A cause and effect relationship between the renin-angiotensin pressor system and elevated blood pressure has been sought persistently since the discovery in 1898 of the enzyme, renin, and the production of experimental renal hypertension some years later by Goldblatt. This relationship is not without experimental foundation since evidence of increased renin content of kidney and the appearance of pressor substances in the blood stream have been demonstrated during alterations in renal artery pressure and in some clinical cases of hypertension. Possibly, technical difficulties inherent in the direct approach to this problem, i.e., the measurement of circulating levels of pressor agent, have prevented investigators from proving the relationship or prevented others from accepting renin as the responsible factor in the pathogenesis of experimental or essential hypertension.

As an alternative approach, antirenin, a neutralizing antibody to renin, may be used to block in vivo the renin-angiotensin pressor system and thereby permit an evaluation of the effect of this system on the various parameters contributing to circulatory homeostasis. This approach is applicable to the dog and several other species of laboratory animals which do not show species specificity of endogenous renin to antirenin formed against a plentiful renin such as bovine or hog. Antirenin formed against hog renin is capable, therefore, of neutralizing the pressor effect of dog renin.

In this report, I wish to summarize data obtained by Wakerlin and his group (of which I was a member for several years) and from work currently being done in my laboratory. We shall present evidence to demonstrate that (a) the renin content of dog kidney increases many-fold in the presence of neutralizing antibody, (b) the increase in renal renin is associated with hyperplasia and granulation of the juxtaglomerular cell, and (c) the kidney of the trained dog with circulating serum antirenin has an altered capacity to compensate for the hemodynamic effects of hydralazine.

A working hypothesis will be proposed, namely, that the renin-angiotensin system is a regulator of intrarenal hemodynamics.

That the renin-blood pressure relationship does not operate through simple cause and effect was indicated some years ago by Wakerlin and associates in the finding that partial constriction of one renal artery in the dog resulted in a fall of renin content of the contralateral kidney to zero or near zero levels (table 1). Partial constriction of the contralateral renal artery resulted in a return of renin concentration to control levels within a few days. Constriction of one renal artery in the dog usually does not produce a sustained increase in systemic blood pressure.

A close and inverse relationship has been demonstrated between blood pressure and antirenin titer. Figure 1 illustrates the effect of hog-renin injections and antirenin formation on blood pressure before and after the production of experimental hypertension by partial renal artery constriction. Blood pressures were obtained by femoral artery puncture at approximately weekly intervals. It is interesting to note that hog-renin injections

*Hydralazine hydrochloride was generously supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.
EFFECT OF ANTIRENIN ON BLOOD PRESSURE OF RENAL ARTERY CONSTRICED DOGS

A. CHRONIC RENAL ARTERY CONSTRUCTION

B. ACUTE RENAL ARTERY CONSTRUCTION

C. PASSIVE TRANSFER

FIGURE 1
Effect of antirenin on blood pressure of renal artery constricted dogs. (Reproduced with modifications from Wakerlin et al. 7)

and antirenin formation had no significant effect on normotensive pressure (fig. 1, record B), prevented the expected rise of pressure to hypertensive levels after renal artery constriction (fig. 1, record B), and reduced the elevated pressure of the experimental renal hypertensive dog (fig. 1, record A). Passive transfer of serum containing a high titer of antirenin from a donor dog to an experimental renal hypertensive animal (fig. 1, record C) resulted in a fall of pressure toward normotensive level, but for only the short period that measurable antirenin was present. These findings suggested that renin played a negligible role in maintaining normal blood pressure in contrast to its dominant role in the production and maintenance of experimental renal hypertension. 7

To explore the relationship between blood pressure, antirenin, and renal renin content, Dr. Brennan and I, in Dr. Wakerlin's laboratory, injected hog renin in three groups of dogs—normotensive, acute experimental renal hypertensive, chronic experimental renal hypertensive—until a wide range of antirenin titers were obtained; the animals were then nephrectomized for renal renin determination. 11 In order to study this relationship during the acute period following renal artery constriction, the acute experimental renal hypertensive group was treated with hog renin prior to renal artery constriction and sacrificed one month after operation. A surprising and very significant increase in renal renin concentration was found in both the normotensive and renal artery constricted dogs injected with hog renin. This increase in renal renin did not show any apparent

