STUDIES ON EXPERIMENTAL HYPERTENSION

XVIII. *Experimental Observations on the Humoral Mechanism of Hypertension**

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THE finding that persistent hypertension may be induced in the dog by constriction of both main renal arteries or by constriction of one main renal artery and extirpation of the contralateral kidney[1] has been fully confirmed by many investigators.[2-8] Hypertension has now been produced by the same method in the monkey,[9] rat,[10-13] rabbit,[14,15] goat and sheep.[16] The development of this method was based upon the assumption that in the most common type of human hypertension in which fairly widespread vascular disease is almost an invariable accompaniment, the arterio- and arteriolosclerosis of the kidney is the primary condition which, in some way, determines the elevation of the blood pressure. Whatever may be the nature of the actual disturbance of hemodynamics which occurs in the human kidney the seat of arterio- and arteriolosclerosis, a similar effect on intrarenal hemodynamics is probably produced in animals by persistent constriction of the main renal artery. The resultant experimental hypertension is strong indication that in man too, the elevation of the blood pressure may be due to the renal vascular disease or any other kind of renal disease which might produce a similar effect on the renal circulation.

The experimental production of a disease process in animals is of value to human medicine in direct proportion to the resultant contribution to the elucidation of the cause and possible cure of its human counterpart. The various studies of experimental renal hypertension produced by constriction of the main renal artery have already brought out

* The investigations by the authors were supported by the Beaumont Trust (L. D. Beaumont) and aided by grants from the Josiah Macy Jr. Foundation, The John and Mary B. Markle Foundation, a group of Cleveland donors, and a special grant to Dr. Lewis from the Dazian Foundation for Medical Research.

This paper was presented in great part by Dr. Harry Goldblatt in the Symposium on Hypertension before the Graduate Fortnight, The New York Academy of Medicine, October 24, 1941.

TABLE I

Subject	*Human Essential Hypertension*	*Experimental Renal Hypertension*
Cardiac rate	Normal	Normal
Cardiac output	Normal [17-19]	Normal [20]
Blood volume	Normal [21, 22]	Normal [23, 24]
Viscosity	Normal [25]	Normal [26]
Peripheral blood flow	Normal [27, 28*]	Normal [29]
Sympathectomy	Does not abolish hypertension [27]	Does not prevent or abolish hypertension [7, 23, 62, 63]
Resection of splanchnic nerves	Does not abolish hypertension [30]	Does not prevent or abolish hypertension [31]
Renal blood flow	Apparently reduced [32, 33]	Reduced [34**]
Renal excretory function	(a) Benign phase normal [35, 36]	Benign phase normal [1, 3, 5, 6, 24, 37]
	(b) Malignant phase reduced [53]	Malignant phase reduced [39]
Cardiac hypertrophy	Left ventricle when uncomplicated by failure [40]	Left ventricle. Rat, [41, 42] Rabbit, [15] Dog [3, 24]
Pulmonary arterial pressure	Not altered when hypertension is uncomplicated by left heart failure, as indicated by normal right heart [36]	Unaltered [43]
Unilateral renal disease associated with hypertension	Cured by nephrectomy when proved unilateral [44]	Cured by nephrectomy [1, 5, 6, 45]
Bilateral nephrectomy	No rise of pressure [46]	No rise of pressure [7, 47-50]
Thyroidectomy	Does not prevent or cure hypertension unless of the type associated with disease of the thyroid [51]	Does not prevent or abolish hypertension [52]
Generalized arteriolar necrosis and necrotizing arteriolitis	In malignant phase only [38, 53, 54]	In malignant phase only [55-57]

* Controversial: Abramson, D. I. and Fierst, S. M.: Resting blood flow and peripheral vascular responses in hypertensive subjects, *Am. Heart J.*, 1942, *23*:84.

** Controversial: Corcoran, A. C. and Page, I. H.: Renal aspects of experimental and clinical hypertension, *J. Lab. & Clin. Med.*, 1941, *26*:1713.

clearly the many similarities of this type to human so-called "essential" hypertension which is so frequently associated with vascular disease, especially involving the kidneys. These similarities are briefly summarized in Table I.

The presence of fairly widespread arteriolosclerosis in cases of persistent benign human hypertension and the absence of this type of change in the arterioles of dogs in the benign phase of persistent experimental renal hypertension for as long as six years, as well as the finding of medial hypertrophy in the small arteries and arterioles in both human and experimental benign hypertension, merely emphasize the probability that in man the arteriolosclerosis precedes and is not caused by the hypertension. Thus, there is adequate indication that experimental renal hypertension and human 'essential hypertension' are closely similar, if not identical, and that results obtained in studies of pathogenesis, prevention or cure of the one may be directly applied to the other.

Much evidence has now accumulated which, both directly and indirectly, shows that the elevation of blood pressure which follows constriction of the main renal arteries is due to a humoral mechanism of renal origin. It has been shown, for example, that renal denervation,[2,4,6,7,58] bilateral supradiaphragmatic excision of splanchnic nerves and lower four thoracic sympathetic ganglia,[31] subdiaphragmatic splanchicectomy with excision of celiac and upper lumbar ganglia,[6] bilateral section of anterior nerve roots from 6th dorsal to 2nd lumbar inclusive,[59] destruction of the spinal cord[60,61] and complete sympathectomy, including denervation of heart,[7,23,62,63] neither prevent nor abolish the hypertension which results from constriction of the main renal arteries. The results of these studies eliminate a nervous reflex from the kidneys as the cause of the hypertension and indicate the probable implication of a humoral mechanism.

