Evidence Against Acetone-Soluble Renin Inhibitors in Normal Human Plasma

ROBERT E. DRUILHET, PH.D., MERRILL L. OVERTURF, PH.D., REX A. HINSHAW, B.S., AND WALTER M. KIRKENDALL, M.D.

SUMMARY The presence of acetone-soluble renin inhibitors in normal plasma has been proposed to explain the variation of plasma renin reactivity (PRR) in samples from normotensive and hypertensive subjects. In our experience, acetone extraction decreased PRR in relation to unextracted control values, an observation which is not consistent with the circulating lipid-renin inhibitor hypothesis. Exposure to acetone at -40°C for 1 minute invariably denatured some endogenous angiotensinogen. The PRR in extracted and unextracted plasma was positively correlated with the concentration of available angiotensinogen, $r = 0.955$ ($p < 0.05$), and $r = 0.964$ ($p < 0.01$), respectively, but the addition of exogenous substrate did not uniformly increase PRR in acetone-treated plasma above control values. These data argue against the use of acetone extraction to demonstrate the existence of circulating lipid-renin inhibitors. Acetone removed 14% to 25% of the normal plasma lipids and although the extract contained most of the major lipid classes, neutral lipids were the most abundant (73% by weight). The presence of acetone-soluble phospholipids appeared to increase angiotensin I formation in the partially purified renin-angiotensinogen system, but phospholipids interfered with the radioimmunoassay and resulted in an overestimation of angiotensin I. Plasma neutral lipids decreased in vitro renin activity by 13% ($p < 0.025$) but this degree of inhibition suggests that lipid-renin interactions may have minimal in vivo physiological significance. In contrast to previous reports, we found the correlation between PRR and endogenous angiotensinogen in normotensive and hypertensive plasmas to be statistically significant ($r = 0.643$, $p < 0.01$). Inactivated human angiotensinogen was also shown to be an inhibitor of renin in vitro. This effect could have possibly influenced PRR values that were determined by others in the presence of inactivated angiotensinogen. (Hypertension 1: 98-105, 1979)

KEY WORDS • renin • human • angiotensinogen • lipid inhibitor

KOTCHEN et al.\(^1\)\(^-\)\(^2\) have concluded that the rate of angiotensin I generation with added renin is accelerated in plasma from uremic patients and patients with renovascular or essential hypertension and decreased in plasma from normotensive subjects. The increased renin activity was reported to be independent of endogenous substrate concentration and was inhibited by the addition of normal plasma.$^1$ These investigators also found that the partial inhibition of renin reactivity in plasma from normotensive control subjects could be negated by acetone extraction.$^7$ Subsequent evidence suggested that neutral lipids from the acetone extracts inhibit the in vitro renin-angiotensinogen reaction.$^4\)\(^-\)\(^6\)

Other explanations for the difference in the angiotensin generation rate between normal and hypertensive plasma have been reported. Sambhi et al.$^9$ demonstrated that plasma from hypertensive patients contains a nonlipid activator of renin; differences in renin substrate were excluded as a possible cause of the variation, and no evidence was found for the existence of a renin inhibitor in normal plasma. Poulsen$^7$ rejected the theory of circulating renin modifiers, both inhibitors and activators. Although Poulsen studied the rat renin-substrate system in untreated plasma, he found that the variation in renin reactivity between normal, salt-depleted, nephrectomized and hypertensive rats was dependent upon the angiotensinogen concentration.

This controversy prompted us to re-investigate the existence of lipid renin inhibitors in human plasma by the procedure of Kotchen et al.$^8$
Methods

Patient Selection

Patients with essential hypertension, renovascular hypertension, and renal insufficiency were selected from the wards and the Hypertension Clinic of the University Hospital. Seven patients with renal insufficiency were on a chronic dialysis program for at least 10 months; blood urea nitrogen (BUN) values before dialysis ranged from 70 to 117 mg/dl and serum creatinine averaged 18.5 ± 2.9 mg/dl. None of the uremic patients was anephric. The diagnosis of renovascular hypertension was made in three patients based upon results obtained from selective renal vein renin samples and the demonstration of renal artery stenosis by angiography. Control subjects were normotensive and free from major disease; diet was not restricted.

