Studies on High Molecular Weight Renin from Hog Kidney

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SUMMARY Previous reports by several investigators on the activation of a high molecular weight renin have not been confirmed. A simple method of extracting hog kidneys by using toluene was modified to yield partially purified renin with a molecular weight of 60,000, exclusively. Attempts to increase the activity of this renin by acidification resulted in a loss of 20-30% of the total activity, although the renin activity remaining was converted to a molecular weight of 40,000 and its specific activity increased 10-fold over that of the original. Attempts to obtain increased activity of the 60,000 molecular weight renin by adding crude kidney extract before the acidification did not result in activation. Activity of renin partially purified by a procedure involving acidification was inhibited by the addition of crude kidney extract. Incubation of increasing amounts of crude kidney extract alone resulted in a progressive reduction in angiotensin produced. When the angiotensin curve was extrapolated to zero extract concentration (be

the inactive renin in his experiments was 43,000 and that the normal active renin in plasma was 41,000.

Renin is most often activated through acidification to a pH below 4. However, in at least two laboratories,12, 14 it was found that storage at low temperatures also can bring about activation of plasma renin. Boyd first reported the occurrence of renin of 60,000 molecular weight in neutral extracts of hog kidney. On acidification, the molecular weight was reduced to 40,000, and the renin became more active. A similar phenomenon was found by Leckie in rabbit kidney extracts, in which the molecular weights were 54,000 before acidification and 37,000 thereafter. In later work, Boyd and McConnell found that the high molecular weight renin consisted of renin bound to a protein in an apparently reversible manner. Whether or not the original material was bound covalently was not definitely established. Day et al. found varying amounts of inactive high molecular weight renin in plasma of only 11 of 28 patients tested, and none at all in normal control subjects. However, Skinner et al. found that inactive renin in the plasma of all their patients and normal control subjects was activated by acidification, and Derkx et al. and Boyd reported similar findings for almost all of their patients. Boyd also noted that the molecular weight of the inactive renin in his experiments was 43,000 and that the normal active renin in plasma was 41,000.

Since the original observation by Lumber's that renin in amniotic fluid is activated by acidification, and Rubin's account of similar activation in extracts of hypertrophied hog kidney,5 there have been a number of reports of renin activation in amniotic fluids,5-6 kidney extracts,5-6,10,16-17 and plasma.1,4,5,11-17 In several cases, it was determined that the renin which underwent activation had a molecular weight higher than normal.5-6,10,17 Day et al. reported that both of two samples of amniotic fluid they investigated by gel filtration had high molecular weight renin, although they found varying amounts of inactive high molecular weight renin in the plasma of only 11 of 28 patients tested, and none at all in normal control subjects. However, Skinner et al. found that inactive renin in the plasma of all their patients and normal control subjects was activated by acidification, and Derkx et al. and Boyd reported similar findings for almost all of their patients. Boyd also noted that the molecular weight of the inactive renin in his experiments was 43,000 and that the normal active renin in plasma was 41,000.

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In our laboratory, a high molecular weight renin was found in small, constant amounts in extracts of hog kidney after acidification.28 Unlike those reported from other laboratories, the component could not be activated, nor
did it change molecular weight from 80,000* by any means we have tried.

Several investigators\(^1\) refer to an earlier work from this laboratory\(^2\) as being an example of renin activation in partially purified kidney extracts. No increase in total activity had ever been noted in that work, nor had molecular weight determinations been carried out. Because of the variation of results among different laboratories and because of the importance of such an activation as a control mechanism for the renin-angiotensin system, a reinvestigation of the problem was carried out to determine the nature of the conversions. The results of these investigations are reported below.

**Methods**

**Hog Kidney Cortex**

Hog kidneys obtained from the slaughter house were frozen and partially thawed. The cortex was sliced off and refrozen for future use.

**Renin Preparation, Method I**

Preparation of the toluene-treated extract was carried out as previously described,\(^3\) except that kidney cortex was used, elution of renin in the DEAE-cellulose batch treatment was at pH 5.0, and the eluates were concentrated by adding solid ammonium sulfate to 2.5 M.