One of the direct indications that a humoral mechanism is involved in the pathogenesis of experimental renal hypertension is the finding[64] that if the renal veins are obstructed at the same time that the renal arteries are constricted, no rise of blood pressure occurs. Another piece of evidence is the finding that when a kidney is transplanted to the neck[6] or groin,[65,66] with no nervous connections with the rest of the body, a rise of blood pressure still occurs when the main artery to the kidney is constricted. Similarly, the demonstration,[67-69] that transplantation of an ischemic kidney from one dog to the neck of a nephrectom-

ized recipient causes an immediate and sustained rise of blood pressure as soon as blood is allowed to flow through the anastomosed vessels, indicates that some substance is washed from the kidney into the systemic circulation. Finally, the actual demonstration that the blood from a kidney with its main artery constricted is actively vasoconstrictor and pressor, whether this constriction is acute or chronic, is proof of the existence of a humoral mechanism.[7,8,70-78]

That renal excretory function remains normal in benign experimental renal hypertension[1,3,5,24] indicates that retention of nitrogenous metabolites is not a causative factor. The observation that bilateral nephrectomy, which results in azotemia, causes no elevation of blood pressure[7,48-50] is additional proof that retention of nitrogenous metabolites plays no part in the pathogenesis of the hypertension but indicates clearly that the presence of the kidneys in the body is a necessary condition for the elevation of the blood pressure.

The Humoral Mechanism

The early studies on the pathogenesis of experimental renal hypertension led directly to a renewal of interest in some old observations made in 1898 by Tigerstedt and Bergman[79] who found that a saline extract of rabbit kidneys had a prolonged pressor effect when injected into another rabbit. They named the active ingredient of the renal extract "Renin." In recent years, especially since the production of experimental renal hypertension, all of the findings of Tigerstedt and Bergman have been confirmed and extended by a large number of investigations which have dealt with the preparation, purification, properties and mode of action of the renal extract which is capable of producing an elevation of blood pressure when it is injected intravenously and for which the term renin has been generally adopted.[50,80-105]

Renin:

Tigerstedt and Bergman found that renin was a protein, not dialyzable and destroyed by heat. They further found that it had a greater and more prolonged pressor effect when injected into a bilaterally nephrectomized animal, and that when renin was injected repeatedly, with intervals of only a few minutes between injections, it elicited decreasing responses of the blood pressure and eventually no response. This pheno-

menon they referred to as tachyphylaxis. Since they found that section of the spinal cord did not affect the pressor response to an intravenous injection of renin, and that renin had no effect on the isolated perfused heart, they concluded that its effect was exerted directly on the peripheral blood vessels.

In the more recent investigations these findings of Tigerstedt and Bergman have been confirmed. The results of further studies on the behavior of renin when it is subjected to ammonium sulfate precipitation and dialysis[85,94,96] suggest that it is a pseudoglobulin, but this cannot be accepted as final until it has been purified and studied by the method of electrodialysis. It has also been found[105] that in an electric field renin migrates to the cathode and that the isoelectric point of renin lies between a pH of 6.5 to 7.5.

Renin has not yet been isolated in pure form but various methods of extraction and purification have been published,[87,91,94,96,101,104] and it has been shown that the purest product so far produced is different from the known sympathicomimetic amines in that its action is not potentiated by cocaine[92] or reversed by ergotamine[93,94,96] or by piperidomethyl-3-benzodioxane.[106,107] It has also been shown that the action of renin differs from that of adrenalin, pituitrin, and tyramine in that the pressor response to an intravenous injection of renin is not associated with a decrease in peripheral blood flow or fall of skin temperature.[86,97]

Out of the early recent studies of the physiological properties of renin came the discovery that renin is not effective unless injected intravenously and that it has no vasoconstrictor properties when added to Ringer's solution which is being perfused through an isolated organ. It is, therefore, not directly a pressor substance. This was a stumbling block to the proper understanding of the mechanism of the pressor effect of renin when injected intravenously. A study by Friedman, Abramson and Marx[92] indicated the answer to this problem, for they found that when Tyrode's solution was used for the perfusion of the isolated dog's tail no vasoconstriction occurred when renin was added, but that when blood plasma was used as the perfusing fluid the addition of renin did produce marked vasoconstriction. This observation was followed by the independent and coincidental finding by Page and collaborators[108,109] and by a group of South American workers[99,100,110] that the interaction of renin and blood plasma or serum in

vitro results in the formation of a new substance which is an effective vasoconstrictor and therefore a pressor substance. Page and collaborators gave the name "renin-activator" to the substance in the blood which interacts with renin and called the resultant vasoconstrictor and pressor substance "angiotonin".[111] The South American workers named the substance in the blood "hypertensin precursor" and the vasoconstrictor and pressor substance "hypertensin".[99]

It is of interest to note here some observations first reported by Dicker[112] and confirmed by Taquini,[113,114] that when the main renal artery or the entire renal pedicle was occluded for varying periods of time, there was a prompt and prolonged rise of arterial blood pressure after the restoration of the renal circulation by release of the renal artery or renal pedicle. This contribution has been fully corroborated by other investigators.[115-119] Taquini[113] also showed that if such a completely ischemic dog's kidney was removed and the clamp on the pedicle not released until the renal vessels were anastomosed to the carotid artery and jugular vein of a nephrectomized recipient, there was a marked rise in the blood pressure of the recipient when the circulation in the completely ischemic kidney was reëstablished. This same effect was observed from the same procedure with partially ischemic kidneys from dogs with chronic renal hypertension. The result indicated that a humoral mechanism, probably identical, was involved in both cases. The study of acute, complete renal ischemia was carried further by Prinzmetal and collaborators[107,118] who demonstrated that renin was the agent present in the saline perfusates of such kidneys, and that the rise in systemic blood pressure which followed the release of the pedicle was therefore due to the liberation of renin into the general circulation. These findings have been recently confirmed.[120,121] Taquini and Braun-Menendez[120] even demonstrated renin in the systemic arterial blood of animals following release of the pedicle of completely ischemic kidneys.

