

THE ETIOLOGY OF HYPERTENSION DUE TO COMPLETE RENAL ISCHEMIA*

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Taquini, in 1938, modifying Goldblatt's epochal experiment (1), showed in dogs that reestablishment of the circulation in kidneys rendered completely ischemic for a period of 4 to 6 hours, was followed by an elevation of blood pressure upon removal of the clamp occluding the renal pedicle (2). This observation has been confirmed in dogs, cats, guinea pigs, and rats (3); but negative results were obtained in rabbits (4).

Previous studies have shown that the rise of blood pressure which follows the termination of temporary, complete renal ischemia is not reversed by 933F (piperidomethyl-3-benzodioxane) (5), proving that the substance which causes the rise of pressure is not an epinephrine-like compound. The elevation of blood pressure is greatly reduced or prevented by the preliminary induction of tachyphylaxis to renin (5), an observation which suggests that the substance responsible for the hypertension is renin or a substance having similar properties of tachyphylaxis. This type of hypertension is not prevented by renal denervation, vagotomy combined with destruction of the spinal cord, or splenectomy (6). According to Taquini (2), blood from a vena cava pocket receiving the return flow of completely ischemic kidneys exhibits a vasopressor action in the Loewen-Trendelenberg preparation. On the other hand, neither Friedman and Prinzmetal (7) nor Mason and Rozzell (8) were able to demonstrate pressor properties in the blood taken from the renal vein of dogs exhibiting Goldblatt hypertension. Extracts of completely ischemic kidneys show greater pressor effects than extracts of normal kidneys (4); similarly, extracts of incompletely ischemic kidneys of dogs (Goldblatt hypertension) contain more pressor material than those of normal kidneys (8, 9).

It would seem that in both the hypertension due to partial, chronic renal ischemia (Goldblatt) and that following the termination of complete, acute renal ischemia (Taquini), a pressor principle of renal origin is released by the ischemic kidneys into the general circulation. Many have con-

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sidered the pressor substance in question to be renin, but hitherto, there has been no satisfactory proof that this or any other particular substance is the cause of experimental renal hypertension (Goldblatt, 11). Whether or not the substances responsible for Goldblatt and Taquini hypertension, respectively, are identical or related, remains for future studies to determine.

In this communication, we wish to report the finding of a pressor substance in perfusates of completely ischemic kidneys. Evidence will be presented proving that this substance causes the elevation of blood pressure occurring upon the termination of complete renal ischemia, and that this pressor principle is renin.

The Pressor Properties of Perfusates of Completely Ischemic Kidneys

The following experiment was undertaken to determine whether perfusates of completely ischemic kidneys contain a pressor substance.

TABLE I
Comparison of Effect of Perfusates from Normal and Ischemic Kidneys of the Same Cat

Animal No.	Duration of ischemia	Rise in B.P. following injection of perfusate of normal kidney	Rise in B.P. following injection of perfusate of ischemic kidney
	<i>hrs.</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
1	7	0	36
2	5	-6	28
3	5	0	56
4	4½	4	48
5	4	0	42
6	4	0	18
7	4	6	32
8	5	0	20
Averages.....	4½	0.5	35

Each of eight cats was anesthetized with ether, and the entire pedicle of the left kidney was ligated after all other attachments were freed. Approximately 5 hours later, each animal was reanesthetized with nembutal intraperitoneally and the blood pressure recorded from the carotid artery. Both kidneys were then removed and each organ perfused with 1 cc. of salt solution (0.9 per cent) per gm. of fresh kidney substance. The salt solution was warmed to 37° C. and injected into the kidney through a cannula in the renal artery by means of a syringe. The perfusate was collected as it came out of the renal vein. No attempt was made to measure the perfusion pressure. In earlier observations the kidneys were perfused only once, but in later experiments, in order to recover a larger amount of active material, each kidney was reperfused three to five times with the original perfusate.

In three instances perfusates of completely ischemic hind limbs were prepared in the following manner. The femoral vessels were isolated and ligated, and a stout cord was tied about the remaining tissues of the leg to eliminate collateral

circulation. 5 hours later, the femoral vessels were cannulated and perfused with warm salt solution, and the perfusates collected from the cannula in the femoral vein.

