to the fact that the increase of pressure occurred only when some necrotic kidney tissue was undergoing absorption within the body. He gave as the conditions under which he observed temporary elevation of blood pressure in dogs, that the total kidney substance be reduced at least 50 per cent and that, in addition a portion of kidney which has been deprived of its circulation be allowed to remain *in situ*.

Although it has been suggested (Fahr (17)) that renal ischemia, by itself, may play an important part in the development of the hypertension which is associated with more or less diffuse vascular disease in man, yet, up to the present time, the validity of this contention has

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not been investigated experimentally in animals by a study designed to test the effect on blood pressure of renal ischemia alone.

In the investigation here reported the working hypothesis adopted was that ischemia *limited to the kidneys* may be the initial condition in the pathogenesis of the hypertension that is associated with nephrosclerosis. If this be true, then renal ischemia, no matter how produced, should be followed by elevation of blood pressure. This report deals with the effect on the blood pressure of dogs of experimentally produced ischemia limited to the kidneys. The simplest method for this purpose being obviously constriction of the main renal arteries, this was the method chosen.

#### *Method*

*Animals and Diet.*—Dogs of various mixed breeds were used. The animals, all females, varied in weight and age. Their exact age was not known but they were all full grown, seemingly normal dogs. Examination of blood pressure, blood and urine during a long control period revealed nothing to indicate renal disease. They were kept in individual roomy cubicles and fed throughout the entire experimental period on purina dog chow, a complete food adequate to maintain adult dogs in good state of nutrition. The amount of water was not limited.

To effect a narrowing of the main renal artery a clamp was devised whereby the degree of constriction of the vessel could be varied and controlled. Various contrivances were devised for this purpose but the clamp illustrated diagrammatically in Text-fig. 1, was finally found the most satisfactory.

*The Clamp.*—The entire clamp is made of one type of pure silver.<sup>1</sup> The sides and back (*B*) are 0.75 mm., the compressing plate (*C*) 0.75 mm. and the removable plate (*D*) 0.5 mm. in thickness. The double acting screw (*A*) whereby the compressing plate is moved is made of round silver wire measuring 3 mm. in diameter. The chamber of the clamp found most suitable for dogs weighing between 10 and 20 kilos measures 3 x 3 x 6 mm.

*Instruments for the Application of the Clamp.*—For the purpose of applying the clamp to the renal artery, which necessitates working at considerable depth, retroperitoneally, special instruments were devised, which are illustrated diagrammatically in Text-figs. 2, 3 and 4. The instrument for holding the clamp while it is being applied to the vessel is shown in Text-fig. 2. The device for the insertion of the removable plate (*D*, Text-fig. 1) which helps to encase the renal vessel in the

<sup>1</sup> Obtained from Baker and Co., Murray and Austin Streets, Newark.

clamp is shown in Text-fig. 3. Text-fig. 4 is a screw-driver with which to screw down the compressing plate (*C*) and turn the retaining screw (*N*) which fixes the clamp in the holder or releases it after application to the vessel.

*Application of the Clamp.*—The clamp, without the removable plate (*D*), is placed with the head of the double acting screw (*A*) pointing downward in the lower part of the clamp holder (Text-fig. 2, position 1) and held firmly in place by means of the retaining screw (*N*). The portion of the renal artery dissected out for the purpose is lifted into the upturned clamp and is then encased in the clamp by the insertion of the removable plate (*D*) into the clamp by means of the special instrument illustrated in Text-fig. 3. The removable plate is held tightly in the jaws of this instrument while it is being inserted in the clamp. In order to be able to compress the vessel to the desired degree by means of the compressing plate (*C*), the clamp holder is so fashioned that pressure upon the knob (*H*) in the handle of the clamp holder (Text-fig. 2) releases the part that holds the clamp so that it turns over on a hinge and inverts the clamp (Text-fig. 2, positions 1, 2 and 3). This brings the head of the screw (*A*) of the clamp uppermost (Text-fig. 2, position 3) and makes it accessible for the purpose of screwing down the compressing plate (*C*). When this has been accomplished, the retaining screw (*N*) is turned back and the clamp, thus released, is easily pushed out of the holder and left on the vessel which it encases and constricts to the desired degree.

#### *Effect on Blood Flow of Constriction of Main Renal Artery*

Several experiments were performed to determine approximately the effect of various degrees of clamping of the main renal artery on blood flow through the kidney. A description of one of these experiments will be given. With the animal under chloralose anesthesia, a T cannula was inserted in the main renal vein which permitted the onflow of blood through the vein or the shunting of the stream and collection of blood for the purpose of measurement of rate of flow. A 3 minute period was adopted for the individual determinations. To avoid possible effects of loss of blood on blood pressure and blood flow, the blood was immediately returned to the body through a cannula in the jugular vein. The mean blood pressure in the carotid artery was determined throughout the experiment by means of a mercury manometer and recording apparatus. A clamp was applied to the renal artery, and, after determinations of flow had been made without compression of the renal artery, the clamp was tightened to various degrees and measurements of blood flow were again made. Table I illustrates the results of one of these experiments. The effects of constriction of the renal artery are obvious. In the animals with

renal ischemia that were permitted to survive, determinations of blood flow through the kidneys could not be made. Since the size of the renal arteries and the blood pressure varied in different animals, even when they were of about equal weight, the same degree of clamping in different dogs undoubtedly had different effects on blood flow. It follows that the degree of ischemia could not be standardized and the exact extent was not known. A rough estimate of slight, moderate, severe, almost complete and complete constriction was made by observation and palpation of the vessel.

*Surgical Procedure for the Production of Renal Ischemia*

All operations were performed under aseptic conditions, with the animal under ether anesthesia, after a hypodermic injection of morphine and atropine. A lumbar incision was made and, by retroperitoneal approach, the main renal artery was identified and dissected out clean, near its origin from the aorta, for a distance sufficient to permit the application of the clamp. No attempt was made completely to denervate the kidney, but the nerve fibers around the artery were sectioned. During the application of the clamp, care was taken in manipulating the artery to avoid at any time complete obstruction of the renal circulation. The method of application of the clamp is described above. Silk sutures were used for closure of the wound.

In most of the animals a clamp was first applied to the main artery of only one kidney and, after an interval of about 2 weeks, or longer, the artery of the other kidney was constricted. In most of the dogs, in the beginning, the clamps were applied so as to produce only moderate constriction of the vessels of both kidneys and subsequently they were tightened one or more times to effect a greater degree of stenosis.

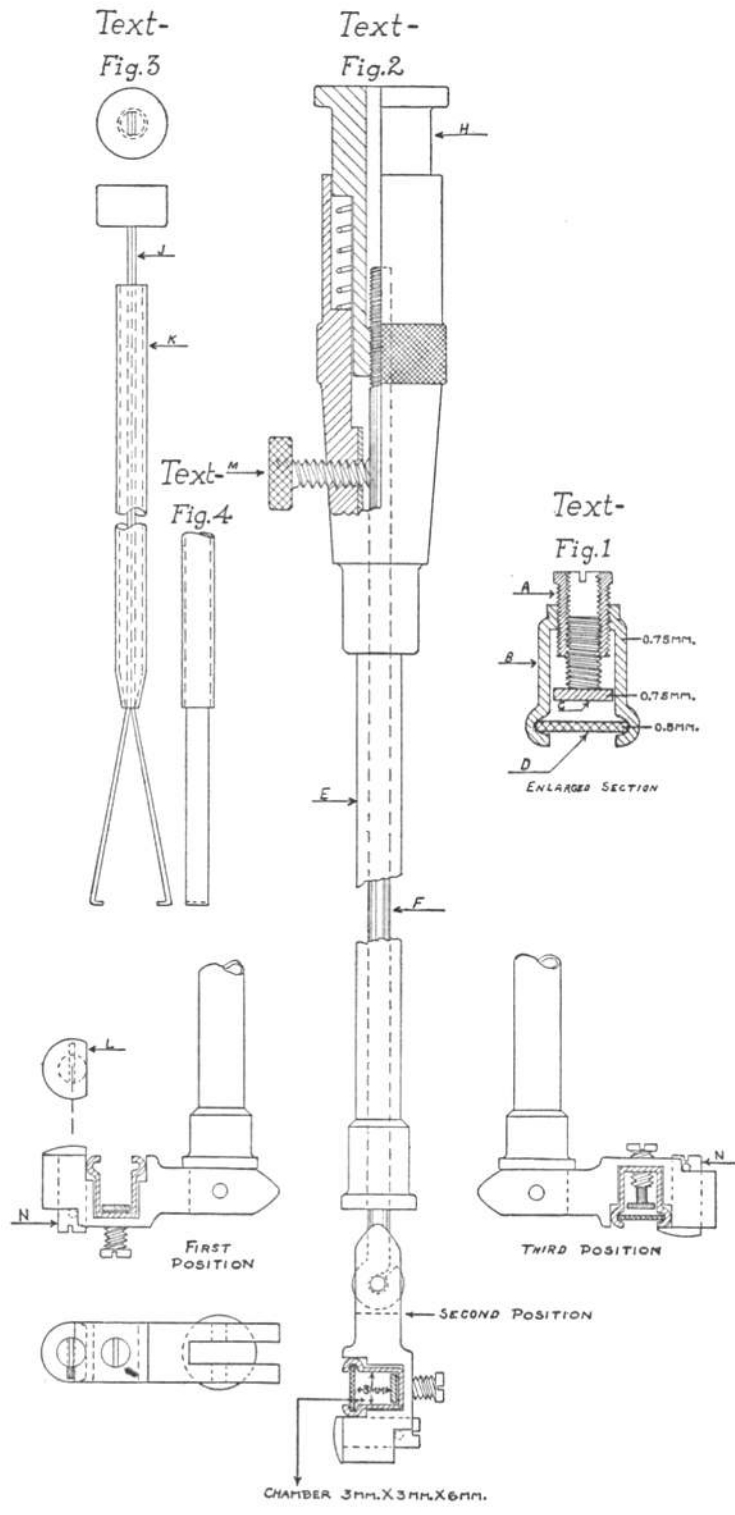
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TEXT-FIG. 1. The clamp. *A*, screw which carries the movable plate; *C*, movable plate; *D*, removable plate.