Specimens used in the study were obtained with a protocol approved by the University Committee for the Protection of Human Subjects. Peripheral venous blood was taken with the subject erect. Samples were collected in a chilled tube or syringe with EDTA (1 mg/ml). Blood from patients with essential hypertension was obtained before treatment. Uremic blood was obtained at the beginning of routine dialysis. A 30- to 40-ml blood sample was collected from control subjects; 5- to 10-ml samples were taken from the hypertensive and uremic patients. Plasma was obtained by centrifugation at 1200 g (4°C) for 15 minutes.

Renin and Substrate Preparation

Kidney renin was extracted from human cadaveric cortical tissue as described in detail previously. Renin substrate (angiotensinogen) was prepared from frozen human plasma by a salt-precipitation method. Renin and angiotensinogen were individually incubated for 1 hour at 37°C with [H]angiotensinogen I and with [11C]angiotensinogen II to detect converting enzyme and angiotensinase contaminants. Radioactive products were separated by electrophoresis and assayed as previously described. Human renin was judged free of converting enzymes and angiotensinases by these methods. Less than 3% of [14C]-angiotensinogen I present (500 pmoles) was hydrolyzed by partially purified human angiotensinogen (385 pmoles). The absence of significant renin-like activity in angiotensinogen preparations was confirmed by incubations with phosphate buffer alone (50 mM, pH 7.4). Aliquots of the substrate, incubated at 37°C, were taken at 1, 3, and 6 hours, and angiotensin I was determined by radioimmunoassay. The mean recovery of angiotensin I from these incubations was 0.08 ± 0.03 ng/ml/hr (n = 6).

Human kidney renin was standardized against the International Reference Preparation of Human Renin (code 68/356) from the Department of Biological Standards, Mill Hill, London. The concentration of angiotensinogen was measured after 1 hour of incubation at 37°C and pH 7.4. The partially purified kidney renin contained 11.87 ± 0.2 International Units (IU/ml) with a specific activity of 0.3 IU/mg protein (n = 6). Renin was lyophilized and stock solutions were prepared such that 10 µl contained a known concentration of renin expressed as International milli-Units (ImU). The concentration of angiotensinogen was estimated by adding human renin (50 ImU) to 50 µl of the substrate preparation in 40 µl of phosphate buffer (50 mM, pH 7.4). The concentration of angiotensinogen I generated after a 4-hour incubation at 37°C was determined by radioimmunoassay. Angiotensinogen values were expressed as pmoles angiotensinogen I formed per ml (pmoles A1/ml = 0.7716 × ng/ml). A similar method was used to estimate angiotensinogen in whole plasma and acetone-extracted samples. The method of Skinner (pH 3.3, 30 minutes at 32°C) was used to inactivate partially purified human angiotensinogen.

Renin Activity

The renin activity or renin reactivity in each plasma or experimental sample was estimated by angiotensin I radioimmunoassay. Dimercapto propanol (10 µl, 0.806 M) and 8-hydroxyquinoline (10 µl, 0.34 M) were added to 1.0 ml of plasma in a polypropylene tube. All samples were adjusted to pH 7.4 with 1.0 M maleic acid. One-half of the sample was placed at 4°C for 1 hour to establish the level of endogenous angiotensin I; the other 0.5 ml was incubated for 1 hour at 37°C to detect renin activity. Two- and 3-hour incubations were used in some experiments. Aliquots of each condition were taken for radioimmunoassay of angiotensinogen. The assays were performed in duplicate. The rates of angiotensinogen I generated in plasma samples with added homologous renin (2 ImU-35 ImU) or angiotensinogen (386 pmoles) and in acetone-treated plasma were determined in the same manner. Renin and angiotensinogen controls were used and the renin value of experimental samples was corrected accordingly. In some instances, a dilution of the incubated sample was required before radioimmunoassay. Plasma renin reactivity (PRR) was expressed as angiotensinogen I (ng/ml) generated in plasma with added homologous renin less the amount generated with endogenous renin.