TABLE 1
Effect of Unilateral Renal Artery Constriction on the Renin Concentration of the Contralateral Kidney*  

<table>
<thead>
<tr>
<th>No. of dogs</th>
<th>Days after constriction</th>
<th>Renin concentration (DU/Gm. tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>2, 2, 2, 2</td>
</tr>
<tr>
<td>7</td>
<td>5-15</td>
<td>0, 2, 2, 2, 2, 2, 2</td>
</tr>
<tr>
<td>15</td>
<td>20-29</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1</td>
</tr>
<tr>
<td>9</td>
<td>30-39</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0</td>
</tr>
<tr>
<td>14</td>
<td>40-69</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0</td>
</tr>
<tr>
<td>12</td>
<td>60-79</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0</td>
</tr>
<tr>
<td>8</td>
<td>80-110</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0</td>
</tr>
</tbody>
</table>

Following constriction of artery to the contralateral kidney

| 7           | 1-5                     | 1, 0, 1, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0 |
| 6           | 5-10                    | 0, 4, 2, 0, 2, 0, 2, 0, 2, 0, 2, 0, 2, 0 |

*Reproduced with modifications from Wakerlin et al., 10 by permission of The Josiah Macy Jr. Foundation, New York, New York.
1 Opposite kidney atrophic.
RELATION OF RENAL RENIN CONCENTRATION TO SERUM ANTIRENIN TITER

FIGURE 2
Relation of renal renin concentration to serum antirenin titer. (From Schmid and Wakerlin.)

relationship to systemic blood pressure levels existing at the time of nephrectomy. It did correlate closely with the amount of antirenin present (fig. 2). Renal renin is reported as Goldblatt Dog Units* per Gm. of fresh kidney tissue and antirenin as Antirenin Units† per cc. plasma. The correlation coefficient between renal renin and antirenin titer for the normotensive group was significant at the 0.1 per cent level and for the combined acute and chronic hypertensive groups at the 1.0 per cent level. A difference was noticed between the normotensive and hypertensive groups in the amount of renal renin per unit of antirenin. The normotensive dogs exhibited renin concentrations higher than the renal artery constricted animals at comparable antirenin titers. This may reflect a greater turnover of renin in the hypertensive groups, stimulated by a lower renal artery pressure.

*Goldblatt Dog Unit (DU) is that amount of renin which will, on intravenous injection into trained, unanesthetized dogs, give a 30 mm. Hg elevation of mean blood pressure.

†Antirenin Unit (AU) is that amount of antirenin contained in plasma capable of neutralizing the pressor response of one Dog Unit of renin.

The dog normally does not exhibit a prominent juxtaglomerular apparatus and granules are not usually present. An excellent correlation between renal renin concentrations and juxtaglomerular cell changes was found (fig. 3). The juxtaglomerular cell change was evaluated on a one to five-pins basis by averaging 50 glomeruli sectioned through their vascular poles. These findings are further evidence that the juxtaglomerular cell is the site of renin formation as originally proposed by Goormaghtigh, suggested by Marshall, and recently strengthened by the microdissection studies of Bing and the fluorescent antibody studies of Hartroft.

Two possibilities are suggested to explain the many-fold increase in renal renin found in the presence of neutralizing antibody. The first could be a blockade of the release of renin by the antibody at the cell or vessel wall. The second, and more acceptable, would be a compensatory stimulation of renin formation by some change secondary to the neu-
EFFECT OF INTRAVENOUS HYDRAZINE ON RENAL HEMODYNAMICS IN TRAINED DOGS WITH AND WITHOUT SERUM ANTIRENIN

Dog A. Dog B. No Antirenin Antirenin Titer - 20 AU

FIGURE 4
Effect of intravenous hydralazine on renal hemodynamics in trained dogs with and without serum antirenin.

FIGURE 5
Effect of oral hydralazine on renal hemodynamics in trained dogs with and without serum antirenin.

enalization of renin and the prevention of its action by antirenin. The finding that renin increases in animals with renal arteries either normal or constricted, without an apparent relationship to systemic blood pressure, suggests that the change common to both may be occurring intrarenally. Tobian 17 in a recent review discussed the possibility that dietary salt or altered renal artery pressure, which are associated with changes in renin formation, may also be associated with systemic and intrarenal hemodynamic and volume changes.