TEXT-FIG. 2. The clamp holder with clamp inserted. *M*, a screw, the loosening of which permits the knob *H* to be pressed down and makes it possible for the part that holds the clamp to assume any one of the three positions illustrated.

TEXT-FIG. 3. Holder for removable plate *D*, Text-fig. 1. The metal tube *K* slides down or up and tightens or loosens the grip of the metal jaws on the removable plate.

TEXT-FIG. 4. Screw-driver for tightening of screw *A*, Text-fig. 1, which carries the movable plate *C*, Text-fig. 1.



*Method of Determination of Blood Pressure*

In all of the early investigations (1-6) the method of determining blood pressure is open to criticism. This does not apply to most of the more recent studies. In this investigation only the systolic blood pressure in a carotid artery was determined. Carotid loops (left side) were prepared according to the method of Van Leersum (18)

TABLE I

*Effect of Various Degrees of Constriction of the Main Renal Artery on the Outflow of Blood from the Renal Vein*

Degree of constriction of renal artery			
None	Moderate	Severe	Very severe
Outflow of blood from renal vein in 10 sec.			
cc.	cc.	cc.	cc.
13.0	8.5	4.5	1.3
14.5	7.5	4.8	1.0
12.5	8.0	3.6	1.2
13.0	8.0	4.0	1.0
Average outflow per sec.			
1.3	0.8	0.4	0.1

This illustrates the effect of various degrees of constriction of the main renal artery on the outflow of blood from the renal vein and gives some estimate of the corresponding degrees of renal ischemia.

Dog 7. Female. Weight 18 kilos. Anesthesia, ether induction followed by chloralose 0.1 gm. per kilo of body weight. Blood heparinized *in vivo*. Clamp on main renal artery. Cannula in renal vein. Moderate, severe and very severe constriction correspond to 2, 2½ and 3 complete turns respectively of screw A in the clamp. (See Text-fig. 1.)

which permitted the daily determination of systolic blood pressure painlessly and with the animal at rest in a specially designed box. Readings of blood pressure were recorded after the animal had been resting in the box for at least 3 minutes. After this, although the pressure varied, it was not falling steadily, as it usually did during the first minute or two after the animal was placed in the box. Instead of arbitrarily choosing the lowest of a number of determinations as the

representative pressure of the day, a questionable procedure recently suggested (19), we used the method of Dominguez (7) and recorded ten observations daily, at one sitting, all made after the period of rest. The arithmetic mean of these ten readings was considered the resting systolic blood pressure of the day. Pressures were measured at about the same time every day. The blood pressure was determined daily for a control period of at least 2 months before the first operation to produce renal ischemia. In this study all of the blood pressure determinations were made by two observers with whom the animals were familiar. In the case of Nos. 5-8, 5-9, 6-0, 6-1, 8-7 and 8-9 all of the determinations were made by one person. In Animals 2-5, 3-8, 4-9, 5-5 and 5-6 all of the determinations during the control period and for a period of several months after the initial establishment of bilateral renal ischemia and consequent elevation of blood pressure were also made by one person. In this group, during the remainder of the experimental period, the determinations were made by the person who made all of the observations in the first group mentioned above.

*Determination of Renal Function before and after the Production of Renal Ischemia*

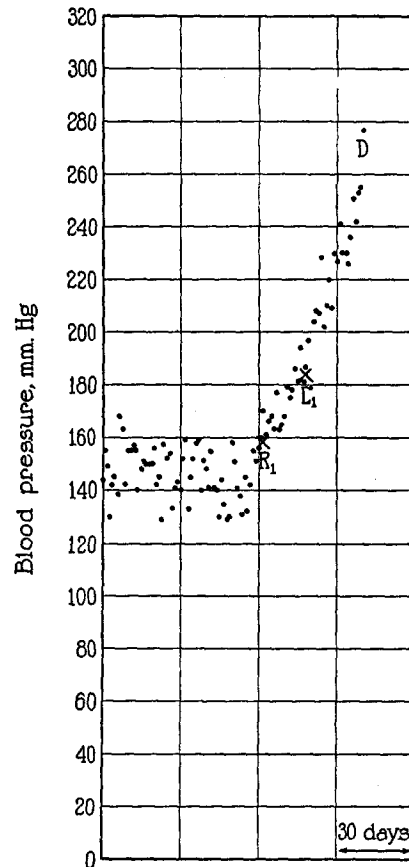
By methods described in detail in a previous study (20) urea clearance and output of phenolsulfonephthalein were determined in all of the animals at frequent intervals before and after the operations. In some of the dogs, in addition to urea, the quantity of total non-protein nitrogen, creatinine and guanidine in the blood was also determined by standard methods.

EFFECT OF RENAL ISCHEMIA ON SYSTOLIC BLOOD PRESSURE AND  
RENAL FUNCTION

Text-figs. 5 to 15 illustrate the individual mean daily systolic blood pressures of all the animals and the urea clearance of some of the animals throughout the entire experimental period. Table II gives the mean systolic blood pressure in monthly periods and Table III gives the mean pressure in the entire control period and in the entire period after the initial constriction of both renal vessels.

*Almost Complete Constriction of Both Renal Arteries with a Short Interval between Operations*

In Animal 2-5 (Text-fig. 5 and Table II) the left renal artery was almost completely occluded at the first operation. Systolic blood



TEXT-FIG. 5. Dog 2-5. Initial weight 12.6 kilos. Final weight 11.2 kilos. R<sub>1</sub>, almost complete constriction of main right renal artery; L<sub>1</sub>, almost complete constriction of main left renal artery; D, died in uremia.

pressure soon rose and remained elevated for 16 days. Then the right renal artery was almost completely obstructed. Text-fig. 5 is a record of the systolic blood pressure before and after the operations. The

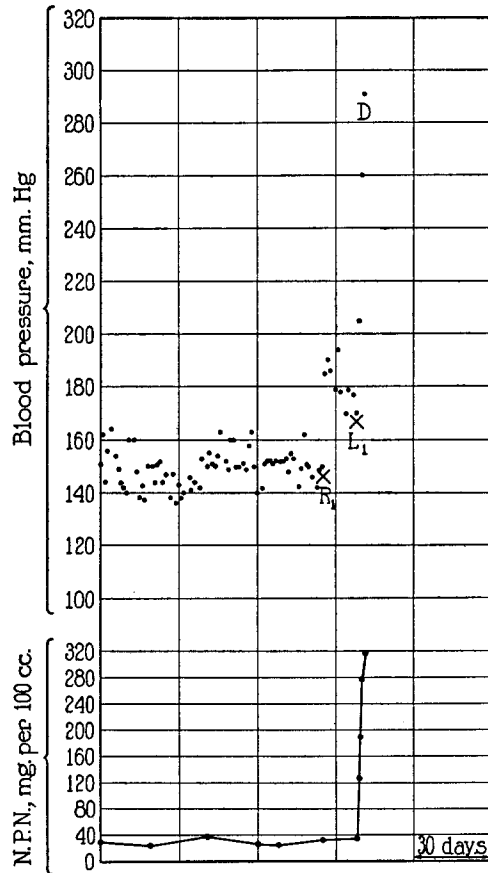


TABLE II  
Mean Daily Systolic Blood Pressures during the Entire Experimental Periods

Dog No.	Months																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
2-5	148	145	R <sub>1</sub> 174	L <sub>1</sub> 225		279	271	274	262	256	253	248	262	238	248	255	250	239
3-8	165	161	L <sub>1</sub> 174	R <sub>1</sub> 196	254	R <sub>2</sub> 216	208	L <sub>2</sub> 206	L <sub>2</sub> 222	211	203	L <sub>2</sub> 235	217	2	3	210		
4-9	157	162	L <sub>1</sub> 184	R <sub>1</sub> 217	209	R <sub>2</sub> 216	R <sub>1</sub> 197	L <sub>1</sub> 200	R <sub>2</sub> 201	L <sub>2</sub> 219	200	190						
5-5	160	154	154	164	169	173	200	L <sub>2</sub> 207	R <sub>2</sub> 214	202	194	L <sub>2</sub> 214	R <sub>2</sub> 214					
5-6	152	157	154	R <sub>1</sub> 188	204	L <sub>1</sub> 208	200	L <sub>2</sub> 207	R <sub>2</sub> 214	202	194	L <sub>2</sub> 214	R <sub>2</sub> 214					
5-8	161	148	140	R <sub>1</sub> 187	200	L <sub>1</sub> 222	219	216	212	201	205	212	N(L) <sub>2</sub> 228	212	204	208		
5-9	172	157	164	176	177	R <sub>1</sub> 232	L <sub>1</sub> 243	226	240	227	R <sub>2</sub> 241	L <sub>2</sub> 228	231	233				
6-0	184	180	175	190	196	S <sub>b</sub> 190	196	F 198	R <sub>1</sub> 239	L <sub>1</sub> 251	221	213	L <sub>2</sub> 231	226	215	215	221	
6-1	188	185	195	S 192	E 198	192	R <sub>1</sub> 199	L <sub>1</sub> 228	R <sub>2</sub> 235	222	214	209						
8-7	154	152	155	R <sub>1</sub> 181	L <sub>1</sub> 262													
8-9	177	169	166	A <sub>1</sub> 166	A <sub>2</sub> 163	R <sub>1</sub> 200	190	L <sub>1</sub> 213	226	212								

The experimental periods are divided into intervals of 1 month. In a few instances, as can be seen in Text-figs. 5 to 15, the period is less than 1 month and usually represents either a shorter interval between operations, or one at the end of the experiment. All of the symbols have the same significance as in Text-figs. 5 to 15. Their position indicates that the procedure which they represent was carried out at the beginning of the period in which they are found.

blood pressure continued to rise following the second operation and finally reached a level almost double the mean pressure during the control period.



TEXT-FIG. 6. Dog 8-7. Initial weight 14.0 kilos. Final weight 14.2 kilos. R<sub>1</sub>, almost complete constriction of right main renal artery; L<sub>1</sub>, almost complete constriction of left main renal artery; D, died in uremia.