Acetone Extraction

Normal human plasma was extracted with 95% acetone by the method of Kotchen et al. Two plasma aliquots (5 to 10 ml) were placed in silanized, 25 × 150 mm glass tubes fitted with teflon-lined screw caps. The plasma was shell frozen in a dry ice-acetone bath and lyophilized. A control aliquot was frozen at −80°C. The acetone-water solution (95:5, v/v) was added to one of the freeze-dried samples (30 ml/g dry plasma). The slurry was shaken vigorously for 1 minute and vacuum filtered through Whatman No. 1 paper. Acetone-soluble material was stored under N2.
at $-80^\circ$C. The plasma powder was placed in a vacuum dessicator for 1 to 2 hours to remove residual acetone. Then, the acetone-extracted sample and the lyophilized control plasma were reconstituted to their original volumes with distilled water. The extracted plasma and the lyophilized and frozen controls were refrigerated at 4°C until dissolved and the pH of each was adjusted to 7.4 with maleic acid. Duplicate 1-ml samples of the three treatments were used to determine the effect of acetone extraction on plasma renin reactivity. The effect of acetone extraction on partially purified angiotensinogen and human renin was determined in a similar manner. Student's $t$ test was used to determine the equality of paired samples and as a test of significance on the difference of means of unpaired samples. Least-squares regression analysis was used to determine correlation coefficients ($r$). Significance levels of $r$ were determined with n-2 degrees of freedom.\textsuperscript{13}

Qualitative Lipid Analysis

Lipids in the pooled acetone-soluble plasma extracts from 12 normotensive subjects were studied. The extracts were concentrated with a rotary evaporator at 25°C and washed repeatedly with dry methanol to remove traces of residual water. Non-lipid contaminants were removed on a Sephadex G-25 column by the method of Wuthier.\textsuperscript{14} The purified total lipids were concentrated \textit{in vacuo}, dissolved in chloroform, and separated into neutral and phospholipid fractions by silicic acid column chromatography.\textsuperscript{16} After the two fractions were brought to near dryness, the neutral lipids were dissolved in chloroform-methanol (2:1, v/v) and the phospholipids in chloroform-methanol-benzene (1:1:1, by volume).

The neutral lipid fraction was qualitatively separated into classes on 0.25 mm Silica gel H plates using a two-dimensional thin-layer chromatography (TLC) system.\textsuperscript{18} Qualitative TLC of phospholipid classes was done on 0.25 mm Silica gel G plates using two-dimensional solvent pairs. Plates were developed with chloroform-methanol-water (65:25:4, by volume) followed by n-butanol-acetic acid-water (3:1:1, by volume).\textsuperscript{17} Iodine vapor was used as a general non-destructive lipid detection reagent.\textsuperscript{18}

Effect of Lipids on Renin Activity

Lipids in the acetone-soluble extracts from 5-ml samples of normal plasma were separated into neutral and phospholipids as described above. These lipids and lipid standards were tested for their ability to inhibit the renin-angiotensinogen reaction. Known amounts of lipid were placed in silanized tubes and the solvent was evaporated under nitrogen. Phosphate buffer (0.6 ml, 50 mM, pH 7.4) was added and the suspension was sonicated at 0°C for three 10-second intervals. Human renin (3 or 6 ImU) and homologous substrate (39 pmoles) were added and the tubes were incubated for 1 hour at 37°C. The amount of angiotensin I formed was measured by radioimmunoassay and compared with the results of control incubations without lipid. Lipid-buffer suspensions (20 µl) were also incubated with human plasma and exogenous renin (35 ImU) to determine the effect of lipids on renin reactivity.