The discovery that renin is not by itself a vasoconstrictor substance and the subsequent finding that it needs a substrate in the blood stream with which to produce a pressor substance has thrown light on the hitherto obscure phenomenon of tachyphylaxis. Page and co-workers[93] showed that the reason for the progressive decrease in the response to consecutive injections of renin was due to exhaustion of renin-activator in the blood. This was confirmed by the South Americans[99] who found in addition that there is an excess of renin circulating in the blood stream

of tachyphylactic animals which, along with the absence of hypertensin precursor adequately explains the reason for failure to respond to further injections of the same substance.

It has also been shown that the action of renin is not affected by hypophysectomy, thyroidectomy, pancreatectomy, gonadectomy, splenectomy, abdominal evisceration, or destruction of the spinal cord.[89,92] It is known, however, that bilateral adrenalectomy is followed by progressive decrease in the response to injections of renin.[122-124] The full significance of this latter finding is not yet completely elucidated. It will be referred to briefly again later in this paper.

It was first noted by Tigerstedt and Bergman that in bilaterally nephrectomized animals renin has a more prolonged and striking pressor effect and that tachyphylaxis is less pronounced in such animals. This has been explained by Page[109] and confirmed by the South American investigators[99] as due to an increase in the amount of renin-activator circulating in the blood stream of such "arenal" animals.

The indications that renin may be the agent which initiates the rise of arterial blood pressure following constriction of the main renal artery are further strengthened by correlating the actual effects of intravenously injected renin on various functions under various conditions with similar functions and conditions in animals with experimental renal hypertension. These are compared in Table II which shows that the physiological effects of renin, when injected intravenously into a normal animal, are identical with the observed hemodynamic state of animals hypertensive due to clamping of the main renal artery.

Even more direct evidence as to the role of renin in initiating the rise of blood pressure which follows constriction of the renal arteries is to be found in the demonstration that extracts of kidneys of animals with chronic renal hypertension[139,140] and of kidneys with acute complete ischemia[118] contain more renin than extracts of the opposite normal kidneys. Direct proof, however, that renin is concerned in the genesis of experimental renal hypertension is furnished by the demonstration of renin in renal vein blood and systemic blood of animals made hypertensive by constriction of the renal arteries.[75,76,141]

Recent studies show that renin plays a part in the maintenance of blood pressure after severe hemorrhage and in shock.[141-146] Renin is liberated from the kidneys and can be demonstrated not only in renal vein blood but also in systemic blood.[141] The liberation of the renin in these

TABLE II

Organ or Condition Studied	*Effect of Intravenous Injection of Renin in Normal Animals*	*Parallel in Experimental Renal Hypertension*
Heart Rate	Unaltered from normal.[79, 92, 98]	Unaltered from normal.[20]
Cardiac Output	Unaltered from normal [88, 125]	Unaltered from normal.[20]
Complete Sympathectomy	Does not reduce B.P. rise due to renin.[126]	Does not reduce B.P. rise in experimental renal hypertension.[7, 23, 62, 63]
Pithing	Does not reduce B.P. rise due to renin.[79, 92, 93]	Does not abolish experimental renal hypertension.[60, 61]
Hypophysectomy	Does not reduce B.P. rise due to renin.[109, 122, 127]	Does not prevent or abolish experimental renal hypertension.[128, 129]
Thyroidectomy	Does not reduce B.P. rise due to renin.[127]	Does not prevent or abolish experimental renal hypertension.[52]
Gonadectomy	Does not reduce B.P. rise due to renin.[122]	Does not prevent or abolish experimental renal hypertension.[130]
Acute Adrenalectomy	Does not reduce B.P. rise due to renin.[93]	Does not reduce rise of B.P. when ischemic kidney of hypertensive dog is grafted into neck of normal dog.[131]
Chronic Adrenalectomy	Abolishes B.P. response to renin.[122, 123, 124]	Prevents or abolishes experimental renal hypertension.[1, 6, 39, 130, 132]
	Response restored by adrenal cortical extract or D.C.S.[124]	Hypertension maintained in adrenalectomized dogs treated with adrenal cortical extract or D.C.S.[39]
Bilateral Nephrectomy	Response to renin greater.[79, 127, 133, 134, 135]	Response to grafting of ischemic kidney of hypertensive dog greater in nephrectomized than normal recipient.[8]
Peripheral Blood flow	No decrease during rise of B.P. due to renin.[86]	No decrease in hypertensive rabbits with renal ischemia.[29]
Renal Hemodynamics	Direct evidence shows decrease of renal blood flow.[89, 92, 136]	Direct evidence shows decrease of renal blood flow.[34]
	Indirect evidence of efferent arteriolar constriction.[89]	Indirect evidence of efferent arteriolar constriction.[159]
Blood Pressure	Infusion of renin causes persistent elevation.[137]	Constriction of renal arteries causes persistent elevation.[1]
	Rise not reversed by 933F.[106, 107]	The rise of B.P. due to release of the pedicle of a completely ischemic cat kidney is not reversed by 933F.[107, 118]
	Repeated injections cause tachyphylaxis.[79, 92, 93, 99]	If a cat is first rendered tachyphylactic to renin, release of pedicle of completely ischemic kidney is not followed by usual rise of B.P.[118, 121]
	Renin causes no rise in pulmonary arterial pressure.[138]	No rise of pulmonary arterial pressure in experimental renal hypertension.[43]

D.C.S. = Desoxycorticosterone acetate.
B.P. = Blood pressure.

circumstances is presumably due to the hemodynamic disturbance which is caused by the fall of blood pressure and which simulates that caused by constriction of the renal arteries.