Quite suddenly, 2 days before death, the animal developed convulsions. Urea clearance, previously not materially affected, was diminished almost to zero. Blood urea and non-protein nitrogen were greatly elevated. There was a large amount of albumin in the urine. The animal became comatose and died in obvious uremia.

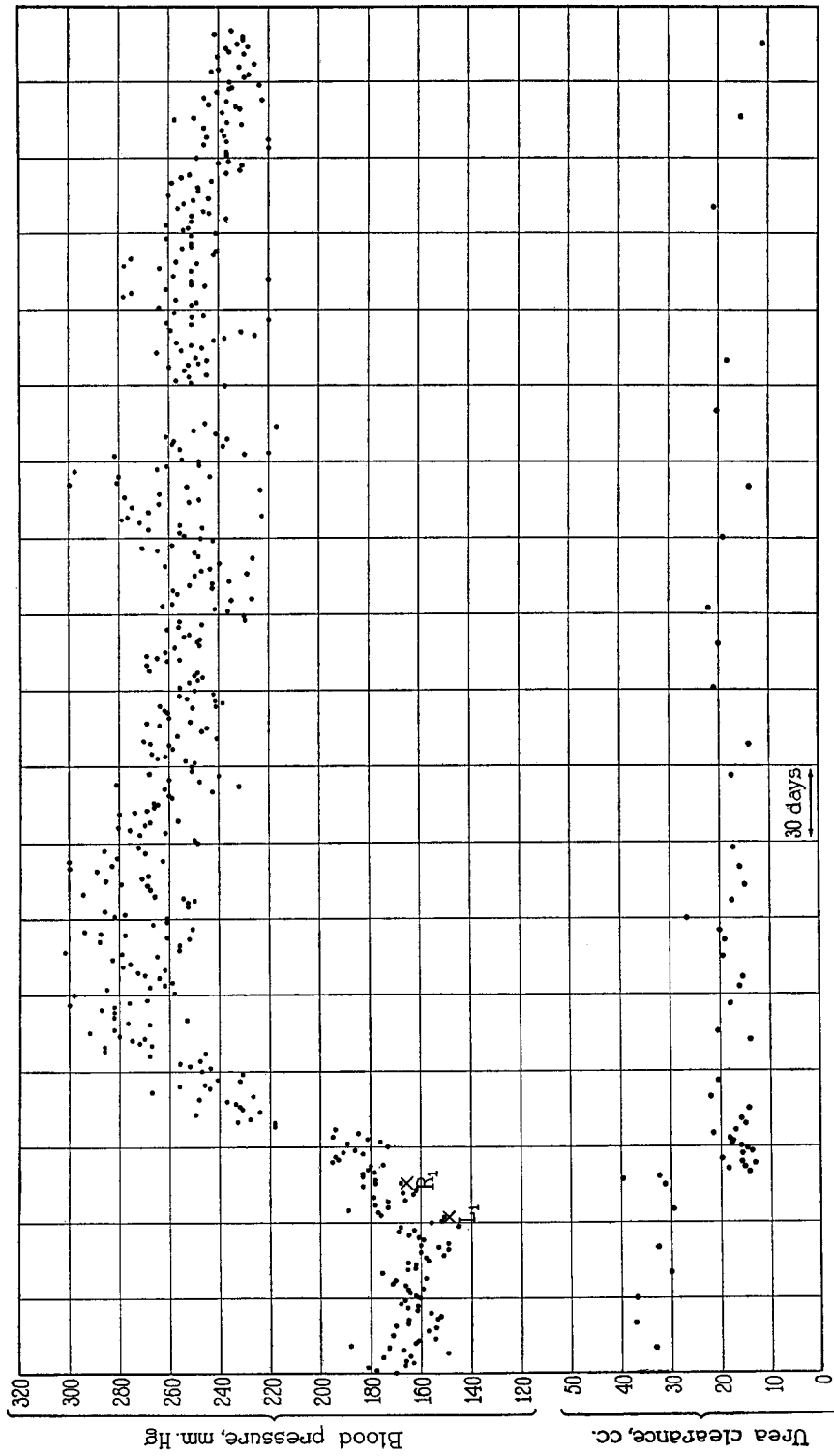
In the gross, the kidneys showed a moderate degree of cloudy swelling and there were two small conical areas of hemorrhagic necrosis in the cortex of the right kidney. Microscopically, in both kidneys, there was severe diffuse parenchymatous degeneration, most severe in the convoluted tubules. The infarcts of the right kidney proved to be very recent. Thrombi in small branches of the renal artery were probably present but were not demonstrated in the sections made. There were no other pathological manifestations of significance in the body.

In Animal 8-7 (Text-fig. 6 and Table II) both renal arteries were almost completely constricted, with an interval of 10 days between the two operations. After almost complete constriction of the right renal artery, the blood pressure rose and remained moderately elevated for a few days but tended then to return toward the original level. However, after almost complete constriction of the other (left) renal artery, the blood pressure soon rose again and kept increasing daily until death which occurred on the 4th day following the constriction of the second renal artery.

Urea clearance was greatly decreased after the clamping of the second renal artery and the quantities of urea nitrogen, total non-protein nitrogen, creatinine and guanidine in the blood kept increasing daily until death. There was a large amount of albumin in the urine. The animal became comatose 2 days before death, had frequent convulsions and died in obvious uremia. The kidneys were similar to those of Dog 2-5 except for the absence of infarcts.

#### *Severe Constriction of Both Renal Arteries with a Short Interval Between Operations*

In Animal 3-8 (Text-fig. 7 and Table II) the main renal artery of the left kidney was first constricted, but the clamping, though severe, was not made as tight as in Dogs 2-5 and 8-7, reported above. For 14 days following this operation there was a barely significant rise of blood pressure. Then the right main renal artery was constricted to about the same degree as the left. Following this, the pressure kept gradually increasing for over a month until it reached a high level. With considerable variations it remained at this level for about 11 months. During the next 4 months it tended toward a lower level. The animal is still alive 15 months after the constriction of both renal arteries and the daily mean systolic blood pressure is still at least 60 mm. Hg higher than the mean pressure during the control period.



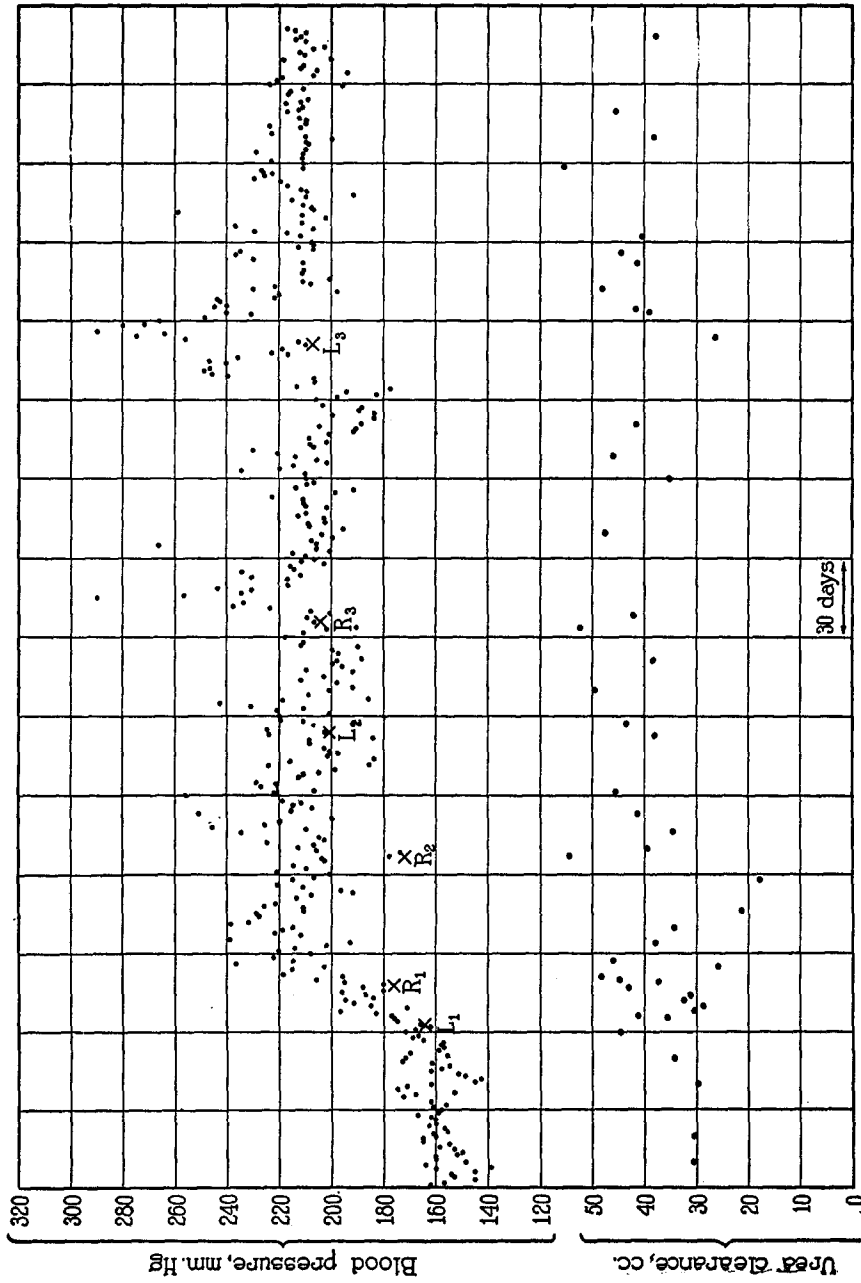
TEXT-FIG. 7. Dog 3-8. Initial weight 11.2 kilos. Final weight 7.6 kilos. L<sub>1</sub>, severe constriction of left main renal artery; R<sub>1</sub>, severe constriction of right main renal artery. The animal is still alive.

After the clamping of the second renal artery, urea clearance promptly became reduced to about 50 per cent of the average value during the control period (Text-fig. 7 and Table II) and, with variations, has remained at this reduced level. However, the output of phenolsulfonephthalein was not significantly affected. Throughout the experimental period the amount of creatinine, total non-protein nitrogen and guanidine in the blood remained within the limits of the control period.