In an attempt to explore the intrarenal effects of antirenin, renal hemodynamic studies were conducted in trained dogs given hydralazine intravenously (fig. 4) and orally (fig. 5). This drug was selected because of its known dilator effect on the glomerular efferent vessels. 18 Intravenous administration of hydralazine (fig. 4) caused a slight fall in systemic blood pressure in both the control and in the dog injected with hog renin, as recorded through indwelling femoral artery catheters. Of primary interest, however, is the finding that the glomerular filtration fractions of these animals differ. Thus, the filtration fraction of the control dog fell slightly (fig. 4, dog A) indicating a postglomerular reduction in resistance, and then returned within minutes to control values. The antirenin dog, however, had a more marked fall in filtration fraction (fig. 4, dog B), which persisted for the duration of the experiment. Although the changes are not as striking in the animals receiving hydralazine orally for a prolonged period (fig. 5), a fall in filtration fraction below control values is suggested by the data in the antirenin group, which persisted for a longer period than in the control group (fig. 5, group A). A marked increase in pulse rate throughout the course of hydralazine treatment in both groups indicated the continued activity of the drug on the cardiovascular system.

We would like to believe that renin is being released in greater amounts in both the control and antirenin animals, stimulated by the effect of hydralazine on postglomerular re-
RENIN, A PHYSIOLOGIC REGULATOR OF RENAL HEMODYNAMICS?

HYPOTHETICAL ROLE OF RENIN-ANGIOTENSIN ON INTRARENAL HEMODYNAMICS

FIGURE 6

Hypothetical role of renin-angiotensin on intrarenal hemodynamics.

sistance, and that the return toward normal in the control animals reflects a compensatory action of the renin-angiotensin system which is blocked in the dogs with serum antirenin. Possibly, a further increase in renin secretion, exceeding the neutralizing effect of antirenin locally, is responsible for the delayed return of the filtration fraction toward control values in the chronically treated antirenin group (fig. 5, group B). A return of renal hemodynamic changes to control values following hydralazine administration has been reported by Moyer.10,20

A working hypothesis (fig. 6) is suggested to explain the effect of antirenin on the renin-angiotensin system in these experiments. Since both the renin-antirenin and renin-substrate reactions are time-dependent, it is possible that the increased formation of renin found in the presence of neutralizing antibody may allow the production of small amounts of angiotensin. If renin is formed at the vascular pole of the glomerulus, as increasing evidence indicates, the most probable site of action of angiotensin would be the smooth muscle of the efferent glomerular vessels. A check and balance mechanism, therefore, may operate normally across the glomerulus between the pressure-sensitive juxtaglomerular cells and the postglomerular vascular resistance. Postglomerular resistance would increase or decrease in accord with an increase or decrease in renin secretion. The accompanying change in intraglomerular pressure, assuming that systemic pressure and preglomerular resistance remained constant, would comprise the return loop of a feedback mechanism to the pressure-sensitive juxtaglomerular cells. In effect, such a mechanism may serve to maintain a constant glomerular filtration pressure, a finding well established for the kidney.21 The presence of antirenin may disrupt this equilibrium by neutralizing renin and preventing the constrictor action of angiotensin. The kidney may in this manner be stimulated by antirenin to "set" its renin secretion at a higher level to maintain intrarenal homeostasis. Similarly, during chronic pathophysiologic conditions associated with alterations in intrarenal hemodynamics, renin may be secreted in excessive amounts and allow the escape of angiotensin into the systemic circulation.

Summary

1. Injections of hog renin and formation of antirenin in the dog will prevent or reduce the elevation of blood pressure associated with constriction of the renal arteries, but will not affect the systemic blood pressure in the normal animal.

2. The kidneys of both the normal and renal artery constricted animal respond with an increase in renin content during hog-renin injections which correlates closely with the serum antirenin titer.

3. The juxtaglomerular cell appears to be the site of renin formation and in this location may affect postglomerular vascular resistance.

4. Dogs with high titers of antirenin ap-
apparently have a reduced capacity to respond to an alteration in postglomerular resistance.  

5. It is proposed that renin formation is controlled normally by a pressure-sensitive feedback mechanism operating across the glomerulus between the juxtaglomerular cell and postglomerular resistance.