The Substrate of Renin [Renin-Activator (Preangiotonin) or Hypertensin Precursor (Prehypertensin)] and the Nature of its Reaction With Renin

The substance in the blood stream upon which renin acts was named renin-activator by Page and co-workers and hypertensin precursor or hypertensinogen by Braun-Menendez and his collaborators. If it is eventually proved that the resultant product of the interaction of these two substances is the cause of elevated blood pressure, then the specific term "hypertensin" of the South Americans will be more pertinent than the non-specific term "angiotonin." However, until the substance in question is actually isolated from the systemic circulation of patients with essential hypertension and/or animals with experimental renal hypertension, it is well to continue to use both terminologies side by side, but to bring them in line we suggest the terms "preangiotonin" for renin-activator and "prehypertensin" for hypertensin precursor, which will be used in the remainder of this paper.

Both Page and co-workers and Braun-Menendez and his co-workers agree upon the identity and the properties of renin-activator (preangiotonin) or hypertensin precursor (prehypertensin). It has been found to be a protein, in all probability a pseudoglobulin rather than a euglobulin, since it is soluble in distilled water and is precipitated from blood serum between 0.34 and 0.6 saturation with ammonium sulfate, findings which have been confirmed in our laboratory by Dr. Yale J. Katz. It is heat labile, non-dialyzable and not ultrafiltrable and is present in blood serum and red blood cells.[99,109] It is undiminished following hyophysectomy[109] but we have found that it decreases and finally almost disappears from the systemic blood of untreated adrenalectomized male dogs. Adequate therapy with adrenal cortical hormones or desoxycorticosterone acetate results in a return of preangiotonin (prehypertensin) to the normal level. We have also found that it disappears from the blood as the result of destruction of the liver. Full details of these studies will be given in a forthcoming publication on the role of the adrenal glands in experimental renal hypertension. Page failed to find any effect on renin-activator (preangiotonin) as the result of bilateral adrenalectomy, but

he has recently reported that it disappears from the blood when the liver is removed or severely damaged by a hepatotoxic substance.[147]

It has been further found both by Page and collaborators, and the South American workers that renin-activator (hypertensin precursor) is increased in the serum of the bilaterally nephrectomized dog[93,99] and exhausted in renin tachyphylaxis. In addition, renin-activator has been reported as increased in experimental renal hypertension and human essential hypertension.[99,148,149]

The South Americans have found, in addition, that the reaction between renin and hypertensin precursor (prehypertensin) is rather specific in that the effect of renin on the substrate in the blood could not be reproduced by other enzymes such as pepsin, pancreatin, papain or extracts of liver or spleen. They also found that hypertensin precursor (prehypertensin) was present only in blood globulins and that the latter could not be replaced by hemoglobin, casein, milk, egg or blood albumin, or by liver, spleen, thymus, testes, lung, heart, skeletal muscle or vegetable proteins.[99]