Results

Plasma samples from 15 normotensive subjects were each extracted with acetone, and renin (3 ImU) was added to duplicate aliquots of the unextracted frozen and lyophilized controls and the acetone-treated portions. The amount of angiotensin I formed at pH 7.4 and 37°C from endogenous angiotensinogen, irrespective of added renin, was less than 7% of that generated in 1 hour by either of the matched, unextracted, controls (fig. 1). The plasma renin activities of frozen, lyophilized, and acetone-treated samples were 2.51 ± 0.96, 2.50 ± 1.36, and 0.15 ± 0.13 ng/ml/hr, respectively. A similar trend was observed in five samples that were incubated for 4 hours. The amount of angiotensin I formed in frozen plasma with added renin (3 ImU) was 3.5 ± 0.3 ng/ml at 1 hour and 8.5 ± 0.5 at 4 hours, an average increase of 3.1 ± 0.06 ng angiotensin 1/ml/hr more than that formed with endogenous renin alone. The mean angiotensin value in acetone-extracted samples with exogenous renin was 0.87 ± 0.4 ng/ml at 1 hour and 1.1 ± 0.9 ng/ml at 4 hours.

**Figure 1.** Effect of acetone extraction on renin activity in normal plasma (mean ± 1 SD, n = 15).
The decrease in plasma renin reactivity (PRR) after acetone extraction, rather than the expected increase, was explained by the data in figure 2. Human renin (35 ImU) was added to each extracted and control plasma from five normotensive subjects. A portion of frozen control plasma was added to an equal volume of reconstituted acetone-treated plasma and all samples were incubated at 37°C and pH 7.4. The PRR was determined at 1 and 2 hours. The theoretical PRR of the mixed frozen-extracted control, shown as a dashed line in figure 2, was calculated as 50% of the frozen control PRR plus 50% of the extracted PRR at each time interval. This value was based on the assumption that renin inhibitors were not removed by acetone. The theoretical PRR of the mixed control for sample B was 38 ng/ml at 1 hour and 74 ng/ml at 2 hours; the measured values were 61 ng/ml and 86 ng/ml, respectively. Similar deviations from the expected PRR value were found with the mixed controls of A, D and E samples. If renin inhibitors were present in the unextracted plasma, the measured PRR of mixed controls should have been lower than the theoretical values, which assumed an absence of inhibitors. This was not observed. The difference in renin reactivity between the various treatments of each plasma sample indicates that acetone extraction had altered some plasma component and that the change in this component modified renin activity.

Table 1 shows the effect of acetone treatment on angiotensinogen concentration in these samples. The variable loss of substrate may explain the decrease in PRR after acetone extraction. No significant difference in PRR (p > 0.05) was detected with the frozen, extracted and mixed control of sample C. Sample C lost only 21% of the initial renin substrate concentration after acetone extraction. These data suggest that PRR is a function of angiotensinogen after extraction since renin reactivity was correlated positively with substrate concentration. Regression analyses of the respective angiotensinogen values (table 1) on the 1 hour PRR of frozen and mixed controls and extracted samples (fig. 2) resulted in correlation coefficients (r) of 0.964, (p < 0.01), 0.985 (p < 0.01) and 0.955 (p < 0.05), respectively.

The effect of exogenous angiotensinogen on renin reactivity in acetone-treated plasma was also studied (fig. 3). Duplicate plasma samples with an average of 1407 ± 356 pmoles angiotensinogen per ml (n = 5) were each extracted with acetone. Human renin (35 ImU) was added to the extracted plasma and two frozen plasma controls from each subject. The PRR was determined at 30 and 60 minutes on one set of frozen and extracted samples and on the duplicate set after adding 386 pmoles of homologous substrate. The amount of angiotensinogen added was based on the loss of substrate from plasma after acetone extraction.