**Renin Preparation, Method II**

Two kilograms of partially thawed hog kidney cortex were ground into an ice-cold stirred mixture of 4 liters of distilled water and 1 liter of toluene. After stirring in an ice-water bath for 2 hours, the mixture was centrifuged 4 hours at 2200 g. The middle layer was withdrawn by suction and the remaining two layers discarded. The extract (between 3 and 4 liters) was then diluted with 5 volumes of cold distilled water, and 15 g of moist, regenerated DEAE-cellulose were added per gram of protein. The pH was maintained between 6.5 and 7.5 by the cautious addition of 1 N acetic acid and the adsorbent, as the mixture was stirred to avoid high local concentrations of acetic acid. After addition of the DEAE-cellulose and acetic acid, the mixture was filtered through muslin with suction in the cold and the filtrate was discarded. The DEAE-cellulose pad was then washed by resuspension in a volume of cold water at least five times as large as the original volume of extract, followed by the careful readjustment of the pH to 5.0 with 1 N acetic acid and filtration. The renin was eluted by suspension of the washed DEAE-cellulose pad in 10 liters of 0.15 M NaCl and titration of the stirred mixture to pH 5.0 by the careful addition of 1 N acetic acid. Stirring was continued for 10 minutes, the mixture was filtered, and the elution step was repeated. The filtrates were then combined and the renin was precipitated by the addition of 400 g of solid ammonium sulfate per liter of stirred solution. Stirring was continued for 2 hours after the ammonium sulfate had dissolved. The precipitate was harvested, taken up in cold distilled water, and dialyzed overnight against 80 volumes of 2.5 M sodium phosphate buffer (pH 7.5), for 8 hours against 80 volumes of 2 M phosphate buffer (pH 7.0) and, finally, overnight against 80 volumes of cold distilled water. After centrifugation of the dialyzed mixture, the supernatant solution was decanted and frozen.

**Crude Extract of Hog Kidney Cortex**

One hundred grams of hog kidney cortex were ground in a small electric meat grinder. The mince was passed through the grinder a second time and into 100 ml of cold distilled water, and the mixture was stirred for 1 hour. After centrifugation for 15 minutes at 500 g, approximately 90 ml of supernatant extract were decanted, stopping the decantation before the loose sediment started over. The residue was resuspended with the aid of 100 ml of cold distilled water and homogenized in portions by one pass with a Teflon-glass homogenizer. Centrifugation of the mixture for 15 minutes at approximately 300 g yielded an additional 100 ml of supernatant solution, which was mixed with the first extract.

**Renin Assay**

Incubation tubes contained 1.7 \(\mu\)mol EDTA, 17.5 \(\mu\)mol phosphate buffer (pH 7.5), 35 \(\mu\)mol NaCl, 350 pmol partially purified hog substrate, 9 \(\mu\)mol phenyl-methanesulfonyl (PMSF), and renin in a total volume of 0.65 ml. After incubation at 37°C, usually for 15 minutes, the reaction was stopped by chilling the tubes in an ice bath and adding 0.35 ml of an acid-phosphate buffer\(^4\) to lower the pH to 5.5. Proteins were denatured by placing tubes in a boiling water bath for 10 minutes. The angiotensin produced was assayed either biologically in an anesthetized rat preparation\(^5\) or by a modification of the radioimmunoassay method of Haber et al.\(^6\) Renin is reported as nanomoles angiotensin per milliliter per 15-minute incubation period. The equivalent of 1 Goldblatt unit in this method is 8.3 nmol angiotensin per 15 minutes.

**Gel Column Chromatography**

Renins of different molecular weights were separated on a Sephadex G-100 column, 2.5 \(\times\) 92.5 cm. Columns were calibrated with bovine serum albumin, ovalbumin, and chymotrypsinogen A. Sample volumes, varying from 1.5 to 4.5 ml, were applied, and the columns were developed by upward flow of 0.05 M sodium phosphate (pH 7.0) in 0.1 M NaCl, at a rate not exceeding 0.3 ml/min. Approximately 5 ml per tube were collected.