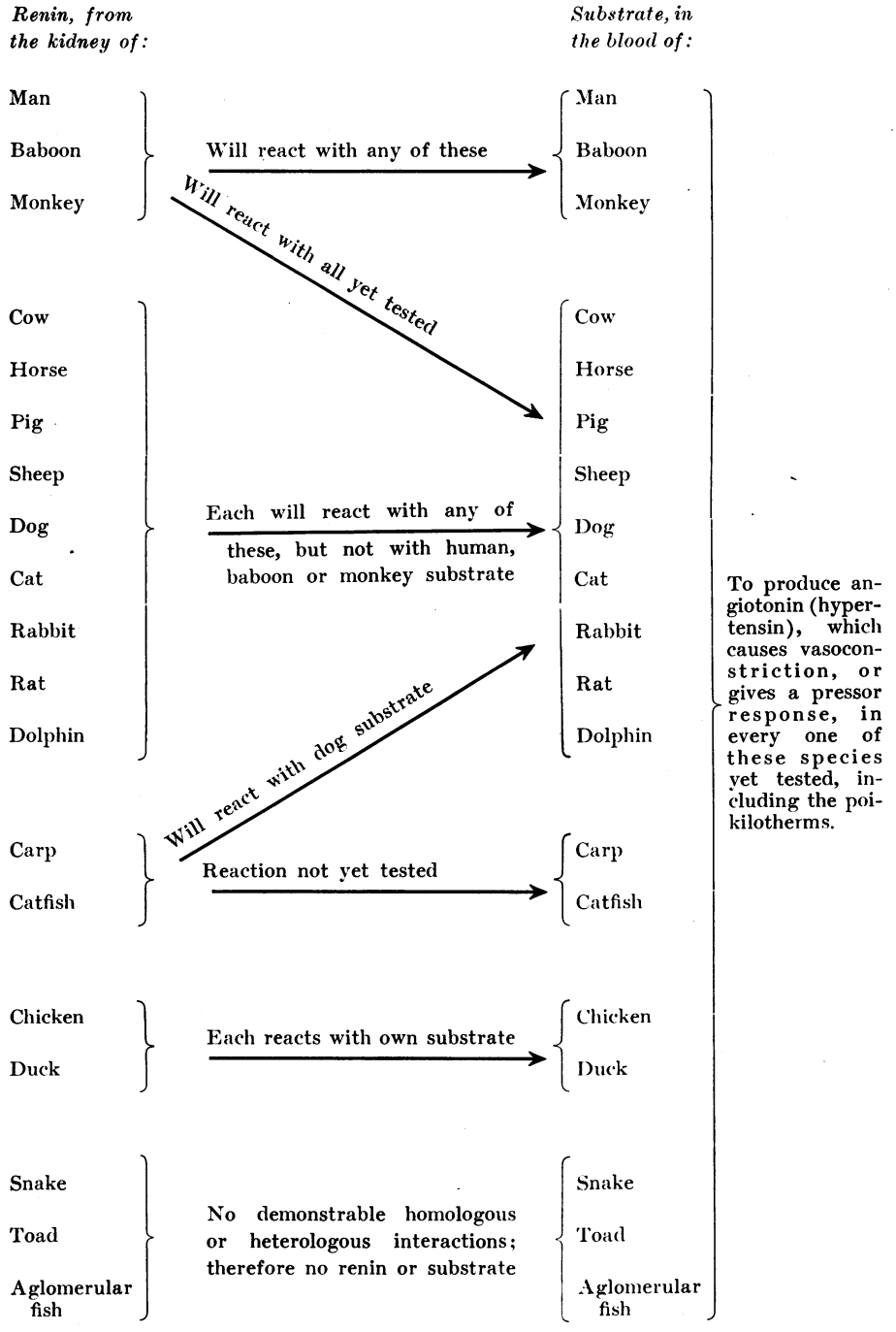
Subsequent studies with pepsin, however, by Croxatto and Croxatto[150] have shown that pepsin will produce a vasoconstrictor substance when it is allowed to act on blood globulins. They showed that the pressor substance produced by the action of pepsin was identical in its behavior to hypertensin (angiotonin) insofar as this could be chemically and pharmacologically determined. This has recently been confirmed by Helmer and Page[151] and by Dr. Yale J. Katz in our laboratory.

Interesting studies on the specificity of renin and its substrate in the blood have been made in a variety of different species by the group of South American workers[152,153] by Corcoran, Helmer and Page[154] and others.[134,155-157] These observations have been conducted on man, baboon, monkey, cow, sheep, horse, pig, dog, cat, rabbit, rat, dolphin, chicken, duck, snake, shark, toad, aglomerular fish, carp and catfish. The results of these studies are best summarized in Table III.

As to the reaction between renin and preangiotonin (prehypertensin), this seems to be enzymatic,[158] as is indicated by the following known properties of these participants and the characteristics of the reaction itself:

1. Renin is a protein, most probably a pseudoglobulin.
2. Renin is non-dialyzable and thermolabile (destroyed above 56° C[94]).

TABLE III


Renin, from the kidney of:
Substrate, in the blood of:
Man
Baboon
Monkey
Will react with any of these
Man
Baboon
Monkey
Will react with all yet tested
Cow
Horse
Pig
Sheep
Dog
Cat
Rabbit
Rat
Dolphin
Each will react with any of these, but not with human, baboon or monkey substrate
Cow
Horse
Pig
Sheep
Dog
Cat
Rabbit
Rat
Dolphin
Will react with dog substrate
Carp
Catfish
Reaction not yet tested
Carp
Catfish
Chicken
Duck
Each reacts with own substrate
Chicken
Duck
Snake
Toad
Aglomerular fish
No demonstrable homologous or heterologous interactions; therefore no renin or substrate
Snake
Toad
Aglomerular fish
To produce angiotonin (hypertensin), which causes vasoconstriction, or gives a pressor response, in every one of these species yet tested, including the poikilotherms.

3. Only a small amount of renin is necessary in proportion to the amount of blood substrate, and the amount of angiotonin (hypertensin) formed is proportional to the amount of blood globulins.
4. The reaction is affected by temperature; hastened by incubation at 37° C. and retarded or stopped at 0° C.
5. The substrate in the blood is also a protein, non-dialyzable and thermolabile.[99,109]
6. The end product of the reaction, angiotonin (hypertensin), is dialyzable and thermostabile, which indicates that the reaction is disintegrative rather than integrative, as is implied by Page's assertion that renin must be "activated."
7. The fact that renin, evidently a proteolytic enzyme, may be actually replaced by pepsin, a known proteolytic enzyme, is further proof of a disintegrative reaction. It may be that renin differs primarily from pepsin in that it acts at the natural pH of the blood and not in the pH range of pepsin activity, while pepsin cannot produce a pressor substance from blood globulins in the pH range of renin activity.

It is for these reasons that we consider renin an enzyme and preangiotonin (prehypertensin) its substrate. Page[159] agrees that this is now his working hypothesis.

Angiotonin (Hypertensin)

The substance formed by the action of renin on preangiotonin (prehypertensin) is the final effector vasoconstrictor and pressor substance of the humoral mechanism. It was independently discovered both by Page and co-workers, who named it angiotonin, and by the South American investigators, who named it hypertensin.