In view of recent observations on the possible rôle played by ischemia of the gravid uterus or its contents in the hypertension of pregnancy (12, 13), and as a further control, perfusates of completely ischemic gravid uteri were prepared as follows: Five pregnant cats were anesthetized with ether, and all vessels supplying the uterus ligated. 5 hours later the animal was reanesthetized, the ischemic uterus removed, the uterine arteries cannulated, and perfusates prepared in the manner described for kidneys.

The pressor effect of all perfusates was tested by intravenous injections into cats anesthetized with nembutal, the blood pressure being recorded from the carotid artery. 0.5 to 1.0 cc. was first injected. If the pressor effect was too great, smaller amounts were subsequently used. If the effect was too small, larger amounts were used. The same amount of each perfusate was always used in comparing the effects of each pair of perfusates. The injections were made at intervals of 15 to 45 minutes, depending on the pressor effect of the preceding injection.

In each instance, perfusates of the totally ischemic kidneys showed marked pressor effects, whereas those of the normal kidneys showed none. The perfusates of ischemic kidneys caused an average rise in blood pressure of 35 mm. of Hg (Fig. 1, Table I).

Following the injection of ischemic renal perfusate, the blood pressure in the test animal usually begins to rise in about 30 seconds, reaches its peak in 1 to 2 minutes, and then gradually drops and reaches its original level within 15 minutes to 1 hour. Preliminary depressor responses were not observed.

The perfusates of the ischemic limbs and gravid uteri contained no pressor material.

Perfusates of Normal Kidneys Do Not Develop Pressor Properties When Incubated Outside the Kidney

The following experiment was performed to determine whether the pressor material found in the perfusates of completely ischemic kidneys is

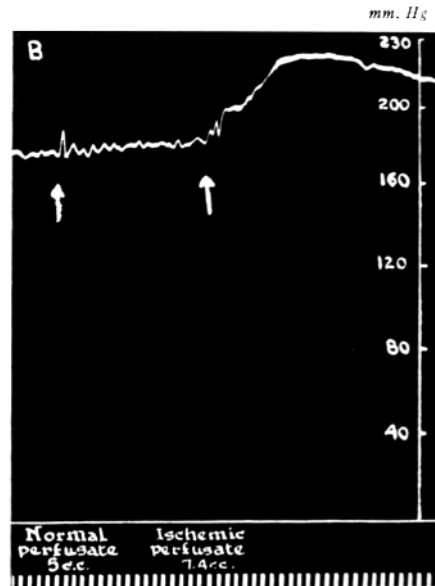


FIG. 1. Pressor effects of perfusates of normal and ischemic kidneys of the same animal. At the first arrow, injection of normal renal perfusate. At the second arrow, injection of ischemic renal perfusate. In this and all subsequent records, all injections were made intravenously and all time markings indicate 15 second intervals. In all the figures pressure is in millimeters of mercury.