**DEAE-Cellulose Chromatography**

Chromatography was carried out on a 1.2- \(\times\) 47-cm column of fibrous DEAE-cellulose which had been equilibrated with 0.025 M sodium acetate buffer, pH 5.5. The sample (adjusted to pH 6.7 and dialyzed against water) consisted entirely of renin with a molecular weight of

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* Determinations carried out on the high molecular weight component found in dialysis precipitates of hog kidney on polyacrylamide gel columns (Biogel P-150) gave a molecular weight of 60,000, as previously reported.\(^8\) Later determinations on cross-linked dextran gel columns (Sephadex G-100) consistently gave a molecular weight of 80,000, even though the normal renin component had a molecular weight of 40,000 on both columns, and the same protein standards were used on both columns.
60,000 prepared by toluene method I. It was pumped on at a rate of 0.6 ml/min. Five-ml fractions were collected. A linear, descending pH gradient was used to develop the column, with 550 ml of 0.025 M sodium acetate buffer, pH 5.5, in the mixer and 0.025 M acetic acid in the reservoir. The acetic acid was pumped into the mixer at a nominal rate of 0.32 ml/min.

Protein Determination

Proteins were assayed by a highly sensitive automated modification of the method of Lowry et al.*

Activation Studies

Renin samples were dialyzed against 40 volumes of 0.05 M glycine-HCl buffer, pH 3.3, in 0.1 M NaCl for 16 hours; this was followed by dialysis against a fresh sample of 40 volumes of the same buffer for 8 hours. They were readjusted to pH 7.0 by a similar program of dialysis against 40 volumes of 0.05 M sodium phosphate buffer, pH 7.0, containing 0.2% neomycin sulfate in 0.1 M NaCl. Control samples were dialyzed for the same period of time against 0.05 M sodium phosphate buffer, pH 7.0. Alternatively, acid treatment was carried out by titrating samples of crude material to pH 3.0 with 1.4 M glycine-HCl buffer, pH 2.0. These samples were kept in a bath at 0°C for 60 minutes and then titrated back to about pH 7 with 2 M Tris, free base. Assayed material was corrected for any change in volume.

Results

Figures 1 and 2 show the results of toluene treatment in the preparation of renin by method I. Although all of the renin obtained had a molecular weight of 60,000 (renin-60)* (Fig. 1), at times as much as 20% of the activity had a molecular weight of 40,000 (renin-40) (tubes 52-60, Fig. 2).

In an effort to inhibit the conversion of renin-60 to renin-40, we added the protease inhibitors used by Murakami and Inagami in their purification of hog kidney renin. The extracting solution contained 5 mM sodium tetradecylate, 0.05 mM diisopropylfluorophosphate (DFP), 2 mM PMSF, and 5 mM EDTA. After the toluene treatment, the inhibitors were added to all solutions at 0.05 times the original concentration. Figure 3 shows results obtained with extracts prepared in the presence of the inhibitors. In this preparation, renin-60 constituted approximately 45% of the total renin activity. In another preparation, 60% of the renin activity recovered was renin-60. The total yield of renin activity per kilogram of original cortex was approximately 30% lower in the preparations to which the inhibitors were added; protein yield also was significantly reduced in these preparations.

* To limit the proliferation of ambiguous terms such as "big renin," "big, big renin," and "prorenin," the last of which has not yet been unequivocally demonstrated, we suggest that renin be designated according to their average molecular weights to the nearest whole 1000 as determined by gel filtration on Sephadex columns. Thus, renin with an elution peak corresponding to 59,500-60,500 molecular weight is referred to as renin-60; renin of 39,500-40,500 is called renin-40. Rounding off for 500 is to the even thousand. This proposed notation will be used in the present paper.
an Amicon PM-30 membrane at 65 psi for gel filtration. The results shown in Figures 5-7 demonstrate that although approximately two-thirds of the main peak (A, Fig. 4) was still renin-60 (Fig. 5), the remaining activity in pools B and C had been converted to renins of lower molecular weight (Figs. 6 and 7).

A sample of renin-60 made by method I and mixtures of renin-60 and crude extract were acid treated by dialysis and assayed for activation. The results in Table 1 show that dialysis at either pH 3.3 or 7.0 resulted in a loss of activity rather than the increase in total activity others have reported.5-7 Acidification of samples from the three concentrated pools from the DEAE-cellulose column also resulted in losses of activity rather than activation.

Although no activation occurred, gel filtration of renin-

Figure 3 Gel filtration of toluene-treated hog kidney renin on Sephadex G-100. Extracts made by method I but in the presence of 5 mM sodium tertthionate, 0.05 mM DFP, 2 mM PMSF, and 5 mM EDTA. All other conditions as described in Figure 1.