Page and Helmer[111] have reported the crystallization of angiotonin in the form of the picrate and oxalate, but not in uncombined pure form, and as yet have not published the melting point of any of these crystals or the probable structure of pure angiotonin. Considerable information is available, however, about the properties of this pressor substance. It is heat and acid stabile, water and alcohol soluble, fluorescent, alkali labile and gives the color reaction for arginine. Its pressor effect is immediate, the maximum rise abrupt, and the entire period of elevation of blood pressure brief, more like the pressor effect of epine-

phrine than that of renin. Renin gives a slow, maximum rise followed by a sustained elevation which may last thirty minutes or longer before returning to normal. This prolonged action of renin is assumed to be caused by the continuous liberation of angiotonin (hypertensin) due to the interaction of renin and preangiotonin (prehypertensin) in the blood stream. Page claims that angiotonin, like renin, also induces tachyphylaxis, and that "it needs its own activator", which he calls angiotonin-activator. He also found that renin destroys angiotonin when incubated with it. In addition, the pressor response to angiotonin was found to be unaffected by cocaine or atropine, or by pithing or adrenalectomy.[111] Other studies showed that the pressor response to angiotonin is greatly increased in nephrectomized dogs and that angiotonin, unlike renin, causes vasoconstriction when perfused with Ringer's solution through an isolated organ.[133] Later studies proved conclusively that the indirect effect of renin and the direct action of angiotonin are exerted on the peripheral blood vessels.[160]

The South Americans confirmed almost all of Page's findings with regard to hypertensin (angiotonin) but could not demonstrate tachyphylaxis to this substance. They also found that it produced a direct vasoconstrictor action, that its action was not reversed by piperidomethyl-3-benzodioxane (933F) and not affected by vagotomy or by excision of the carotid bodies, splanchnic nerves, liver, or adrenals, by evisceration or by destruction of the medulla.[161] They found that it was dialyzable, soluble in glacial acetic acid, liquid phenol and ethylene glycol, and that it was destroyed by pepsin.[99] Destruction by pepsin indicates that hypertensin (angiotonin) may be a polypeptide, although Page and Helmer[111] claim a negative biuret for angiotonin. Braun-Menendez and co-workers[100] also found that hypertensin was destroyed by trypsin and extracts of liver and spleen as well as by fresh normal blood serum, and that this ability of fresh serum to destroy hypertensin was decreased following bilateral nephrectomy. This substance which destroys hypertensin has been named hypertensinase by the South Americans.[99,162]

Many studies have now been reported on the physiological effects of angiotonin on the blood pressure, heart rate and output, coronary blood flow, venous pressure, viscera and renal blood flow and clearance.[163-175] In general, these correspond to the effects of the intravenous injection of renin in intact animals. Any differences that have been

found between the effects of intravenously injected renin and angiotonin may be due to the fact that the angiotonin formed from renin in vivo differs in some respects from the angiotonin produced in vitro.

The Evidence for the Existence of "Angiotonin-Activator". Page has stated,[176] "Since renin requires a second substance in order for it to exhibit pressor action, it follows that there is some reaction between these two substance to produce a third." Although Page demonstrated that renin and renin-activator form a new substance, angiotonin, yet he failed even to postulate the existence of a second new substance as the reaction product of angiotonin and angiotonin-activator.

In reference to angiotonin Page also stated,[133] "It does not appear to be the end product of the reaction between renin and renin-activator, for it itself is destroyed by further contact with renin." This statement was invalidated by the work of the South Americans,[162] who showed that another enzyme contained in renal extract, which they named hypertensinase, was responsible for the destruction of angiotonin (hypertensin) and that highly purified renin does not do it. These findings we have verified. They indicate that angiotonin (hypertensin) is the end product of the reaction between renin and renin-activator and thus further indicate that there is no need for angiotonin-activator.

In regard to the question of angiotonin tachyphylaxis, which Page observed and which he blamed partially on the exhaustion of angiotonin-activator, the South Americans[99] do not find this phenomenon in intact animals, nor do we. The South Americans and ourselves used the natural form of angiotonin produced by incubation of renin with serum. The difference in results may be due to the use of crystalline salts of angiotonin by Page and collaborators.

Again in reference to angiotonin-activator, Page said[133] "We employ the name, as in the case of renin-activator, to connote that neither renin nor angiotonin exerts a pressor action in the absence of their respective activators." It is of interest in this connection to note that angiotonin (hypertensin) will cause vasoconstriction in all species so far tested, including poikilotherms. Every species, from the kidneys of which renin has been extracted, whether this renin was effective in the same or in other species, has been found also to possess prehypertensin (preangiotonin) in its own blood. The renal extract of toad and snake,[153] prepared by the method used for the extraction of renin, is not effective

in the toad, snake or any other species. Thus the toad and snake contain no demonstrable renin or prehypertensin. Yet hypertensin (angiotonin) is effective in raising the blood pressure in both snake and toad.[153] It seems far fetched, therefore, to assume the existence of "angiotonin-activator" in animals which do not possess any of the other elements of the humoral mechanism of hypertension, yet give a definite vasoconstrictor and pressor response to the injection of angiotonin.

The difficulty of correctly ascertaining just what function Page assigns to "angiotonin-activator" or what its place in the humoral mechanism is, may well be gathered from his recent statements that "Angiotonin may be an intermediate in a series of reactions which ultimately lead to its destruction, i.e., renin + renin-activator → angiotonin + angiotonin-activator→vaso-inactive substance,"[159] and "It is my guess that angiotonin is only one in a series of substances, although probably a key substance which are formed when renin is allowed to act."[176]

It may be stated, therefore, that further evidence is needed for the existence of angiotonin-activator, and that even the demonstration of angiotonin tachyphylaxis[175] in isolated organs must be differentiated from a possible inhibitory toxic or other effect of the injected material on the blood vessels.