**Figure 2.** Plasma renin reactivity (PRR) in the various treatment samples of plasma from five normotensive subjects (A–E). The mixed control consisted of equal volumes of acetone-extracted and frozen plasma; theoretical values were calculated from the PRR in extracted and frozen samples assuming that acetone did not remove renin inhibitory factors.
The 30- and 60-minute renin reactivity determinations of paired samples of unextracted and extracted normal plasma with and without added angiotensinogen is shown in figures 3A and 3B. These results seem to indicate that the decrease in PRR after extraction is not altered by the addition of angiotensinogen. However, if the PRR in unextracted samples with endogenous angiotensinogen is compared with the PRR in duplicate samples with added angiotensinogen (fig. 3C), PRR of the acetone-treated plasma apparently increased in two samples after 30 minutes but only one remained elevated after 60 minutes. The renin reactivity at 60 minutes decreased in two extracted samples and remained relatively constant in the other two samples. The plasma sample which showed a sustained increase in renin reactivity lost only 16.8% of the endogenous renin substrate during acetone treatment and 46% of the substrate was destroyed in the sample that showed the greatest decrease in PRR.

The role of angiotensinogen as a mediator of PRR was also evaluated in unextracted plasma from nine normotensive and 14 hypertensive subjects (fig. 4). Renin reactivity in plasma of renovascular hyperten-

### Table 1. Effect of Acetone Extraction on Renin Substrate Concentration (pmoles/ml)

<table>
<thead>
<tr>
<th>Plasma sample</th>
<th>Frozen control</th>
<th>Extracted</th>
<th>% Loss</th>
<th>Mixed control*</th>
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<tbody>
<tr>
<td>A</td>
<td>783</td>
<td>81</td>
<td>90.7</td>
<td>432</td>
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<td>B</td>
<td>772</td>
<td>270</td>
<td>66.0</td>
<td>521</td>
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<tr>
<td>C</td>
<td>968</td>
<td>761</td>
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<tr>
<td>D</td>
<td>752</td>
<td>319</td>
<td>57.0</td>
<td>535</td>
</tr>
<tr>
<td>E</td>
<td>1036</td>
<td>517</td>
<td>35.3</td>
<td>658</td>
</tr>
<tr>
<td>Mean</td>
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<td>390</td>
<td>53.8</td>
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<tr>
<td>SD</td>
<td>87</td>
<td>259</td>
<td>26.5</td>
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</tr>
</tbody>
</table>

*Estimated from the substrate concentration of frozen and extracted samples; 0.5 ml of each were combined as a mixed control sample.

This value was 216 to 895 pmoles/ml with an average of 441 and a SD of 271 pmoles/ml. After the addition of angiotensinogen, the combined substrate concentration in each milliliter of unextracted and extracted samples was 1810 ± 346 and 1764 ± 154 pmoles, respectively.
sive patients was significantly greater than that observed in plasma of either normotensive subjects, essential hypertensive patients, or uremic patients. The PRR in patients with essential hypertension or uremic plasma was not significantly different from the normotensive control values. The angiotensinogen concentration in all plasma samples was correlated positively with renin reactivity, \( r = 0.643 \) (\( p < 0.01 \)). The individual correlation coefficients were: 0.848 (\( p < 0.05 \)) for normotensive plasma, 0.599 (\( p > 0.05 \)) for plasma from essential hypertensive patients, 0.775 (\( p < 0.05 \)) for patients with renal insufficiency, and 0.999 (\( p < 0.05 \)) for plasma from renovascular hypertensive subjects.