Figure 5 Gel filtration of fraction A after DEAE-cellulose chromatography. Tubes 51-80 (Fig. 4) were pooled and concentrated to 20 ml through an Amicon PM-30 membrane. One ml was applied to the column. Conditions and development were as in Figure 1.

60, after dialysis to pH 3.3 (Fig. 8), showed that essentially all the activity in the sample emerged in a volume consistent with a molecular weight of 42,000, whereas an extremely small peak was present at 82,000 molecular weight.

Renin-40, made by a method which included an early acidification step to remove angiotensinases,26 was apparently inhibited by the addition of crude extract of hog kidney cortex (Fig. 9).

Figure 10 shows renin activity in increasing amounts of crude extract incubated as described under Methods. Results are plotted as renin activity per milliliter against the actual volume of extract incubated.

Because these results could be attributed either to the

Figure 4 Chromatography of renin-60 on fibrous DEAE-cellulose with a descending pH gradient. The pH gradient was generated by pumping 0.025 M acetic acid from a reservoir at a nominal 0.32 ml/min into a mixer containing 550 ml of 0.025 M sodium acetate buffer, pH 5.5. Pumping from the mixer to the column was at a nominal 0.60 ml/min. Volumes collected were approximately 5 ml/tube. △ = pH.
FIGURE 6 Gel filtration of fraction B after DEAE-cellulose chromatography. Tubes 81–130 (Fig. 4) were pooled and concentrated to 14 ml. Two ml were applied and filtered as in Figure 1.

FIGURE 7 Gel filtration of fraction C after DEAE-cellulose chromatography. Tubes 130–190 (Fig. 4) were pooled and concentrated to 18 ml. Filtration of 2.5 ml of the concentrated solution was carried out as described in legend to Figure 1.

TABLE 1 Effect of Low pH and Crude Hog Kidney Extract on the Activity of Renin-60

<table>
<thead>
<tr>
<th>Material</th>
<th>Treatment</th>
<th>angiotensin/ml original per 15 minutes</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin-60</td>
<td>None</td>
<td>104</td>
<td>-20</td>
</tr>
<tr>
<td></td>
<td>pH 7.0 dialysis</td>
<td>83</td>
<td>-26</td>
</tr>
<tr>
<td>Renin-60 + 0.1 volume crude extract</td>
<td>pH 7.0 dialysis</td>
<td>77</td>
<td>-26</td>
</tr>
<tr>
<td>Renin-60</td>
<td>pH 3.3 dialysis</td>
<td>64</td>
<td>-38</td>
</tr>
<tr>
<td>Renin-60 + 0.1 volume crude extract</td>
<td>pH 3.3 dialysis</td>
<td>80</td>
<td>-23</td>
</tr>
</tbody>
</table>

For the assay of the treated extracts, each tube contained 1.7 μmol EDTA; 17.5 μmol phosphate buffer, pH 7.5; 35 μmol NaCl; 30 μl PMSF in absolute alcohol; and 350 pmol partially purified hog substrate in an incubation volume of 0.65 ml. Incubation was for 15 minutes at 37°C.

FIGURE 8 Gel filtration of renin-60 after dialysis to pH 3.3. For procedure, see legend to Figure 1.

Discussion

The role of the renin-angiotensin system in the onset and maintenance of the various forms of experimental or human hypertension has not been clearly delineated. Therefore, the concept of an inactive precursor of renin or a renin inhibitor might serve to explain many of the apparent contradictions in the data found in the literature.

In examining the role high molecular weight renin plays in the organism, it seemed to us convenient to have a method of preparation which would allow production of the material with no admixture of renin-40. Method II, which we developed by modifying the original toluene
effect of one or more inhibitors or to the activity of angiotensinases, the crude extract was incubated for 15 minutes or 2 hours with added angiotensin but in the absence of substrate. As can be seen in Table 2, angiotensinase activity is not evident at low concentrations of added extract in 15-minute incubations; at higher concentrations however, angiotensinase activity is not inhibited by EDTA, PMSF, or DFP.