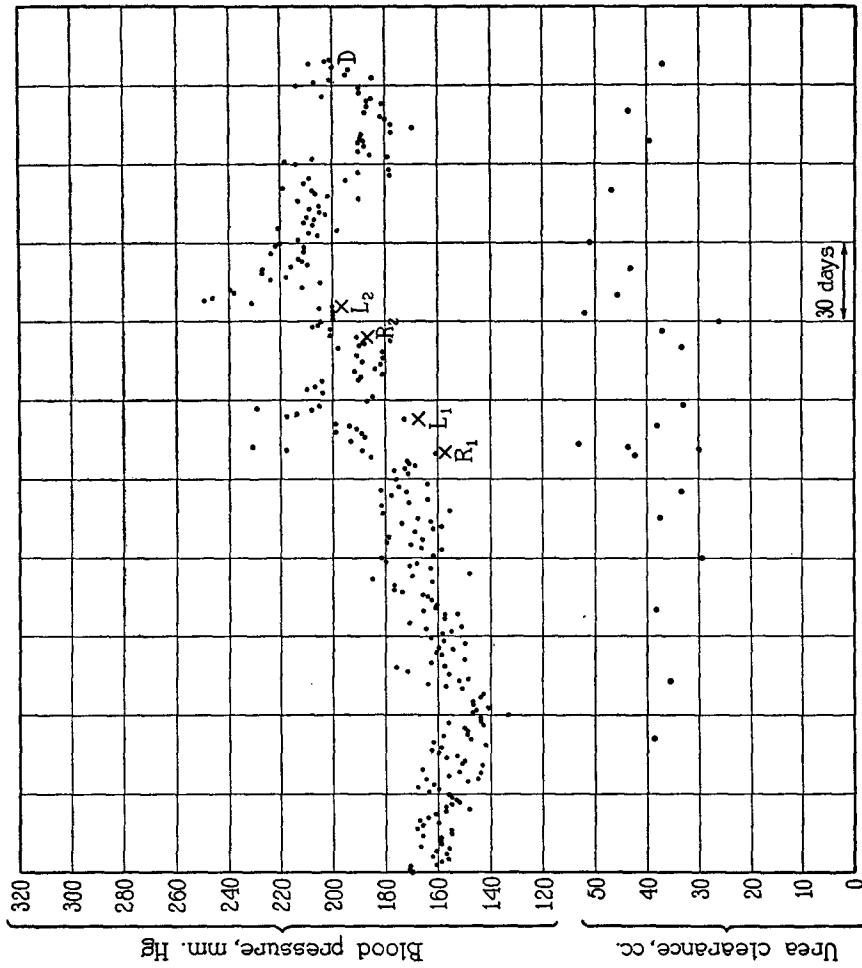
*Moderate Constriction of Both Renal Arteries, Subsequently Increased*

In Animals 4-9, 5-5, 5-6, 5-9 and 6-1 (Text-figs. 8 to 12, and Table II) the initial constriction of one renal artery was moderate. After an interval which varied from 11 days to 7 weeks the other main renal artery was constricted to about the same degree. In Animal 6-1 (see *S*, Text-fig. 12) at the first operation, clamps were applied to both main renal arteries, but the vessels were not constricted. Later the animal was again given anesthesia for a period of 2 hours (see *E*, Text-fig. 12). There was no elevation of blood pressure following either of these procedures. In this animal, and in the others in this group, following the constriction of one renal artery, there was a significant but variable rise of systolic blood pressure which tended to return to a lower, if not to the original level. Following the constriction of the artery of the other kidney, there occurred further increase of systolic blood pressure which remained elevated as compared with the normal but in some, after varying intervals, tended to return to a lower though not to the original level. Subsequently, after varying intervals, whether or not there was a tendency for the elevated pressure to become lower, both clamps were tightened one or more times. In one animal, No. 5-6, the stenosis of both vessels was finally made complete. (Text-fig. 10 and Table II). In all of these animals the daily blood pressure remained elevated above not only the mean pressure of the entire control period but also well above the highest values obtained during this period. These animals have been observed for periods varying from 5 months to 1 year, following the initial production of bilateral renal ischemia.

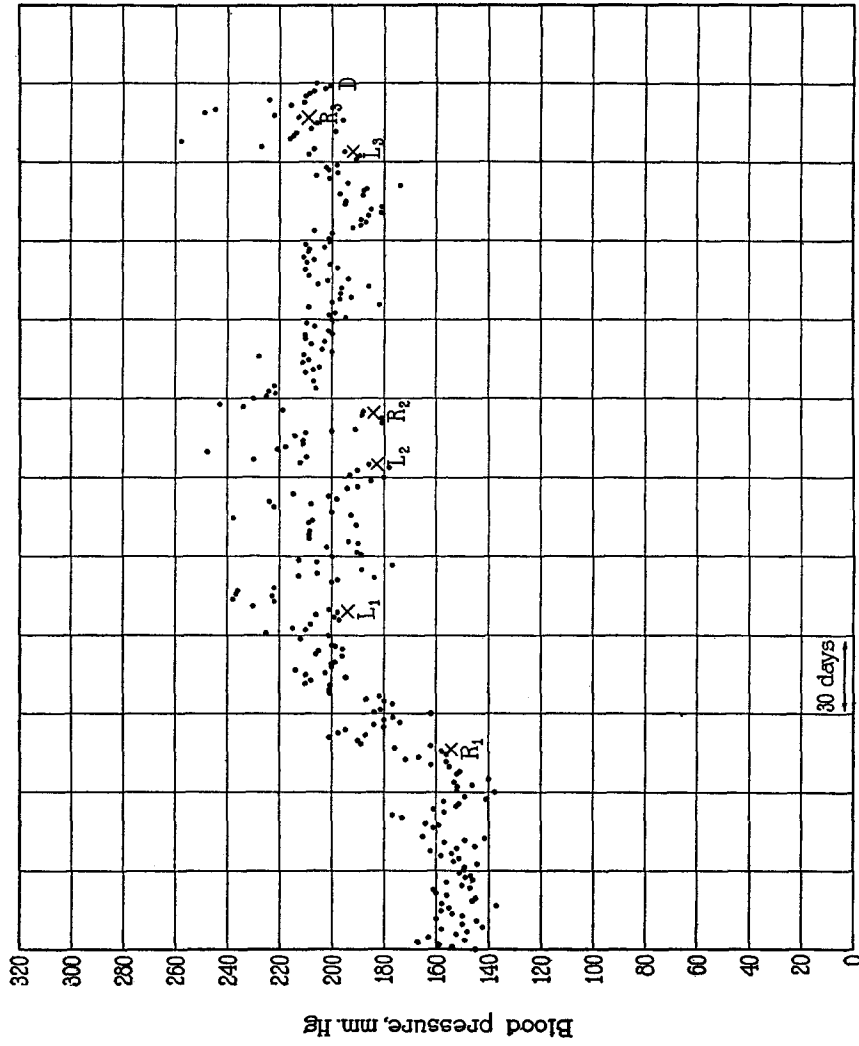
In No. 5-6, urea clearance and output of phenolsulfonephthalein were not studied because collection of urine was very difficult. In the other animals urea clearance varied greatly following the clamping of the vessels, but there was no permanently significant change and, at the end of the experimental period, the level



TEXT-FIG. 8. Dog 4-9. Initial weight 16 kilos. Final weight 17.6 kilos. L<sub>1</sub> and R<sub>1</sub>, moderate constriction of left and right main renal arteries; L<sub>2</sub> and R<sub>2</sub>, constriction of left and right main renal arteries increased to severe; L<sub>3</sub> and R<sub>3</sub>, constriction of right and left main renal arteries again increased to almost complete. The animal is dead. It was used for the experiment to test the effect of compression of the carotid loop on systemic blood pressure.