Hypertensinase (Angiotoninase)

Early in the course of their studies on the humoral mechanism the South Americans discovered that normal blood serum would destroy hypertensin in vitro, and they advanced the name "hypertensinase" for the responsible destructive agent. They demonstrated that hypertensinase, which was destroyed by heat (60° C.) could be separated from renin and prehypertensin, both of which are also heat labile, by incubating at 37° C. at a pH of 3.9 for fifteen minutes. Thus they showed that if the hypertensinase in renin and serum were first destroyed by this method, which leaves renin and prehypertensin unaltered, prolonged contact of renin and prehypertensin then resulted in a maximum yield of hypertensin which remained constant despite the length of incubation. The optimum pH of hypertensinase activity was found to be between 7.5 and 8.5, and its action to be affected by temperature, for at 0° C. its action stops. Thus they believe it to be an enzyme. It can be precipitated by half-saturation with ammonium sulfate, but not by dialysis.

In their study of the hypertensinase content of various tissues Fasciolo et al[162] found that kidney cortex had 1500, intestinal mucosa 1200-1600, pancreas and spleen 200, hemolyzed red cells and liver 100 units per gram. Serum and plasma (without hemolysis) were found to have only about 1 unit per gram.

That the kidney may be the main source of the hypertensinase in normal blood is indicated by the finding that there is almost a complete disappearance of hypertensinase from the blood stream of bilaterally nephrectomized dogs.[162]

Harrison, Grollman and Williams[177-179] were the first to attempt the treatment of experimental renal hypertension with renal extracts, although they had made no in vitro studies of the action of these extracts on angiotonin (hypertensin). Their approach was based on the observations that the normal kidney, by some humoral means, might play a part in eliminating the hypertensive effect of a contralateral ischemic kidney, since the blood pressure of such an animal tends to return to normal, and also that when the normal kidney is removed, in the presence of the ischemic one, the blood pressure ascends to and remains at a much higher level.

The idea that the normal kidney might play a part in the elimination of the "chemical mediator" in experimental hypertension was, incidentally, studied by Katz and Rodbard,[180-182] who confirmed the fact that the blood pressure falls to normal in six hours after the removal of a single ischemic kidney[6] and also observed that it takes five times as long for it to reach normal after removal of both kidneys when one or both were ischemic. They believed that the more rapid fall of the blood pressure to normal levels in the presence of normal kidney tissue, as contrasted with the slow fall in its absence, might be due to the excretion or in vivo destruction of the "chemical mediator" by the remaining normal kidney. By constructing a uretero-venous fistula on the side of the normal kidney they showed that removal of the opposite ischemic kidney was still followed by a rapid, six hour fall of the blood pressure to normal, and concluded that the normal renal tissue destroyed the "chemical mediator," since its excretion back into the blood stream would have kept the blood pressure elevated for a length of time comparable to that necessary in the absence of any renal tissue.[182] Since then, Dexter and Braun-Menendez[183] have demonstrated that the renal threshold of renin is too high for excretion of renin to account for the

stabilizing role of the normal kidney.

Independently of Harrison and collaborators, Page and his collaborators[184-186] reported the treatment of hypertension with renal extracts containing angiotonin-inhibitor. The basis of their approach was different from that of the South Americans and of Harrison and co-workers, for they[133] had previously published their findings that renin and angiotonin tachyphylaxis were both due to more than simple exhaustion of renin-activator and angiotonin-activator, and had postulated the presence of renin-inhibitor and angiotonin-inhibitor. They believed that it was the loss of these two inhibitors after nephrectomy that accounted in large measure for the increased sensitivity of nephrectomized animals to renin and angiotonin. Although in their first publication they claimed that the blood of hypertensive animals treated with their renal extracts no longer contained renin-activator or angiotonin-activator,[184] neither this finding nor the existence of renin-inhibitor has been confirmed. All of their work was done in vivo, but recently they[187,188] have confirmed the results of Fasciolo and collaborators[162] on the ability of extracts of various organs to destroy angiotonin in vitro, which we have also confirmed.

Since the existence of a substance which destroys hypertensin (angiotonin) was first described by the South Americans and shown to be due to an enzyme which they named hypertensinase, we believe that to keep the terminologies similar, until a final one is adopted, the corresponding term angiotoninase is apropos for "angiotonin-inhibitor," for the action of the enzyme in question is not that of inhibition of angiotonin, or suppression of renin- or angiotonin-activator, but the actual destruction of angiotonin itself. Because of the inconsistency of their results, the South Americans[141] did not publish the effect of treatment of experimental renal hypertension with renal extracts containing hypertensinase. Since it has not yet been shown that the possible antipressor effect of renal extracts of various kinds that have been used for the treatment of hypertension is actually due to hypertensinase (angiotoninase) it is well for the present to refer to such extracts as antipressor renal extracts.

Friedman[164] demonstrated that renal vein blood from an ischemic dog's kidney contained much less hypertensinase than renal vein blood from normal kidneys. This throws light on the earlier experiments by Freeman[189] who found that normal dog's blood would reduce the blood

pressure of dogs hypertensive due to constriction of the main renal arteries, whereas blood from hypertensive dogs gave no such effect. In this case the antipressor effect may have been due to the presence of hypertensinase in normal blood and its diminution in the blood of hypertensive animals, just as other previously cited results in bilaterally nephrectomized animals may have been due to the absence of hypertensinase in this condition.