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treatment, resulted in a preparation containing renin-60 exclusively. The total yield of renin activity per kilogram of kidney cortex is somewhat reduced with this method, presumably because of a less efficient maceration of the tissue. However, the simplicity of the extraction and the assurance that all the activity is renin-60 make it advantageous to use. Haas et al.\textsuperscript{27} discovered early that freezing and thawing increased the amount of renin that can be extracted from rabbit kidneys. In the light of recent investigations into the activation of renin, it cannot be overlooked that the increase of renin activity might have been due to a gradual conversion of an inactive precursor to a more active renin, as was reported by Sealey et al.\textsuperscript{28} in a preliminary note.

The finding that approximately half the activity was renin-60 (Fig. 3) was also unexpected. Previous reports from Rubin\textsuperscript{1} (hog kidney) and Leckie\textsuperscript{8} (rabbit kidney) lead one to expect at least a 2.5-fold increase in activity when the conversion from renin-60 to renin-40 occurs. Since each molecule of renin-40 should be 2.5 times as active as one of renin-60, it was unclear why the yield of renin activity in this case decreased by 30\% per gram of hog kidney cortex, unless the losses, due to denaturation in the presence of the added protease inhibitors, were extremely large. Murakami et al.\textsuperscript{29} in a preliminary note, reported that renin in crude extracts in a medium containing protease inhibitors was exclusively in high molecular weight forms. Although no details are yet available, it is possible that their protease inhibitors were not the same as those used previously\textsuperscript{25} and in our experiments. Another possibility is that the toluene purification method we used, in combination with the protease inhibitors, had a deleterious effect which resulted in the destruction of a great deal of the postulated binding protein.

The chromatography on DEAE-cellulose (Figs. 4–7) demonstrates that some conversion of renin-60 to renin-40 does take place on the column, as reported by Boyd\textsuperscript{18} and Leckie and McConnell\textsuperscript{19} for crude preparations. However, whereas these investigators found that phosphate buffer of low ionic strength apparently elutes the more active renin of lower molecular weight first, we found, using a descending pH gradient, that the first main peak completely eluted from the column at pH 5.2 (A, Fig. 4) contained all of the renin-60 which still remained (Fig. 3); the more tightly bound activity consisted of lower molecular weight forms. Although no details are yet available, it is possible that their protease inhibitors were not the same as those used previously\textsuperscript{29} and in our experiments. Another possibility is that the toluene purification method we used, in combination with the protease inhibitors, had a deleterious effect which resulted in the destruction of a great deal of the postulated binding protein.

![Figure 10](http://circres.ahajournals.org/)

**Figure 10** Renin-specific activity as a function of crude extract incubated. Increasing volumes of crude extract of hog kidney cortex were incubated for 15 minutes at 37°C under standard conditions (see text). Renin activity was calculated per milliliter of original extract.

TABLE 2  Effect of PMSF and DFP on Angiotensinase Activity of Water Extract of Hog Kidney Cortex

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Volume of extract incubated (ml)</th>
<th>Additions*</th>
<th>pmol angiotensin remaining per sample</th>
<th>After 15 minutes</th>
<th>After 120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>None</td>
<td>64</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>PMSF</td>
<td>55</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>DFP</td>
<td>47</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>PMSF and DFP</td>
<td>57</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>None</td>
<td>79</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>PMSF</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>DFP</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>0.20</td>
<td>PMSF and DFP</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*Each tube contained 70 pmol angiotensin I, 1.7 μmol EDTA, 17.5 μmol phosphate buffer (pH 7.5), 35 μmol NaCl, and crude extract as indicated in a final incubation volume of 0.65 ml. Where indicated, 10 μl of 0.3 μl PMSF in absolute alcohol and 10 μl of 0.3 μl DFP in isopropanol were added.

for human plasma. However, the fact that we found renin-60 consistently and exclusively when method II was used indicates that the freezing and thawing we carried out was insufficient to bring about a change in molecular weight, despite any other effect it may have had.

The addition of the inhibitors used by Murakami and Inagami\textsuperscript{29} as preservatives of renin activity in their preparation of hog renin resulted in a reduction of the total renin yield per kilogram and a decreased amount of protein in our experiments. The reason for this is not immediately apparent, unless the renin-60 is a combination of renin and a bound protein, as reported by Boyd\textsuperscript{18} and Leckie and McConnell\textsuperscript{19}. If this is the case, it may be that the presence of the protease inhibitors stabilized some other protein which is involved in the conversion of renin-60 to renin-40. If the binding protein in the presence of the protease inhibitors is more labile than renin (as it is at pH 3.3,\textsuperscript{18} 19 or possibly even in the cold\textsuperscript{12}–13), relatively more of it would be denatured, thus leaving an insufficient amount to bind all the remaining renin. This could account for the fact that not all the renin was renin-60.
therefore may not be directly comparable to the crude extract.