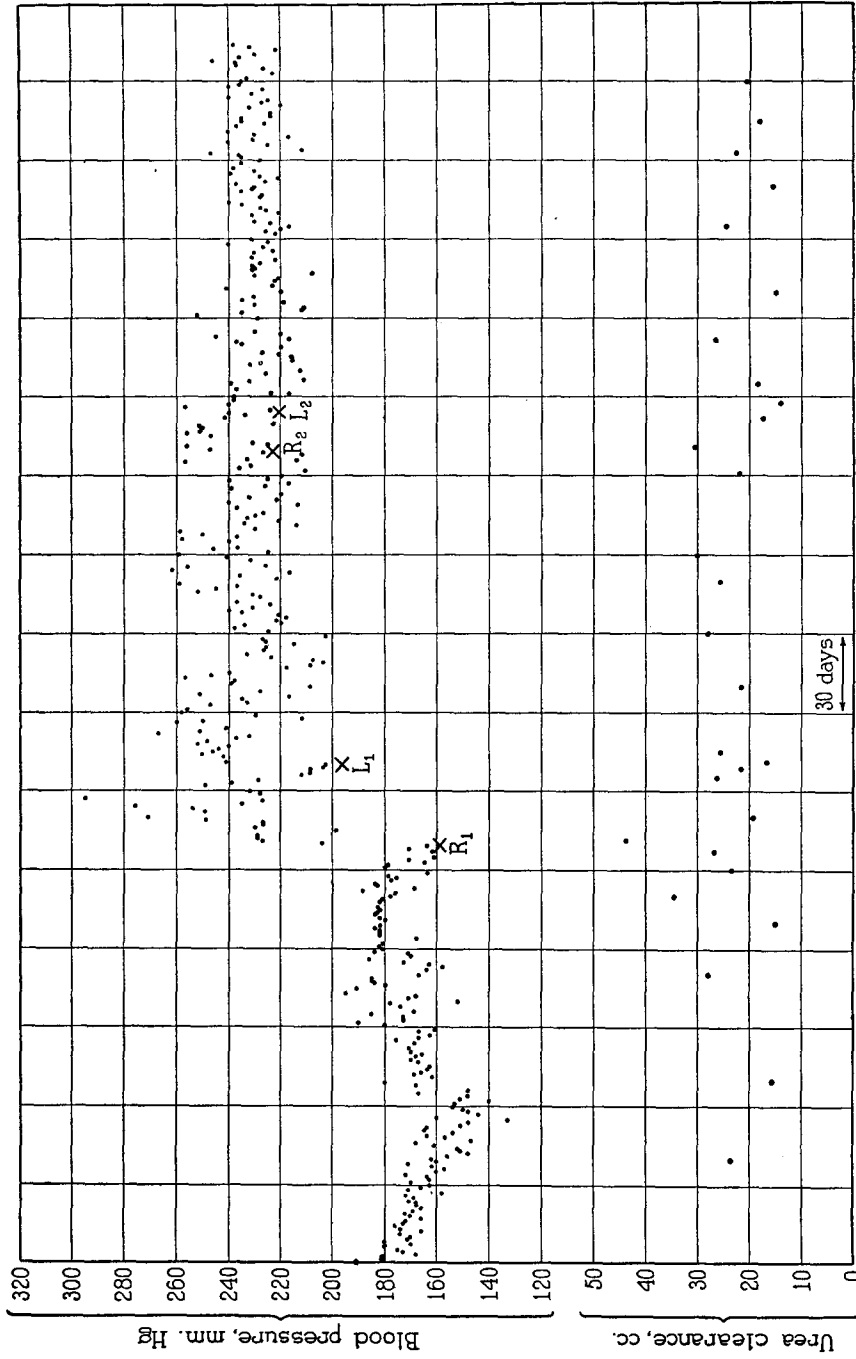


TEXT-FIG. 9. Dog 5-5. Initial weight 14.4 kilos. Final weight 16.4 kilos. R<sub>1</sub> and L<sub>1</sub>, moderate constriction of right and left main renal arteries; R<sub>2</sub> and L<sub>2</sub>, constriction of right and left main renal arteries increased to very severe; D, the animal died during anesthesia for proposed unilateral nephrectomy.

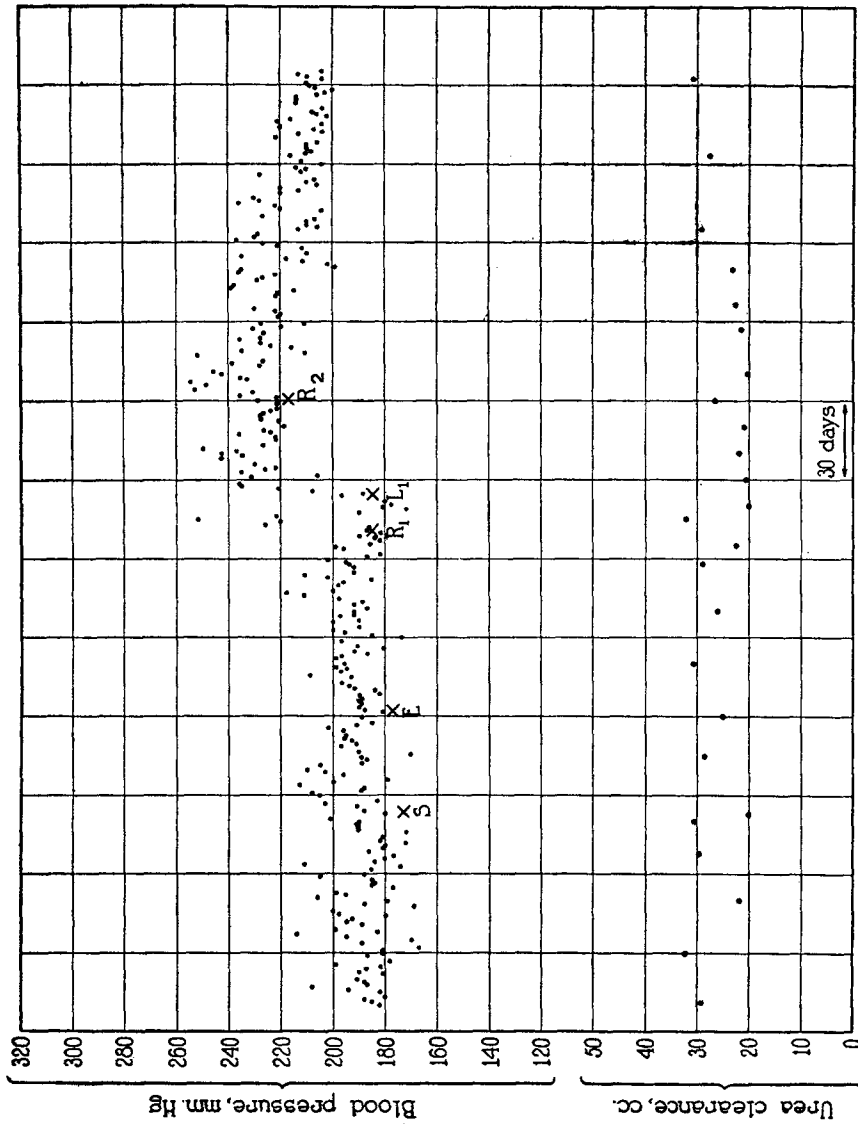


TEXT-FIG. 10. Dog 5-6. Initial weight 12 kilos. Final weight 11.6 kilos.  $R_1$  and  $L_1$ , moderate constriction of main right and left renal arteries;  $L_2$  and  $R_2$ , constriction of both arteries increased to severe;  $L_3$  and  $R_3$ , constriction of both arteries made complete; D, the animal died during anesthesia for proposed unilateral nephrectomy.





TEXT-Fig. 11. Dog 5-9. Initial weight 12.4 kilos. Final weight 13.2 kilos. R<sub>1</sub> and L<sub>1</sub>, moderate constriction of right and left main renal arteries; R<sub>2</sub> and L<sub>2</sub>, constriction of right and left main renal arteries increased to very severe. The animal is still alive.



TEXT-FIG. 12. Dog 6-1. Initial weight 21.4 kilos. Final weight 20.6 kilos. S, surgical operation under anesthesia, for exposure of renal arteries and application of clamps without constriction of vessels; E, ether anesthesia for 2 hours; R<sub>1</sub> and L<sub>1</sub>, moderate constriction of right and left main renal arteries; R<sub>2</sub>, constriction of right main renal artery increased to very severe. The animal is still alive.

was within the limits of the control values or only slightly reduced. The quantities of urea, creatinine, total non-protein nitrogen and guanidine in the blood varied but were within normal limits throughout the entire experimental period. The output of phenolsulfonephthalein was not significantly affected in any of these dogs following the production of renal ischemia.

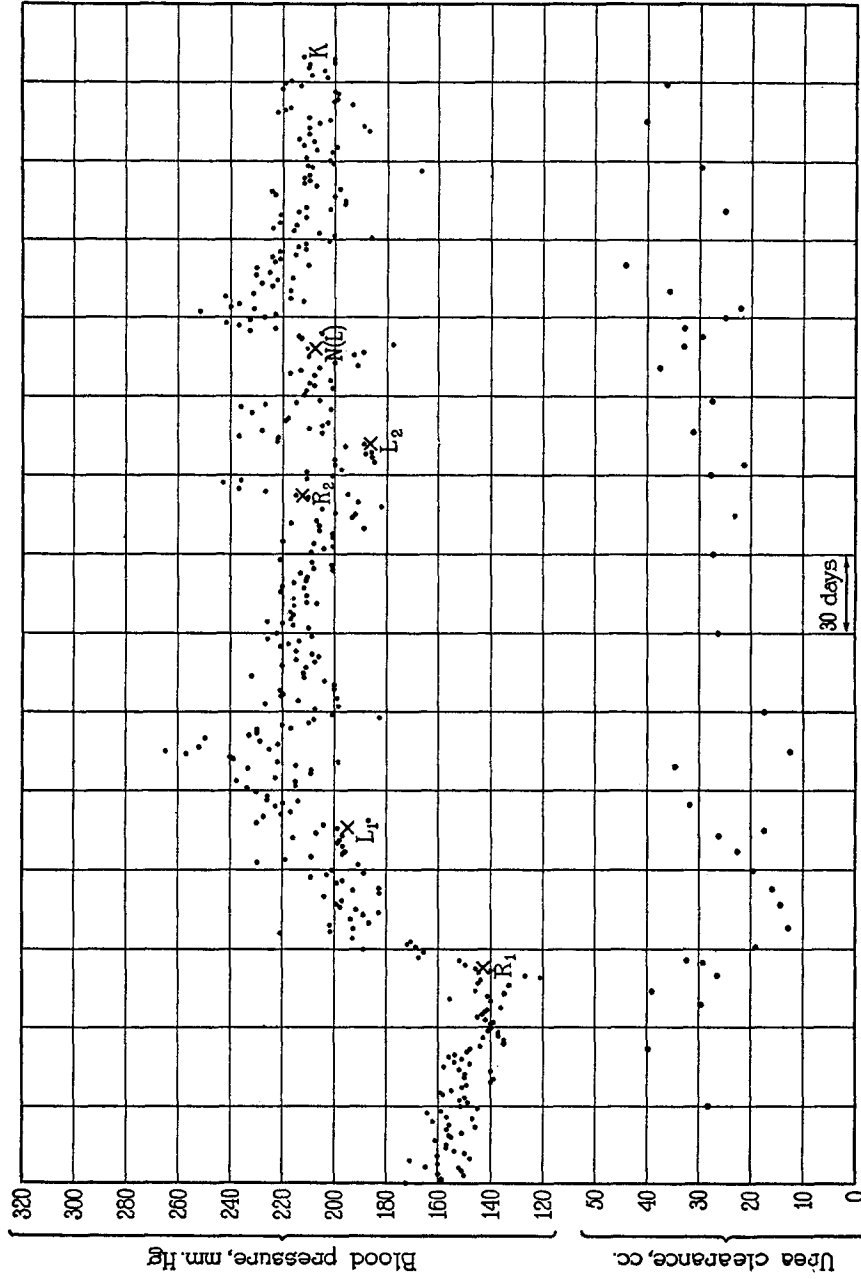
Animals 4-9, 5-5 and 5-6 are dead. Animal 4-9 was sacrificed in the experiment described at the end of this paper for the purpose of determining the effect of compression of the carotid loop on systemic blood pressure. At autopsy, the left kidney weighed 39 gm. and the right 33 gm. The capsule was moderately thickened and quite adherent in both. The microscopic examination has not yet been made.