Previous workers have excluded differences in angiotensinogen as a cause of PRR variations. This contention was based on the destruction of endogenous renin substrate by the Skinner method\(^{13} \) and the addition of exogenous angiotensinogen before the determination of PRR. We propose that the presence of inactive angiotensinogen in the plasma samples may have influenced renin reactivity. This possibility was evaluated by incubating partially purified human angiotensinogen (355 ± 43 pmoles) and human kidney renin (35 ImU) with increasing amounts of angiotensinogen inactivated at 32°C and pH 3.3 for 30 minutes. The substrate inactivation method was approximately 80% effective; 710 pmoles of active angiotensinogen per ml was decreased to 137 ± 15 pmoles. The results of this study (fig. 5) indicate that angiotensinogen, inactivated by this method, may be an inhibitor of renin activity in vitro. If this occurs in whole plasma, attempts to remove the influence of substrate concentration on the variability of PRR would tend to compound the differences in angiotensin generation rate between samples with varied amounts of angiotensinogen.

Acetone-soluble lipids were used to further evaluate the existence of renin inhibitors in normal human plasma. Extracts of plasma from 12 normotensive subjects were pooled and the acetone-soluble lipids were recovered. The amount of total lipids purified on Sephadex G-25, 111 mg, indicated that acetone removed about 1.0 mg of lipid from each ml of plasma extracted (112 ml). The acetone-soluble lipids were composed of 27% (w/w) phospholipids and 73% (w/w) neutral lipids. Ninety-eight percent of the total lipid mass was recovered from the silicic acid column. Qualitative thin-layer chromatographic analyses showed that most of the major lipid classes were present in the acetone extract.

The total lipid extract (1 to 5 mg) and 0.5, 1, 2 and 3 mg of the phospholipid and neutral lipid fractions were incubated for 1 hour at 37°C and pH 7.4 with normal plasma (1.0 ml) and 35 ImU of human renin. Plasma was added to the dried lipid and a suspension was prepared by sonication. Controls consisted of plasma and renin without lipid and with 0.5 mg to 3 mg of the synthetic triacylglycerides, tripalmitin, tristearin and trilinolein. None of these lipids inhibited renin reactivity when added to whole plasma.
The effect of synthetic triglycerides and the acetone-soluble neutral and phospholipids from 5 ml of normal plasma on the partially purified human renin-angiotensinogen system was studied. Tristearin had no effect on renin activity in this system but tripalmitin, trilinolein and the plasma lipids did alter renin activity. The amount of angiotensin I generated in the renin-angiotensinogen reaction with tripalmitin and the plasma phospholipids and neutral lipids is shown in figure 6. Tripalmitin (2 mg) inhibited 20% of the renin activity, the acetone-soluble phospholipids increased renin activity by a factor of 1.23 ($p < 0.10$) and 13% ($p < 0.025$) of the renin activity was lost in the presence of plasma neutral lipids. The renin-inhibitory effect of trilinolein ranged from 3.4% inhibition with 0.5 mg to 21% inhibition with 3 mg of the triglyceride.

The interaction of lipids with angiotensin I was used to assess the validity of altered renin activity in vitro. Neutral and phospholipid fractions from 5 ml of plasma and the triglycerides (2 mg/ml) were added to each of six angiotensin I standards (50 to 500 pg/ml) before radioimmunoassay. The presence of triglycerides and plasma neutral lipids did not affect the recovery of angiotensin I. However, added phospholipid caused the percent of antibody-bound angiotensin I to decrease an average of 5.5 ± 0.6 percentage points ($n = 18$) in the radioimmunoassay estimate of each angiotensin I standard. Thus, the values determined for the six standards in the presence of phospholipids were 2.6 ± 0.5 times greater than those obtained without phospholipid. These data indicate that phospholipids affect the radioimmunoassay estimate of angiotensin I and not renin activity in vitro.

![Figure 6](http://hyper.ahajournals.org/)

**Figure 6.** Effect of phospholipids (PL) and neutral lipids (NL) from 5 ml normal plasma on the reaction of partially purified renin and angiotensinogen, comparison with the effect of tripalmitin. Samples were incubated 1 hour at 37°C and pH 7.4 (mean ± 1 SD, $n = 5$).