References


Discussion

Dr. Friedman: I think every year we rediscover that the Goormaghtigh cell is producing renin, and this is very encouraging until we remember that we don't find it necessary to discover every year that penicillin cures pneumonia. I myself worked in 1940 and 1941 on trying to find out where renin came from. We came to the conclusion that renin came from the proximal tubule. I think Braun-Menendez also came to that conclusion. This work might have been very obscurely printed in the Journal of Experimental Medicine, but it isn't often quoted today. When Dr. Goormaghtigh visited our laboratory, we indicated to him that one of the richest sources of renin is the fetal kidney. If one wants to go back far enough, one can find renin in the mesonephron. We pointed out that the latter was very rich in renin yet had no recognizable juxtaglomerular apparatus, and we asked Dr. Goormaghtigh if he would like to take a look at the sections. He said he couldn't explain this detection of renin in fetal kidneys, but he was quite aware of its presence, and thought that perhaps Goormaghtigh cells were distributed throughout the arteries of such animals without any condensation of these cells. One can go back as far as one wishes in the embryo kidney, these cells cannot be found—yet the kidneys are rich in renin. We thought we had some relatively cogent evidence that perhaps the convoluted tubule might have renin. Until our findings are explained, re-proving the same thing every year indicates to me that no one is quite sure what they are saying about the Goormaghtigh cell and its role in renin production.

Dr. Schmid: I am aware of your work with the fetal kidney and until recently did not come to a conclusion as to where renin may be located. I have been impressed, however, with the reports by Tobian, Gross, Hartroft, and others which relate renal renin to the juxtaglomerular cell. In our experiments, when we use the Bowie stain to study specimens from kidneys with a 20- to 30-fold increase in renin, low power reveals large dark spots in the specimen due to the intense staining of the juxtaglomerular areas. I have never seen heavily granulated juxtaglomerular cells in the normal dog. In the dog injected with hog renin, we find marked granularity of these cells, a finding which correlates well with the renin content of the kidney. Although we have stained only 11 kidney specimens, I am confident that the correlation will continue to hold true for additional specimens. This excellent correlation between juxtaglomerular-cell granularity and renal content of renin leads me to believe that the juxtaglomerular cell is very likely the site of renin formation.

Dr. Friedman: Have you ever done this on the fetal kidney?

Dr. Schmid: No. I have no answer for the findings with the fetal kidney except to point out that we are working with an unusual experimental situation which results somehow in a marked increase in the renin content of kidney and allows us to demonstrate the increased juxtaglomerular-cell granularity. Possibly, the hemodynamics are such in the fetal kidney that there is not a stimulation of these cells. We are proposing that the increase in renal renin is due to an altered hemodynamic situation within the kidney.

Dr. Pago: In the past few years angiotensin has been out of the dog house, so now I feel impelled, for a change, to be the devil's advocate. Many of you never knew that Dr. Corcoran years ago did much to start the ball rolling by studying the efferent and afferent resistance changes during infusion of what was then called angiotonin in dogs and man. It was apparent to us, if not to others, that the filtration pressure, and thus intrarenal pressures, could be under the control of such a substance. As we pointed out, this was a way of keeping filtration normal even in the face of reduced tubular blood flow. About this time Goormaghtigh suggested that renin might be present in the juxtaglomerular cells (which have now deteriorated into JGA, thus putting us all in the class of inarticulate
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enzymologists). But the notion didn't stick because no evidence was produced to substantiate it. A decade later opinion had softened and more circumstantial evidence has appeared to suggest this localization of renin. Even now it is not altogether convincing. Thus the suggestion that renin is in a strategic location and in control of intrarenal pressure is still "iffy."

By the same line of crochety reasoning, I didn't think we should accept the too-glib term "renin content of the kidney." The burden of proof is on those who use this term to demonstrate its validity. Enzymes in tissues are extraordinarily difficult to quantitate. Almost none of these measurements have fulfilled the ordinary requirements of chemical quantitative analysis. You don't know how extractable the enzyme is; you don't know whether the extraction process alters the amount; you don't know the sort of electrolyte environment to work in, nor the nature and quantity of co-substances necessary for maximum activity. In short, with the present saline extracts of ground kidney, you will be very lucky if your measurements ultimately turn out to be right. I am not sure either what the relation is between the "renin content" and the amount of renin secreted. Having done the legal work for the Devil, I now can say that I personally like the idea of angiotensin being concerned in the mechanism of intrarenal hemodynamic control. This is too important, however, not to subject each step in this theory to rigid analysis. I don't care much which way the cat jumps, but I want to be sure we all are clear that this is really the path that was chosen by that mythical feline.