Animal 5-5 died during anesthesia for proposed unilateral nephrectomy. At autopsy, the kidneys were about the same size as they were when observed at the preceding operations. The right weighed 44 gm. and the left 40 gm. Microscopically, both kidneys showed only moderate parenchymatous degeneration and slight thickening of Bowman's capsule and of the basement membrane of some of the glomeruli. There were more and larger vessels in the renal capsule than is usual in a dog's kidney, which probably served as accessory circulation.

Animal 5-6 also died during anesthesia for proposed unilateral nephrectomy. At autopsy, both kidneys were reduced in size. The left weighed only 15 gm. and its size was about one-fourth of what it was when observed at the first operation for the production of ischemia 7 months before death. The right kidney weighed 32 gm. In both kidneys the capsule was thickened. Microscopically, there were many large vessels in the capsule, at least some of which entered the renal substance, and evidently must have acted as a substantial accessory circulation. In both kidneys, but more in the left, there was severe diffuse parenchymatous degeneration which affected most severely the medullary parenchyma, and also diffuse increase of connective tissue. In most of the glomeruli of the left and in many of the right kidney there were greatly thickened basement membranes of the glomerular tufts and thickened Bowman's capsules. In the left kidney many of the interlobular and afferent vessels had thickened walls and the size of the lumen was greatly reduced. In some of the arterioles there was hyaline degeneration of part or of all of the thickened wall. Interstitial fibrosis was greater in the left kidney.

*Bilateral Moderate Renal Ischemia, Later Increased, and, Still Later, Unilateral Nephrectomy*

In Animal 5-8 (Text-fig. 13 and Table II) the original constriction of both main renal arteries and the subsequent tightening of the clamps were of about the same degree as in the preceding group of dogs. Systolic blood pressure showed a significant rise, which persisted, but with a tendency to return to a lower though not the original level.



TEXT-FIG. 13. Dog 5-8. Initial weight 12.6 kilos. Final weight 10.3 kilos. R<sub>1</sub> and L<sub>1</sub>, moderate constriction of right and left main renal arteries; R<sub>2</sub> and L<sub>2</sub>, constriction of right and left main renal arteries increased to very severe; N (L), left nephrectomy; K, animal killed.

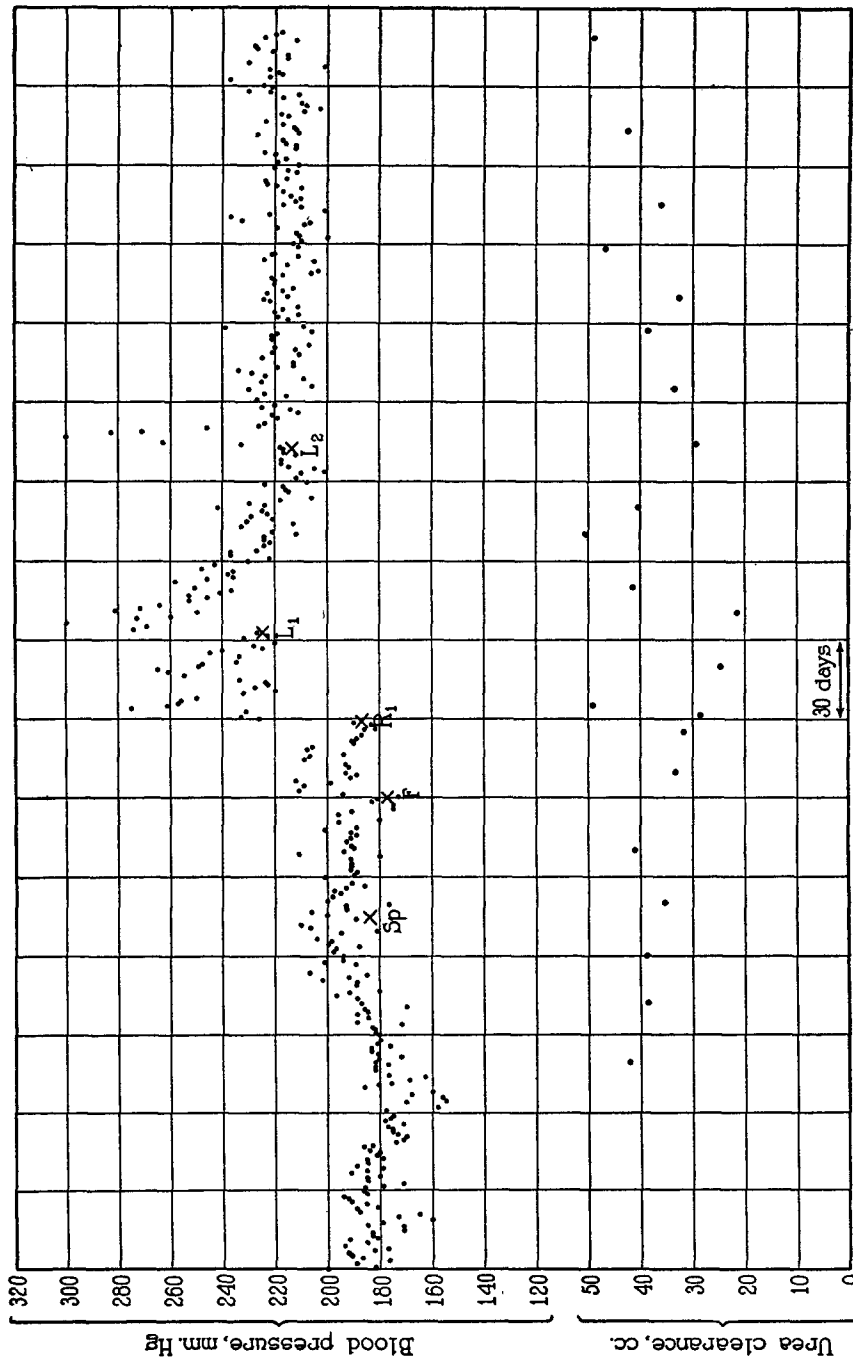
6 weeks after the last tightening of the clamps the left kidney was removed. This was followed by a significant but slight further increase of blood pressure which did not persist at that level. However, the blood pressure continued to be definitely elevated as compared with the control period.

Urea clearance was moderately reduced following the initial clamping of the right renal artery but soon returned to normal. It was not significantly affected by any of the other procedures and, at the end of the experimental period, the values for the clearance and for urea, creatinine, total non-protein nitrogen and guanidine in the blood were well within the limits of the control period. The output of phenolsulfonephthalein was not significantly affected by any of the procedures. Following the tightening of the clamp a moderate degree of albuminuria developed. This became more severe after removal of the left kidney and persisted to the end. Animal 5-8 was killed 4 months after the removal of the left kidney and 10 months after the initial production of bilateral renal ischemia.

The left kidney, removed surgically, and the right, removed at autopsy, showed gross and microscopic changes similar in most respects to those of Dog 5-6. In the gross, the kidneys were only moderately reduced in size compared with their appearance at the first operation. The right weighed 30 gm. and the left 35 gm. The capsule was moderately thickened. Microscopically, the thickening of Bowman's capsules was particularly striking but, as in No. 5-6, there was also thickening of the basement membrane of glomerular tufts and of the walls of the smaller arteries. There was much less increase of interstitial connective tissue than in the left kidney of No. 5-6.

*The Effect on Blood Pressure of Severe Ischemia in Other Organs,  
Followed by Renal Ischemia*

In Animal 6-0 (Text-fig. 14 and Table II) after the usual control period, the splenic artery was almost completely occluded. 6 weeks later, both femoral arteries were very greatly constricted immediately below Poupart's ligament. There was no significant alteration of systolic blood pressure due to any of these procedures. 1 month later, the main right renal artery was moderately constricted, and 1 month after this the left renal artery was similarly constricted. About 10 weeks later the clamp on the left renal artery was tightened so that the constriction of the vessel was almost complete. A significant but moderate elevation of blood pressure occurred following the initial constriction of both main renal arteries. There was a tendency for the pressure to return to a lower level. It was temporarily in-



TEXT-FIG. 14. Dog 6-0. Initial weight 18.0 kilos. Final weight 19 kilos. Sp, almost complete constriction of main splenic artery; F, very severe constriction of both femoral arteries immediately below Poupart's ligament; R<sub>1</sub> and L<sub>1</sub>, moderate constriction of right and left main renal arteries; L<sub>2</sub> constriction of left main renal artery increased to almost complete. The animal is still alive.

creased by the subsequent tightening of the clamp on the left renal artery. Although it soon returned to a lower level, yet this continued to be significantly but only slightly higher than during the control period. Fig. 1 is a roentgenogram of Animal 6-0, taken during life, with all of the clamps applied.

Urea clearance, the quantity of urea, creatinine, total non-protein nitrogen and guanidine in the blood, and the output of phenolsulfonephthalein were not significantly affected by any of these procedures. At the end of the experimental period their values were about the same as during the control period.