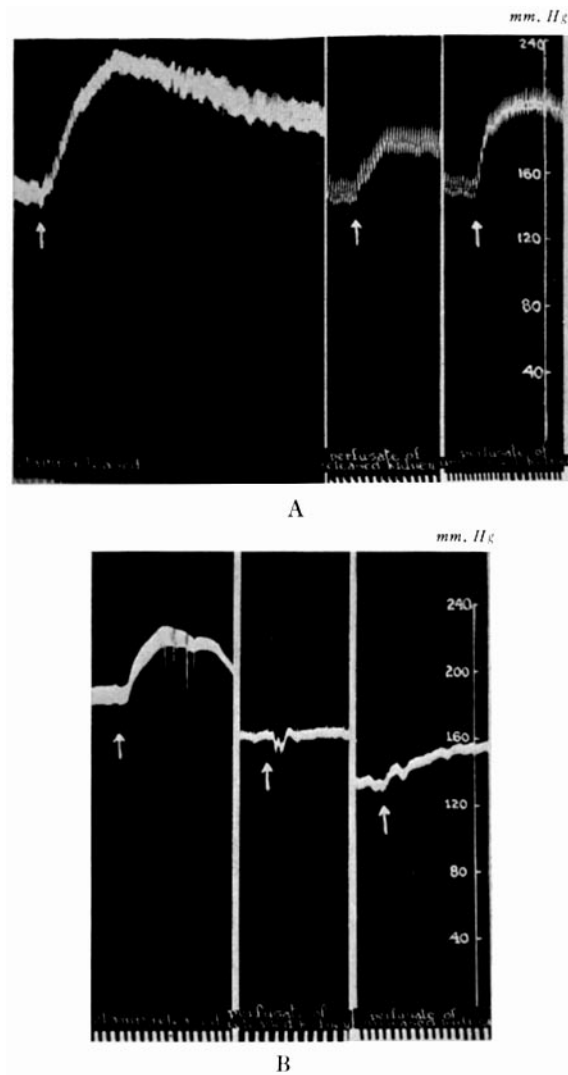


FIG. 2. Pressor effects of perfusates of released and unreleased ischemic kidneys of the same animal. A and B illustrate two such experiments. At the first arrow, the clamp occluding the pedicle of one ischemic kidney was released, reestablishing the circulation to that kidney. At the second arrow, the perfusate of this released ischemic kidney was injected. At the third arrow, the perfusate of the opposite, unreleased ischemic kidney was injected.

actually produced by the kidney during the period of complete ischemia, or whether the intravascular contents (perfusate) of the normal kidney con-

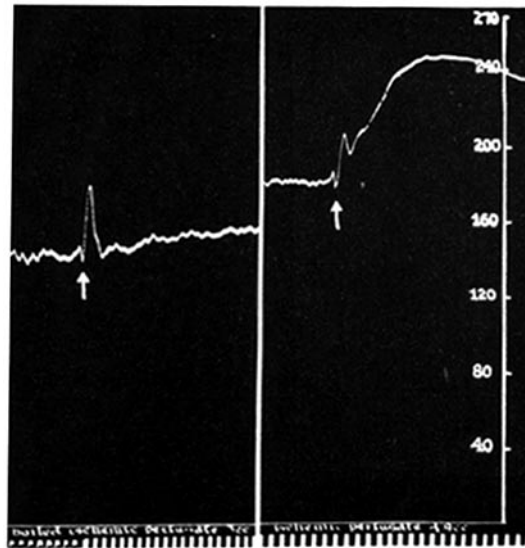


FIG. 3. Destruction of pressor substance in perfusates of ischemic kidneys by boiling. First arrow, injection of 3 cc. boiled ischemic renal perfusate. Second arrow, injection of 4.9 cc. unboiled ischemic renal perfusate. The pressor response of the heat-stable pressor substance seen at the first arrow is discussed in the text.

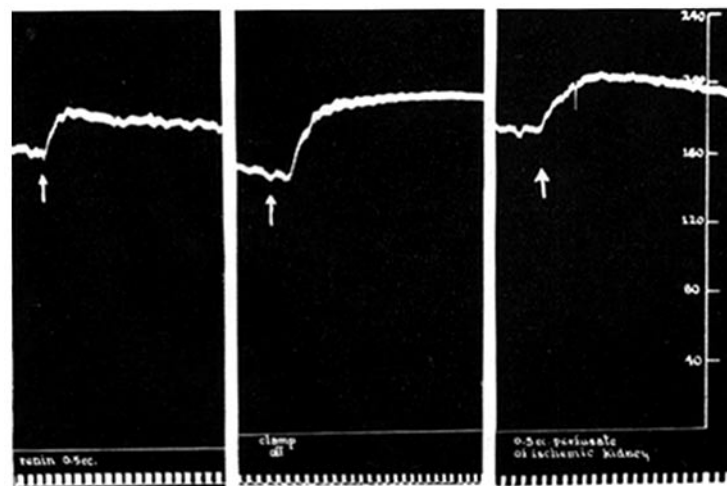


FIG. 4. Similarity of pressor curves following injection of renin, reestablishment of circulation of a completely ischemic kidney, and injection of ischemic renal perfusate. At first arrow, injection of 0.5 cc. of renin made from cat's normal kidneys. At second arrow, removal of clamp occluding cat's renal pedicle after 5 hours of ischemia. At third arrow, injection of 0.5 cc. of perfusate of cat's ischemic kidney.