One last word: must we start adding abbreviations to the language of our trade union? I notice many British journals simply bristling with them. I really can't imagine what they save, unless it is the reader from understanding what the author is trying to impart.

Dr. Laragh: I was surprised that the hydralazine failed to increase the renal blood flow in the normal dog. We have not done this in dogs, but in normal human beings, that isn't what has been observed. Secondly, I was not clear about whether the effect on renal blood flow was permanent or the depression came back to normal after a while, in the antirenin dog. I would expect that after a while the renal blood would tend to return to normal anyway.

Dr. Schmid: The dose of hydralazine selected for intravenous use is relatively low and gives only minimal effects on renal blood flow in normal dogs. I have used higher doses and there is a greater and more prolonged increase in renal blood flow. If the systemic pressure falls markedly from the higher dose, we may set into play another factor known to influence renin secretion. It is true that very little change in flow occurred in the control animals receiving hydralazine. Other investigators, notably Moyer, have found that the flow returns to control levels after acute or chronic administration of hydralazine to dogs and human beings. I hope I am not misstating Dr. Moyer's experience. In our experiments, in the antirenin-treated dog the flow did not return to normal after intravenous hydralazine during the three-hour period of the study. The orally treated animals, however, showed a slow return of filtration fraction to control value in the antirenin group, which may be due to an increase in renin formation overcoming the local blocking action of antirenin. These experiments with hydralazine are somewhat preliminary, and more are under way.

Dr. Masson: I would like to extend the comments made by Dr. Page and draw attention to the difficulties of interpreting data on renin content. It has been said that renin content represents the difference between synthesis and secretion without being a measure of either. Recently, we made temporal studies on renin content and renin secretion following treatment with desoxycorticosterone plus salt. There is an early and abrupt cessation of secretion while content remains normal; then content decreases gradually to low lev-
It is therefore possible to have a dissociation of these two variables so that determination of renin content may not necessarily reflect activity of the pressor function of kidneys.

**Dr. Schmid:** Interpretation of the renin content of kidney poses many problems. Data of this type must be carefully evaluated. We are trying to get around this difficulty by studying several parameters at once and by forming antirenin in dogs. This elevates the renal renin content in a somewhat linear fashion. We can then superimpose a renal hemodynamic change on this kidney, either surgically or pharmacologically, and see what happens to the increased renin content. One parameter is to remove the kidney and measure the renin content by bioassay. Possibly, changes in the increased amount will allow a more significant evaluation. Also, the Bowie stain may help to demonstrate whether an acute degranulation may have occurred from the experimental procedure in the hyperplastic juxtaglomerular cells. Following the serum antirenin titer may provide an indirect measurement of renin secretion. Simultaneous hemodynamic studies are important.

**Dr. Hollander:** During hydralazine studies, did you notice any difference in excretion of sodium and water in response to intravenous Apresoline in your control group of animals and in your treated group of animals?

**Dr. Schmid:** I have no information on this point.

**Dr. Hollander:** How about water?

**Dr. Schmid:** There was a drop in urine flow in these experiments during the period of hydralazine treatment. The significance of this is difficult to evaluate since this factor was only semi-controlled, in that we gave each animal a standard amount of water by stomach tube before the clearance study. This change was noticed when the data were analyzed and I don’t have an answer for it. There was a fall in inulin clearance in some animals which would indicate a fall in glomerular filtration rate, which I believe Selkurt has found related to urine flow.

**Dr. Corcoran:** I think Dr. Laragh’s remarks were important. We collected data on response to hydralazine in normal dogs and hypertensive dogs in doses of 0.2 mg./K. The usual response was a considerable increase in CPAH of the order of 150 or 200 per cent of control levels, often a fall in filtration rate and a fall in urine flow and electrolyte output.

**Dr. Schmid:** How long did you follow your animals after the administration of hydralazine?

**Dr. Corcoran:** Usually about an hour after the injection. There were three 10- to 20-minute periods of urine collection.

**Dr. Schmid:** We have done this in four trained dogs without antirenin and in some instances the filtration fraction stayed down for three to four clearance periods of 10 minutes, but it has always returned toward control values within the three-hour period of observation following intravenous injection of hydralazine.