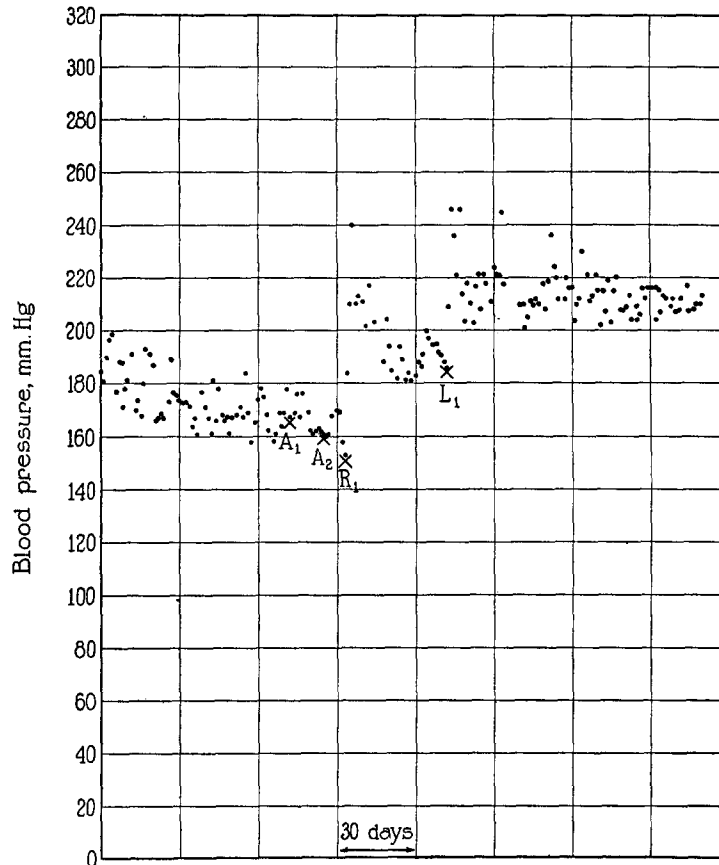
*Renal Ischemia Following Excision of Right Suprarenal Body, Section of Left Splanchnic Nerves, Denervation of the Left Suprarenal Body and Destruction of Its Medulla*

In Animal 8-9 (Text-fig. 15 and Table II) after the usual control period, the right suprarenal body was removed. Under ether 2 weeks later the left major and minor splanchnic nerves were cut, all of the nerve fibers to the left suprarenal body that were recognizable in the gross were severed, and the medulla of the left suprarenal body was removed as completely as possible by means of a burr. The blood pressure remained practically unchanged after these procedures. Then, at separate times, both main renal arteries were moderately constricted by clamps. Following this the systolic blood pressure showed a moderate rise which persisted with some tendency to return to a lower but not to the normal level for this dog. In No. 8-9, for the same reason as in No. 5-6, urea clearance and output of phenolsulfonephthalein were not determined. The quantity of urea, creatinine, total non-protein nitrogen and guanidine in the blood varied but was not significantly affected by any of the procedures.

*The Possible Effect of Compression of the Carotid Loop on Systemic Blood Pressure*

An experiment was performed which was designed to cover the possibility that the elevated systolic blood pressure found in all of the animals during the period following the constriction of both renal arteries was really due in some way to compression of the carotid loop by the cuff used in making the determinations. Although the same method was used during the control period, yet it might be averred

that the clamping of the renal arteries altered the body in some way so as to account for this difference of reaction to a possible reflex from the compressed carotid artery, or to the partial reduction of cerebral



TEXT-FIG. 15. Dog 8-9. Initial weight 13.6 kilos. Final weight 16.2 kilos.  $A_1$ , excision of right suprarenal body;  $A_2$ , destruction of medulla and denervation of left suprarenal body. Section of left major and minor splanchnic nerves;  $R_1$  and  $L_1$ , moderate constriction of the right and left main renal arteries. The animal is still alive.

circulation caused by the repeated, momentary occlusion of the one carotid artery during the determination of blood pressure.

Animal 4-9 was used. At this time this animal was still showing a significant though slight elevation of blood pressure as determined by the carotid loop method.



Under ether anesthesia, after a hypodermic injection of 1/2 grain morphine sulfate and 1/300 grain atropine sulfate, a cannula was inserted in the right femoral artery and connected with a mercury manometer for the purpose of making a kymographic record of the mean blood pressure in this vessel. Through a small incision in the neck, the right common carotid artery was exposed and separated from surrounding tissues. Some determinations of pressure in the carotid loop (left common carotid) were made and the cuff was then left loosely applied to be in

TABLE III

*Mean Daily Systolic Blood Pressure during the Control Period before Clamping Either Renal Artery and during the Entire Period after the Initial Constriction of Both Main Renal Arteries*

Dog No.	Control period			Period after initial constriction of both renal arteries		
	Mean systolic blood pressure	No. of days	No. of observations	Mean systolic blood pressure	No. of days	No. of observations
	<i>mm. Hg</i>			<i>mm. Hg</i>		
2-5	146	62	62	174	21	21
3-8	160	63	63	252	454	345
4-9	159	63	63	215	363	314
5-5	161	161	145	202	138	116
5-6	154	76	76	205	201	172
5-8	150	83	83	214	294	219
5-9	169	148	148	232	274	235
6-0	185	134	121	224	227	194
6-1	191	182	156	221	161	137
8-7	152	86	68	262	4	4
8-9	173	72	65	212	98	87

By number of observations is meant the number of days on which determinations of blood pressure were made. During the control period, in many of the dogs pressures were taken every day of the week, so that the number of days and number of observations coincide. The mean daily systolic blood pressure is the mean of ten observations made at one sitting.

readiness for the compression of the loop. A record of blood pressure in the femoral artery was then made. The mean pressure in the femoral artery immediately before the compression of the carotid loop was 185 mm. of mercury and 205 mm. in the carotid loop. This is about the normal difference between the pressures in these two vessels when both are obtained by direct methods. The carotid loop was then quickly compressed, by pumping air into the cuff around it, so as completely to obstruct the flow of blood through the left carotid artery for 80 seconds. During the compression of the carotid loop (see 1, Fig. 2) the mean

pressure in the right femoral artery showed an insignificant elevation of not more than 5 mm. Hg. After an interval, the freshly exposed right common carotid artery was completely obstructed by compression with a bulldog clamp, the jaws of which were covered with rubber, and, at the same time, the carotid loop (left common carotid) was compressed so as completely to obstruct blood flow through the vessel. There was a prompt but slight (15 mm. Hg) rise of pressure in the femoral artery, which persisted while the carotids were obstructed but promptly returned to the original level when the compression of both vessels was released (see 2, Fig. 2). After an interval of a few minutes the left carotid alone was again completely obstructed by compression of the carotid loop. This time there was practically no effect on the mean blood pressure in the femoral artery (see 3, Fig. 2). The compression of the carotid loop was released and, after an interval, the freshly exposed right carotid alone was clamped. There was a prompt but slight (15 mm. Hg) elevation of mean blood pressure in the femoral artery (see 4, Fig. 2) which persisted until the clamp was removed from the artery, when it promptly returned to the original level. The interesting finding is that the increase of pressure during the obstruction of this freshly exposed right carotid artery alone was exactly equal to the elevation which occurred following the previous compression of both carotid arteries. It follows, therefore, that the compression of the carotid loop contributed little or no effect when both vessels were compressed at the same time.

All of these observations were repeated with exactly the same results (see 5-9, Fig. 2). Even ten quickly repeated occlusions of the left carotid artery in the carotid loop (see 6, Fig. 2) failed to have a significant effect on the pressure in the femoral artery. Constriction of the freshly exposed right carotid artery with or without coincident compression of the carotid loop again had an equal effect on the blood pressure in the femoral artery.

It is safe to conclude, therefore, that the elevation of blood pressure (of the order found following the constriction of the renal arteries Text-figs. 5 to 15) cannot be attributed to any effects of compression of the carotid loop in making the determination.

#### DISCUSSION

The results of the foregoing experiments show that the constriction of the main arteries of both kidneys of dogs was followed invariably by elevation of systolic blood pressure, determined in a carotid artery, which persisted for as long as 15 months. Great, permanent constriction of large arteries in other parts of the body did not induce persistent significant elevation of systolic blood pressure. The in-

creased values cannot be accounted for by the compression of the carotid loop in making the determinations.

The mechanism of elevation of the blood pressure of these animals has not been elucidated by these experiments. Bell and Pedersen (10), in attempting to explain the temporary increase of systolic blood pressure which they observed in rabbits, following the experimental production of venous stasis in one kidney, used the teleological argument that: "When there is increased resistance in the renal circulation the blood pressure must be increased in order to maintain the normal blood flow." They referred to this as a compensatory process. Such an argument could also be applied to the case of experimental renal ischemia and has the advantage as well as weakness of all teleological reasoning. It fails to explain just why the normal blood flow must be maintained and gives no clue to the specific mechanism whereby this is effected. It merely takes advantage of the fact that in those animals that survived the blood pressure actually was raised and, *as a consequence*, blood flow was most probably improved; but it does not explain the mode of development of the elevation of blood pressure.

It is a simple matter to outline a number of possible mechanisms whereby the blood pressure may be raised as a result of renal ischemia.

(a) Afferent impulses from the affected nerve endings in the ischemic kidneys to the sympathetic ganglia or vasomotor center may result in general vasoconstriction and consequent elevation of blood pressure.

Complete denervation, if possible, of both kidneys, before the application of the clamps, would give some clue to the existence of such impulses but would not distinguish between a direct, purely nervous, and an indirect humoral mechanism brought into play by such afferent impulses from the kidney. This experiment has not yet been performed.

(b) Afferent impulses from the ischemic kidneys may, in some way, bring about increased output of some internal secretion which, by peripheral or central action, may effect general vasoconstriction, and thus raise the blood pressure.

Sufficient experiments to determine this were not made. That the suprarenal medulla probably plays no part in the process is indicated by the experiment on Dog 8-9 in which the right suprarenal body was removed, the medulla of the left destroyed and the left splanchnic

nerves and nerve fibers to the left suprarenal body sectioned. The blood pressure was not significantly affected by these procedures. Subsequently, production of moderately severe bilateral renal ischemia was followed by moderate elevation of systolic blood pressure. The possible part played by the pituitary body was not investigated.

(c) There may be an accumulation or new formation of some substance, or there may occur a disturbance of chemical equilibrium between substances present in the blood which may effect a pressor action like that of a hormone.

The existence in the blood of subjects with hypertension of a substance or substances capable of raising the blood pressure and differing either quantitatively or qualitatively from any found in subjects with normal blood pressure, has not been demonstrated. In those animals (Nos. 2-5 and 8-7) in which the constriction of the vessels was almost complete from the beginning, that showed great elevation of blood pressure but did not survive very long, there was an accumulation of urea, creatinine and total non-protein nitrogen in the blood. However, it has not been shown that these substances have a pressor effect and they did not increase in the blood of the animals in which the clamping of the renal arteries was less severe but in which, nevertheless, an elevation of pressure did occur. In all of the animals, except No. 2-5, the quantity of guanidine in the blood was determined at intervals. Animal 8-7 is the only one that showed an appreciable elevation of this substance above the normal. This occurred during the 4 days of survival following the almost complete clamping of the second renal artery, during which time the animal was in uremia. In all of the other animals, even in No. 3-8, which had the longest period of persistent hypertension, during which pressures even higher than those of Animal 8-7 were attained, no increase of guanidine in the blood was found. Major (21) has shown that there is decreased excretion of guanidine in the urine of patients with arterial hypertension. This was not studied in these animals. Major and collaborators (22-27) have shown that various guanidine compounds, when injected into the blood stream of animals, cause a rise of blood pressure which persists longer than after injections of adrenalin. It has been shown (28) that the site and mode of action of guanidine compounds are like those of adrenalin. However, no one has found an increase of guanidine in

the blood of patients with vascular hypertension. The exact part, if any, played by the metabolism of guanidine in patients with hypertension, and in these animals with experimentally elevated blood pressure, still remains to be determined.