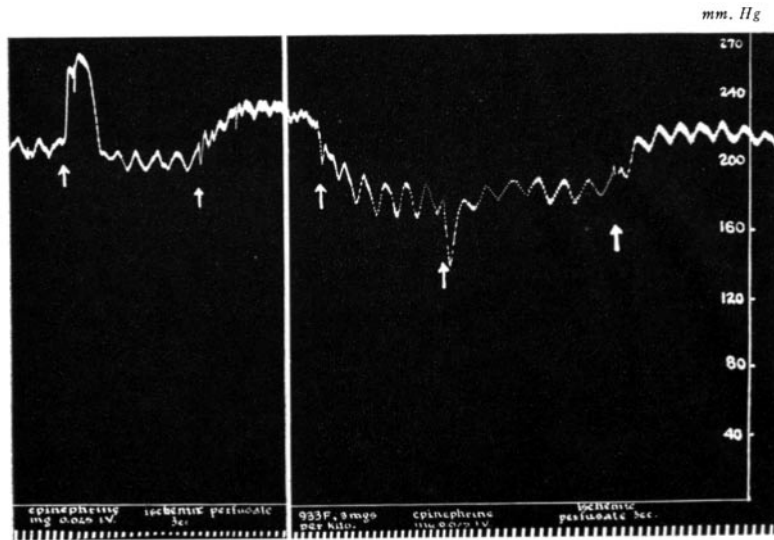


FIG. 5. Effect of 933F on the pressor response to epinephrine and ischemic renal perfusate. At first arrow, 0.025 mg. of epinephrine. At second arrow, 3 cc. of ischemic renal perfusate. At third arrow, 933F (3 mg. per kilo). At fourth arrow, 0.025 mg. of epinephrine. At fifth arrow, 3 cc. of ischemic renal perfusate. The pressor response to epinephrine has been reversed by 933F, whereas that to ischemic renal perfusate has not been affected.

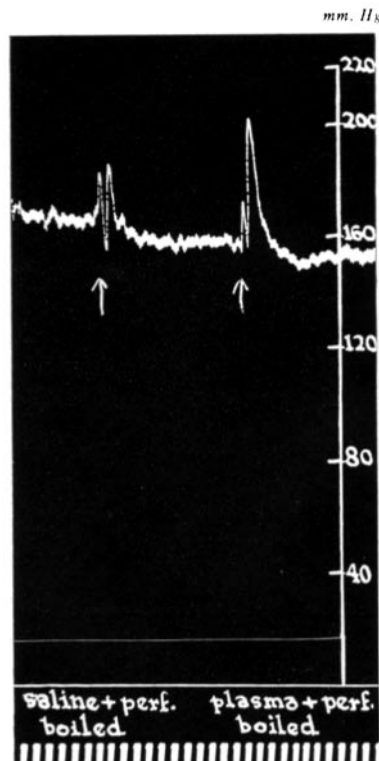


FIG. 6. Demonstration of the heat-stable pressor substance formed by incubation of ischemic renal perfusate with plasma. At first arrow, injection of ischemic renal perfusate incubated with normal saline for control. At second arrow, injection of ischemic renal perfusate incubated with plasma. Both solutions were boiled for 5 minutes before injection.

tain a non-pressor precursor which develops pressor properties during the period of circulatory suspension (incubation). In six instances perfusates of normal kidneys were incubated at 37°C. for 5 hours and then tested as above. In no instance was a pressor response obtained. This experiment proves that the normal kidney does not excrete a substance into the blood stream which assumes pressor properties during the period of ischemia and that the pressor substance is therefore formed directly by the kidney as a result of ischemia.

Comparison of the Pressor Effects of Perfusates of Released and Unreleased Totally Ischemic Kidneys

The finding of pressor material in perfusates of completely ischemic kidneys does not prove that it is the cause of the hypertension which follows termination of the complete renal ischemia. The following experiment was devised to determine whether or not this substance is the cause of the hypertension.

Under ether anesthesia, both renal pedicles of each of fourteen cats were completely occluded by bulldog clamps. In most instances, one kidney was occluded about 10 minutes before its fellow. Approximately 5 hours later the animals were reanesthetized with nembutal intraperitoneally, and the blood pressure recorded from the carotid artery. In each experiment the clamp occluding the pedicle of the kidney first operated upon was now released, and the circulation to that kidney reestablished. This procedure resulted in the usual rise of blood pressure. The opposite kidney was not unclamped. Both released and unreleased kidneys were now removed and perfusates from each prepared and tested in the manner previously described.