Morris and Lumbers suggested that acidification to pH 3.3 may activate an enzyme which in turn activates the renin. On grounds that treatment of the crude extract with toluene followed by the batch DEAE-cellulose step might have removed or inactivated such an enzyme, the partially purified renin-60 was acidified both alone and with added crude extract (Table 1). The resultant lack of activation (Table 1) indicates that what takes place in the crude kidney extract may very well differ from what occurs in amniotic fluid. In our experience, dialysis of crude kidney extract to pH 3.3 and back to neutrality results in the denaturation of relatively large amounts of protein, something that presumably does not occur in amniotic fluid, in which protein concentration is relatively low. On the other hand, Inagami and Murakami reported the activation of a purified high molecular weight precursor of renin. It is possible that our using toluene in the preparation resulted in a product that is not strictly analogous to a renin of 60,000 molecular weight prepared by other means. However, it should be pointed out that, although acidification gave no activation (Table 1), the molecular weight of most of the product of this treatment was reduced to 40,000, with a very small amount at 82,000 (Fig. 8). The data in Figure 9 show that increasing amounts of crude extract apparently increasingly inhibit partially purified kidney renin until a plateau at 80% inhibition is reached. This precludes the existence of an activator but is consistent with the presence of an inhibitor. Renin-60, prepared by method II, was similarly inhibited by the addition of crude extract.

Increasing amounts of extract incubated with a fixed amount of protein substrate yield an activity curve that falls off by several orders of magnitude with increasing amounts of crude extract incubated (Fig. 10). An extrapolation of the activity curve to the ordinate indicates renin specific activity of approximately 20 nmol/ml per 15 minutes, a level which is about twice as great as the 11 nmol/ml per 15 minutes found in the crude extract after acidification. When we replaced the PMSF with 3.4 mm 8-hydroxyquinoline, there was no great change in the specific activity of approximately 20 nmol/ml per 15 minutes. Neither PMSF nor DFP exerted an inhibitory effect. Addition of 3.4 mm 8-hydroxyquinoline in place of PMSF gave somewhat better protection against angiotensin destruction in 15-minute incubations but did not change the overall results. The fact that proteolytic destruction of angiotensin occurred in crude extracts tends to conceal any possible concomitant inhibition of renin activity and makes the latter difficult to demonstrate. Several investigators have referred to earlier findings from this laboratory as an example of activation, using as a basis for their conclusion the difference in charge and increased specific activity when renin I was acidified and rechromatographed on DEAE-cellulose. However, no increase of total activity had been observed in this laboratory, and the increase in specific activity was interpreted as being the result of a purification, based on the charge on the molecule brought about by acidification. The present experiments confirm the postulate of Boyd and Leckie that renin 1 is actually an active renin of 60,000 molecular weight; they also bear out earlier observations from this laboratory that no increase in total activity occurs. It should be pointed out that renin 1, found previously, is different from the four forms found on a DEAE-cellulose chromatogram after acidification, since the latter all have a molecular weight of 40,000.

A comparison of the data in Figures 1, 3, and 8 illustrates that an increase in specific activity alone does not necessarily imply activation. A large proportion of the protein in the preparations is in the 65,000–75,000 molecular weight class, and renin-60 filters on the gel column at the trailing edge of this broad peak. In our studies, conversion to renin-40 either during preparation (Fig. 3) or after acidification (Fig. 8) did not result in a change of size of a significant portion of the protein peak in which the renin-60 traveled. Thus, the conversion itself resulted in a 10-fold increase in specific activity of the renin peak on the column, despite the fact that the total activity was not increased, and, indeed, an actual loss of enzyme activity occurred during the dialysis (Table 1). The apparent activation and the change in molecular weight, therefore, are unrelated phenomena, at least in crude kidney extracts. Whether the renin-60 in such extracts is adventitious or serves a function in the organism remains to be determined.

References
Studies on high molecular weight renin from hog kidney.
M Levine, K E Lentz, J R Kahn, F E Dorer and L T Skeeggs

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