#### RÉSUMÉ

In eleven dogs, by the carotid loop method of Van Leersum, systolic blood pressure was determined daily for at least 2 months before, and for from 3 days to 15 months after, the constriction of both renal arteries by means of a special clamp devised for the purpose. The clamp permitted the degree of constriction of the vessel to be varied and increased at will. In some of the animals the constriction was made great from the beginning; in others, it was made moderate at first and subsequently increased one or more times. Constriction of one renal artery was followed by a moderate or slight rise of blood pressure which tended to return toward the level of the control period. Following the production of bilateral renal ischemia, the systolic blood pressure rose to a varying degree in all of the animals. During a variable period following the constriction of the second vessel very high values were common. No attempt was made in this series of animals to reduce or remove the accessory circulation through the capsule of the kidney. This is being done in another series of animals. In those animals in which the constriction was made moderate at first, and later increased, the amount of this accessory circulation probably became quite considerable and very likely was responsible for the tendency of the elevated pressure eventually to decrease in most of the animals. However, in all of the animals, the systolic blood pressure remained elevated well above the normal range.

In two animals, (Nos. 2-5 and 8-7), in which the clamping of both arteries was made almost complete from the start, the rise of blood pressure which followed was accompanied by the development of uremia which rapidly proved fatal. In these animals, the amount of urea nitrogen, total non-protein nitrogen and creatinine in the blood kept increasing and the urea clearance and output of phenolsulfone-phthalein kept decreasing until death. In the remaining animals that survived for many months the only renal function test which indicated some renal damage in a few of the animals was the urea clearance.

Urea, total non-protein nitrogen, creatinine and guanidine remained within normal limits. In one animal (No. 3-8), that has had a persistently elevated systolic blood pressure for 15 months following severe constriction of both main renal arteries, the urea clearance remained reduced throughout to about 50 per cent of the mean value obtained during the control period. In the remaining animals in which the constriction was made moderate at first and then increased, urea clearance showed either slight reduction, with rapid return to normal, or practically no change from the normal.

In one animal (No. 6-0), the splenic artery and both femoral arteries were greatly constricted at different times before the clamps were applied to the renal arteries, but no rise of blood pressure occurred until after the renal arteries of this animal had been constricted.

In one animal (No. 8-9), the right suprarenal body was removed, the left suprarenal body was denervated and its medulla mechanically destroyed, and the left major and minor splanchnic nerves were sectioned. The blood pressure of this animal showed no significant change until after the renal arteries were constricted, when a moderate rise promptly occurred and persisted.

Of the nine animals in which the systolic blood pressure remained elevated for 4 months or longer following the initial constriction of both renal arteries, five are still alive. The examination of the kidneys of three of the dead animals (Nos. 5-5, 5-6 and 5-8) in this group (one kidney of No. 5-8 was removed surgically) indicates that ischemia may be able to induce significant changes in glomeruli, parenchyma and vessels of the kidney. Gross infarction of kidney substance was not observed in these kidneys, and microscopically massive necrosis was not present. The changes in the tissues of the animals with persistent hypertension and without signs of uremia were therefore abiotrophic rather than necrobiotic. Thus, necrosis of kidney substance was not a necessary condition for the development of elevated blood pressure in these animals. It is to those abiotrophic changes in the kidneys that the elevation of blood pressure is probably attributable because it is well known that in acute experiments clamping even of both renal vessels has little or no immediate effect on blood pressure. The pathological changes in the kidneys as well as possible pathological changes in other parts of the body will be studied at autopsy in the remaining animals and will be the subject of another report.

Throughout this study cardiograms of all the animals were made at regular intervals as an aid to determine the development of cardiac hypertrophy in these animals. The results of the study to determine the existence of hypertrophy by roentgenographic and morphologic methods will be included in the report which will deal with the anatomical findings in all of these animals.

#### CONCLUSIONS

These experiments indicate that, in dogs at least, ischemia localized to the kidneys is a sufficient condition for the production of persistently elevated systolic blood pressure. When the constriction of both main renal arteries is made only moderately severe in the beginning, the elevation of systolic blood pressure is unaccompanied by signs of materially decreased renal function. In this respect the hypertension in these animals resembles the hypertension which is associated with so called benign nephrosclerosis in man. Subsequent increase of the constriction of the main renal arteries does not materially damage renal function, probably because of adequate development of accessory circulation. More delicate methods for detecting a change may yet prove that some damage does occur. Almost complete constriction of both main renal arteries, from the beginning, results in great elevation of systolic blood pressure which is accompanied by severe disturbance of renal function and uremia. This resembles the type of hypertension which is associated with so called malignant nephrosclerosis, in the sense of Fahr (17). In several of the animals with persistent elevation of systolic blood pressure, anatomical changes were observed in the glomeruli, vessels and parenchyma of the kidneys which are most probably directly referable to the ischemia.

It is hoped that these investigations will afford a means of studying the pathogenesis of hypertension that is associated with renal vascular disease.

To Professor T. J. Hill we are greatly indebted for advice and assistance in the construction of the first model of the clamp. We wish to record our sincere thanks to Professor J. M. Rogoff who performed the surgical operations on the suprarenal bodies of Animal 8-9. To Professor H. T. Karsner we are very grateful for his continued interest in and furtherance of this investigation.

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## EXPLANATION OF PLATES

## PLATE 23

FIG. 1. Dog 6-0. Roentgenogram, showing an arterial clamp on the splenic, both renal and both femoral arteries.

## PLATE 24

FIG. 2. Dog 4-9. Ether anesthesia drop method, following hypodermic injection of morphine and atropine. Experiment to determine the effect of compression of the carotid loop on systemic blood pressure.



The numbers 1 to 9 indicate the beginning of obstruction of one or both carotid arteries. The release of the signal indicates when the compression was stopped.

(1) Left common carotid artery completely obstructed by compression of carotid loop by means of air pumped into a cuff around the loop.

(2) Freshly isolated right common carotid and the left common carotid in the loop simultaneously obstructed.

(3) Same procedure as (1).

(4) Freshly isolated right common carotid alone completely obstructed.

(5) Same procedure as (1).

(6) Left common carotid obstructed by compression of loop as (1) but repeated ten times. Each obstruction lasted 10 seconds, with an interval of 10 seconds between occlusions.

(7) Same procedure as (4).

(8) Same procedure as (1).

(9) Same procedure as (2).



FIG. 1

(Goldblatt *et al.*: Hypertension. I)

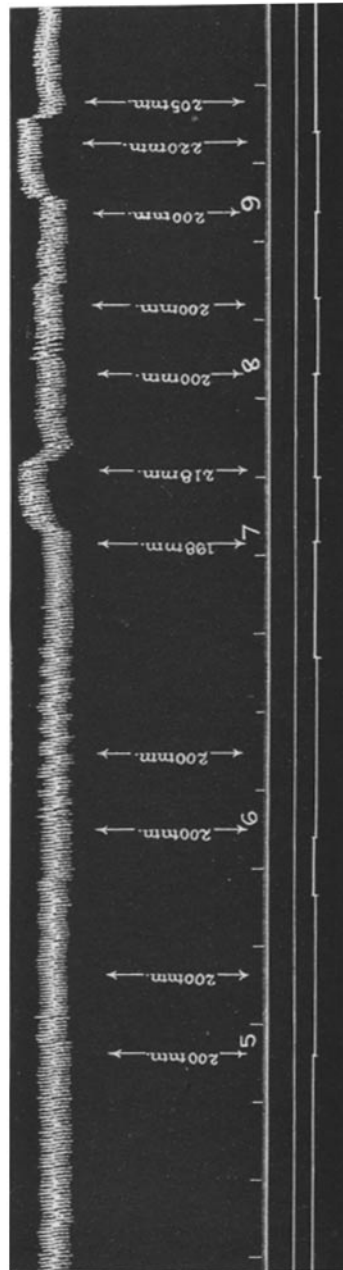
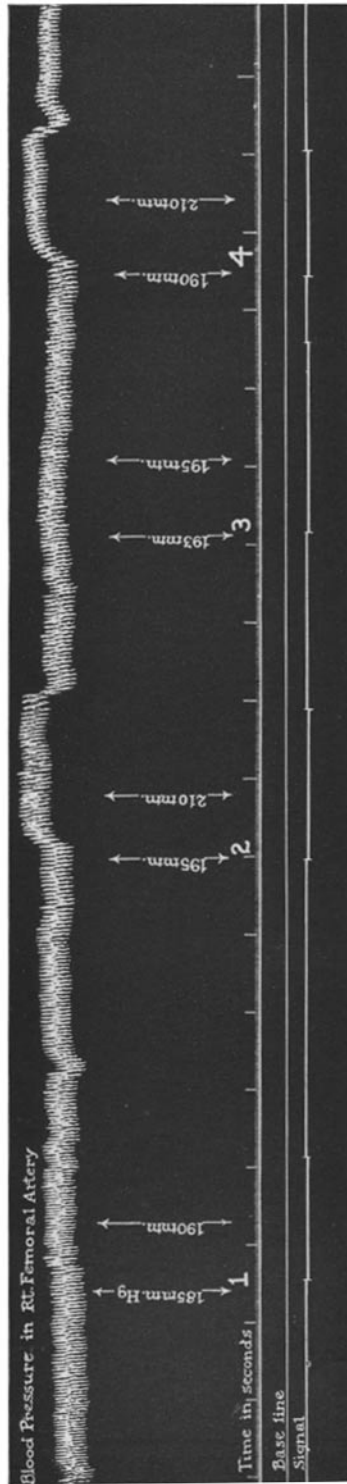


FIG. 2

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