In each experiment, both kidneys were made equally ischemic, the only difference between them being that the material causing the rise in blood pressure was washed out of the released kidney upon restoration of its circulation, whereas none was removed from the unreleased kidney. If the pressor material in perfusates of completely ischemic kidneys is the same as that which causes the hypertension occurring upon the termination of complete renal ischemia, there should be less pressor material in the perfusate of the released ischemic kidney of the same animal. This is exactly what was found. In thirteen of fourteen experiments the perfusates of the released ischemic kidneys exhibited smaller pressor effects than those of the unreleased ischemic kidneys of the same animals (Fig. 2, Table II).

In the above experiments, it was found that the pressor substance in perfusates of completely ischemic kidneys had the property of tachyphylaxis. For this reason the perfusate of the released kidney was injected first in each instance. Had the injections been made in reverse order, *i.e.*, had the perfusate of the unreleased kidney been injected before that

of the released one, objection might have been made that the smaller pressor response of the latter was due, not to a lesser content of pressor material but to the tachyphylactic effect. Were it not for this property of tachyphylaxis the difference between the two perfusates would have been appreciably greater.

The smaller pressor response of perfusates of released kidneys is due to the washing out into the general circulation of some (or all) of the pressor substance from the released kidney, leaving behind less pressor substance

TABLE II
Comparison of Effect of Perfusates from Ischemic Kidneys after Reestablishment of Circulation and Control Ischemic Kidney of the Same Cat

Animal No.	Duration of ischemia	Rise in B.P. following reestablishment of circulation in one ischemic kidney	Rise in B.P. following injection of perfusate of ischemic kidney after reestablishment of circulation	Rise in B.P. following injection of perfusate of control ischemic kidney
	<i>hrs.</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
1	5	46	10	20
2	3½	56	22	42
3	4½	38	0	22
4	4	8	30	40
5	5	14	18	34
6	5	60	0	16
7	5	98	28	56
8	5	24	12	54
9	7	42	30	54
10	3½	26	0	16
11	4½	18	46	34
12	4½	34	0	12
13	5½	52	10	22
14	5	48	24	44
Averages	4.8	40	16.4	33.3

to be recovered by perfusion. The greater pressor effect of the perfusate of the unreleased kidney is explained by the fact that none of the pressor substance which was formed was discharged into the systemic circulation. This warrants the conclusion that the pressor material found in the perfusate of ischemic kidneys is the cause of the hypertension which occurs upon the termination of complete renal ischemia.

Experiments in an Endeavor to Identify as Renin the Pressor Substance in Perfusates of Completely Ischemic Kidneys

Evidence in favor of the belief that the substance causing the hypertension due to complete renal ischemia is renin may be summarized as follows:

1. The pressor activity of ischemic renal perfusate is destroyed by boiling for 5 minutes (twelve experiments). Renin is also destroyed by boiling for 5 minutes (14, 15). The briefly acting pressor response of boiled ischemic renal perfusate as seen in Fig. 3 is frequently found and is due to a heat-stable substance which will be discussed later.

2. As pointed out above, the perfusate of ischemic kidneys has the property of tachyphylaxis. An example is cited in Table III. Renin also exhibits tachyphylaxis (14, 15).

3. The configuration of the pressor curve of ischemic renal perfusate is similar to that of renin prepared by the method described by Pickering and Prinzmetal (15), (Fig. 4).

TABLE III
Effect of Repeated Injections of Ischemic Renal Perfusate in the Anesthetized Cat (Tachyphylaxis)

Time of injection	Pressor effect
<i>p.m.</i>	<i>mm. Hg</i>
3:24	40
3:50	32
4:17	28
4:27	24
4:33	14
4:47	14
5:05	12

4. The pressor effect of renin is not affected by 933F (5, 16). The following tests demonstrate the failure of 933F to reverse the pressor response of ischemic renal perfusate.

In six experiments, perfusates of ischemic kidneys were prepared as previously described. Cats were anesthetized with nembutal intraperitoneally, the carotids cannulated, and blood pressures recorded. Epinephrine and ischemic renal perfusate were then injected intravenously, followed by 933F in dosages of 1 to 3 mg. per kilo. Epinephrine and ischemic renal perfusate were then retested.

Before the administration of 933F, both epinephrine and ischemic renal perfusates caused a rise in blood pressure. Following 933F, that of ischemic renal perfusate still caused a rise, whereas the pressor effect of epinephrine was invariably reversed (Fig. 5).