Non-Specific Pressor and Anti-Pressor Substances

An indication of the part which the kidney might play in the elaboration of at least one of the substances involved in the humoral mechanism of hypertension has been given by a series of important studies by Bing and collaborators.[190-193] They have demonstrated that an ischemic kidney is capable, by effecting decarboxylation, of converting l-dopa (l-dihydroxyphenylalanine), a substance with no pressor properties, into hydroxytyramine, a powerful pressor substance. The amount of hydroxytyramine formed in the kidney from dopa, only under conditions of oxygen lack, was found to be proportional to the reduction of blood flow through the perfused kidney.[191] On the contrary, l-dopa added to normal blood being perfused through a kidney with normal blood flow was not converted into hydroxytyramine. It is of special significance that although liver and intestine also contain l-dopa-decarboxylase, yet these organs were unable, even when their circulation was reduced, to produce hydroxytyramine from dopa added to the blood. Similarly, it is only when the main renal artery is constricted that experimental hypertension results in the dog.[194,195] In further experiments on artificially perfused kidneys, it was found that the ischemic human kidney also converted dopa to hydroxytyramine.[190] The above experiments were done with kidneys in vitro, but Bing was also able to show that partially or completely ischemic cat kidneys converted dopa into hydroxytyramine in vivo.[192] As the result of these studies Bing has not concluded that hydroxytyramine is the cause of, or is in any way involved in, the pathogenesis of experimental renal hypertension, for he has demonstrated that angiotonin (hypertensin) is destroyed by a different fraction of renal extract than is hydroxytyramine, and that these two pressor substances are destroyed by different mechanisms.[193] This fact, along with his finding that renin was unable to effect decarboxylation of dopa and convert it to hydroxytyramine,[190] helps

to emphasize Bing's contention that he does not claim that hydroxytyramine is the cause of experimental renal hypertension. In this respect he has already been misquoted.[196] He has merely intended to show that when the blood flow to a kidney has been reduced the organ behaves differently than when the circulation is normal and that under these conditions it is capable of converting an amino acid, itself without pressor properties, into a powerful pressor amine.

Schroeder and Adams[197,198] have found that tyrosinase, a phenolic oxidase, has the power to destroy angiotonin in vitro. In all of their tests in which tyrosinase alone was added to angiotonin, and the two shaken together, very little oxygen was absorbed and the angiotonin lost none of its pressor activity. When a small amount of blood serum, however, was added to the mixture of tyrosinase and angiotonin, oxygen was rapidly absorbed and the angiotonin was completely inactivated. Schroeder and Adams concluded that tyrosinase destroyed the angiotonin, but in the light of our present knowledge it is only fair to conclude that the effect may have been due to the hypertensinase in the blood serum and not to the tyrosinase itself. Nevertheless, Croxatto and Croxatto[199] found that tyrosinase from mushrooms and amine oxidase from the liver of the squid, did destroy the vasoconstrictor activity of hypertensin as tested by Laewen-Trendelenberg technique on the toad.

The presumable destruction of angiotonin by tyrosinase led Schroeder[198] to postulate that angiotonin, like adrenalin and tyramine, "may accordingly contain a phenolic group." On this basis he undertook the treatment of experimental and human hypertension with tyrosinase and found that it reduced the blood pressure. Martin and collaborators[196] also found that tyrosinase will lower the blood pressure of hypertensive dogs, but added that this effect of tyrosinase was greatly enhanced by the addition of catechol.* How these results are related to the antipressor effects of renal extracts in experimental renal and in human hypertension is far from clear. When it is realized that such substances as typhoid vaccine and sterile milk[200] can also cause a reduction of blood pressure in experimental renal and human hypertension, the intricacy of this phase of the problem of hypertension becomes obvious. The present status of the treatment of experimental renal hypertension is reviewed in full in a forthcoming publication.[200]

* In a personal communication from Dr. Myron Prinzmetal[201] we have learned that a mushroom tyrosinase preparation, in which the tyrosinase was inactivated by heat, was also effective in producing a significant lowering of blood pressure and remission of other symptoms of human hypertension, when the extract was administered by intramuscular injection.

Summary

It has been demonstrated that after constriction of the main renal arteries to a sufficient degree to produce experimental renal hypertension, renin, an enzyme, is liberated into renal vein blood. It has been shown that this enzyme, renin, interacts with preangiotonin (prehypertensin) a pseudoglobulin substrate in systemic blood, probably produced by the liver in the presence of an adequate amount of functioning adrenal cortical tissue, to form the final effector vasoconstrictor and pressor substance, angiotonin (hypertensin). It has also been demonstrated that the physiological effects of the intravenous injection of renin are identical to the hemodynamic alterations found in experimental renal hypertension and in human essential hypertension. It follows, therefore, that the humoral mechanism noted above may also be responsible for the elevation of blood pressure in human essential hypertension. Although the demonstration in hypertensive blood of vasoconstrictor substances which may possibly be related to this humoral mechanism, has been reported, the proof that angiotonin is the responsible agent, awaits its isolation from the systemic blood of human beings with hypertension.

In addition to renin, preangiotonin (prehypertensin) and angiotonin (hypertensin) which are involved in the humoral mechanism of experimental renal hypertension, a fourth factor, hypertensinase (angiotoninase) has been discovered. Although this substance, an enzyme, destroys angiotonin (hypertensin) in vitro, there is no proof yet available that it is effective in lowering blood pressure in man or animals with hypertension. It has not yet been shown that the anti-pressor renal extracts with which hypertension is said to have been lowered in man and animals are rich in hypertensinase and inactive when this enzyme is destroyed. The exact nature of the effective substance in anti-pressor renal extracts which have been used in the treatment of hypertension, remains to be elucidated.

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