5. Ischemic renal perfusate incubated for 15 minutes with plasma yields a heat-stable pressor substance (Fig. 6). Page and Helmer (17) and Braun Menendez *et al.* (18) have demonstrated that incubation of renin with plasma yields a similar substance.

6. The pressor effect of ischemic renal perfusate is not diminished or nullified by a previous injection of cocaine (five experiments). The pressor effect of renin is likewise not affected by a previous injection of cocaine (19, 20), a substance which abolishes the pressor response of tyramine (21).

7. The following experiment is a more direct attempt to establish the identity of the pressor substance in ischemic renal perfusate with renin. It has been previously shown that the amount of extractable renin is increased in completely ischemic kidneys (5). If the pressor material in ischemic renal perfusate is renin, there would necessarily be less renin in the extract of the released ischemic kidney than in the unreleased ischemic kidney of the same animal.

Under ether anesthesia, both renal pedicles were clamped in each of nineteen cats. Approximately 5 hours later the animals were reanesthetized with nembutal intraperitoneally, and one renal pedicle unclamped for 15 minutes. The usual rise in blood pressure took place following the reestablishment of circulation. In no instance was the clamp on the opposite kidney removed. The kidneys were removed and without being perfused were extracted and assayed for renin by the method previously described (15).¹

If renin is the substance that causes the rise in blood pressure following reestablishment of the circulation of completely ischemic kidneys, there should be more renin in the extracts of unreleased ischemic kidneys than in released ischemic kidneys. Analogous reasoning was employed to explain the greater quantity of pressor material in perfusates of unreleased ischemic kidneys.

In seventeen of nineteen experiments the extracts of the unreleased kidneys exhibited greater pressor effects than extracts of the released kidneys. In one instance, extracts of both kidneys were the same, and in the other, the extract of the released kidney had a greater pressor effect than its unreleased fellow (Table IV). The finding of an increased quantity of renin in extracts of unreleased kidneys is additional evidence that the pressor substance in perfusates of ischemic kidneys is renin.

In a preliminary report (23) we named this pressor material ischemin, with the reservation that this term would be dropped if it proved to be renin. The evidence presented in this paper strongly suggests that it is renin which we will hereafter assume it to be. It is realized that final proof cannot be obtained until chemical identification is possible. If chemical analysis proves the pressor substance in ischemic renal perfusate to be different from renin, the term ischemin may be reemployed.

¹ Tachyphylaxis does not occur if the procedure described by Pickering and Prinzmetal (15) is followed. The experiments in which Landis *et al.* (22) found tachyphylaxis in rabbits were performed in a different manner.

The Rôle of the Heat-Stable Substance and the Effect of Blood on the Formation of Renin

Williams and Grossman (24) found that a heat-stable pressor substance having certain qualities similar to epinephrine was formed in the kidney when this organ was perfused. They termed this substance perfusin. Kohlstaedt, Helmer, and Page (25) and Friedman, Abramson, and Marx (20) showed that renin itself is not a pressor substance, and Page and Helmer (17) showed that renin incubated with plasma forms a heat-stable substance which they termed angiotonin. Braun Menendez *et al.* (18) independently made similar observations and termed this substance hypertensin.

TABLE IV
Assay of Renin in Released and Unreleased Ischemic Kidneys of Cats

Animal No.	Pressor effect of renin extracted from released kidney	Pressor effect of renin extracted from unreleased kidney
	<i>mm. Hg</i>	<i>mm. Hg</i>
1	7	25
2	0.5	8.5
3	5	7
4	10	14.5
5	7	14
6	0	13
7	7	18
8	7	17
9	7.5	10
10	23	19.5
11	6	10
12	12	21
13	12	12
14	8	10
15	2	11
16	2	11
17	0	11
18	1	12
19	5	29

Victor, Steiner, and Weeks (26) more recently found a heat-stable pressor substance in anaerobic autolysates of the kidneys. It seems that hypertensin and angiotonin are the same substance and it is quite possible that perfusin is of identical character.

The rôle of this heat-stable pressor substance in hypertension due to acute, complete renal ischemia was determined in the following experiments. As pointed out above (section 1), when ischemic renal perfusate is boiled, the bulk of the active material is destroyed, and a small amount of briefly acting, heat-stable pressor substance usually remains (Fig. 4). Having demonstrated above (section 5) that the latter substance is formed when ischemic renal perfusate is incubated with plasma, it became necessary to determine whether the absence of blood in the kidney during the

period of ischemia prevented the formation of this substance. Accordingly the following experiments were performed.

Effect of Blood on the Formation of Renin

Ten cats were anesthetized with ether, both renal pedicles ligated, and the kidneys immediately removed. One kidney was quickly perfused with warm salt solution (0.9 per cent) until the washings were crystal clear. This comparatively blood-free kidney, and the unperfused blood-containing kidney, were then incubated in the same animal's abdomen for 5 hours. For the purpose of this experiment these kidneys were the same as completely ischemic kidneys with their pedicles clamped *in situ*, except that one of them was comparatively blood-free. After 5 hours of incubation (or ischemia), the blood-free and the blood-containing kidneys were removed and perfusates from each prepared in the manner described above.

It was found that the perfusates of the comparatively blood-free kidney contained more renin than that of the blood-filled kidney of the same animal. In a long series of experiments which will be reported elsewhere, it was found that this effect was due to a factor in red blood cells which inhibits the formation of renin.

This procedure of incubating blood-free kidneys with various substances can be used to determine whether they inhibit or enhance the formation of renin in the ischemic kidney.

Effect of Blood on the Formation of the Heat-Stable Substance

In eight instances perfusates of blood-free and blood-containing kidneys were prepared in the manner described above. These were boiled for 5 minutes and the supernatant fluid collected and tested. It was found that the perfusate of the blood-filled kidney contained relatively large quantities of the heat-stable substance, whereas that of the comparatively blood-free kidney contained little or none of this material.

Since the perfusates of blood-free ischemic kidneys contain very large amounts of the substance causing the hypertension (renin) and little or none of the heat-stable material, and since a large amount of the heat-stable substance is present in perfusates of ischemic blood-filled kidneys, it is concluded that during the period of complete renal ischemia a small amount of the renin liberated reacts with the stagnant plasma in the blood vessels to form the heat-stable substance. This does not take place in kidneys which are washed free of blood.

DISCUSSION

The sequence of events occurring during complete renal ischemia and following the reestablishment of the renal circulation can now be reconstructed as follows: As a result of complete renal ischemia and absent

pulsations in the renal vessels (27), more renin is formed in the kidney, or that which is present becomes more readily available for extraction (5). Normally, the kidney secretes little or no renin, since perfusates of normal kidneys are not pressor. As a result of complete ischemia the renin passes from the depots of storage and manufacture through the endothelial cells of the vessels. A small amount of the renin reacts with some of the plasma contained in the renal vessels to form a minute amount of the heat-stable substance described above, but the bulk of the transported renin remains unaltered during the period of ischemia. When the renal circulation is restored, the renin enters the systemic circulation and causes (Taquini) hypertension, presumably through its transformation in the peripheral blood stream into a heat-stable pressor substance, which may be angiotonin or hypertensin.

CONCLUSIONS

1. Perfusates of totally ischemic kidneys of cats contain a pressor substance which is not present in the perfusates of normal kidneys, ischemic hind limbs, or ischemic gravid uteri.
2. The pressor material in ischemic renal perfusates originates directly in the kidney as a result of complete ischemia.
3. The pressor principle contained in ischemic renal perfusates is the cause of the hypertension which follows the reestablishment of circulation in completely ischemic kidneys, since perfusates of unreleased completely ischemic kidneys contain more pressor material than perfusates of released ischemic kidneys of the same animal.
4. The pressor principle in ischemic renal perfusates is presumed to be renin for the following reasons. (a) Both substances are destroyed by boiling. (b) Both substances induce tachyphylaxis. (c) The configuration of both pressor curves is identical. (d) The pressor action of both is not reversed by 933F, proving they are not epinephrine-like substances. (e) When incubated with plasma, both form a heat-stable pressor substance. (f) The pressor effect of both is uninfluenced by a previous injection of cocaine. (g) Unreleased, completely ischemic kidneys yield more pressor material on extraction than do released ischemic kidneys of the same animal.
5. The perfusates of blood-free ischemic kidneys contain more renin than those of blood-filled ischemic kidneys.
6. A method is described by which the power of various substances to inhibit or enhance the production of renin in the ischemic kidney may be tested.

7. A small amount of the heat-stable pressor substance, presumably angiotonin or hypertensin, is formed by the reaction of the pressor material (renin) and plasma in the vessels of the kidney during the period of complete ischemia.

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