Discussion

We have previously demonstrated the inhibitory effect of purified neutral and phospholipid classes from human kidney on the human renin-tetradecapeptide renin substrate reaction in vitro and approached the present study as an extension of that investigation. However, repeated attempts to increase renin reactivity in normal human plasma by acetone extraction, the method originally used to suggest the presence of circulating lipid-renin inhibitors, resulted in a substantial decrease in the rate of angiotensin I generation. Observed differences in extracted versus unextracted PRR were apparently the result of loss of angiotensinogen in acetone-treated plasma. Extraction of plasma with acetone at 0°C was reported to have an infrequent but variable destructive effect on renin substrate which could be minimized by using −40°C acetone. The lower extraction temperature was used exclusively in our study but some angiotensinogen was denatured in all instances. Extracted samples lost between 21% and 90% of their pre-extraction substrate value, measured in terms of angiotensin I equivalents. We found that a direct correlation existed between PRR in both control ($r = 0.964, p < 0.01$) and extracted ($r = 0.955, p < 0.05$) plasma and available angiotensinogen in the sample.

The PRR in mixed controls, prepared from equal volumes of an acetone-extracted sample and the corresponding frozen plasma, indicated that normotensive plasma did not inhibit renin reactivity. In fact, the increase in estimated PRR detected in mixed controls suggests that acetone destroyed some plasma component that was required for optimum angiotensin I formation. Possibly the unextracted portion of the mixed control partially compensated for the denaturing effects of acetone. The lack of a renin inhibitory factor in normal plasma and the deleterious effect of acetone on PRR was further substantiated by the results obtained with plasma sample C (fig. 2). In sample C, PRR did not change after acetone extraction presumably because angiotensinogen in this sample was least affected by acetone. However, attempts to restore angiotensinogen in acetone-extracted plasma to pre-extraction levels with exogenous substrate did not uniformly restore renin reactivity (fig. 3C). The one extracted sample supplemented with exogenous substrate that showed a substained increase in PRR retained the largest amount of endogenous substrate after extraction. Other extracted samples with added angiotensinogen had lower or unchanged PRR values when compared to unextracted plasma; these samples lost significant quantities of angiotensinogen. Most authors have attempted to obviate the influence of different angiotensinogen concentrations on PRR by inactivating endogenous substrate. In the present report, we have found the correlation between the unextracted PRR and the renin substrate concentration in all samples (normal, hypertensive and uremic) to be significant ($p < 0.01$). Additionally, we have
demonstrated that the presence of angiotensinogen inactivated at 32°C and pH 3.3 for 30 minutes decreased the rate of angiotensin I generation by the renin-angiotensinogen system in vitro. This presumably could be due to the inhibition of renin by the inactivated substrate and might explain many of the reported variations in PRR.

We are unable to explain the lack of correlation between our data and previous reports, but our study clearly does not support the view that acetone extraction of normal plasma will increase renin activity. We feel that a circulating renin activator did not influence our results since Sambhi et al. have indicated that the activator is not lipid soluble and is absent from normal plasma. Furthermore, our results strongly suggest that renin activity in either extracted or unextracted, normal or hypertensive plasma is dependent upon variations in angiotensinogen concentration. This finding is in agreement with the report of Poulsen and others.

In this regard, we have examined the acetone extract of normal plasma for evidence of lipid-renin inhibitors. Based on normal plasma lipid values (400–700 mg/100 ml), acetone removed from 14 to 25% of the lipids in normotensive plasma and most of the major neutral and phospholipid classes were recovered. Neutral lipids composed nearly three-fourths of the acetone-soluble lipids and phospholipids accounted for the remainder. Neither the total acetone-soluble extract from 5 ml of plasma nor the neutral or phospholipid portions of the extract inhibited renin activity in whole plasma. The amount of angiotensin I generated in the renin-angiotensinogen reaction was slightly increased (p < 0.10) in the presence of plasma phospholipids but the apparent change in renin activity was shown to be the result of erroneous estimates of angiotensin I in the presence of phospholipids. This effect of phospholipids on estimates of angiotensin I has been suggested by others.

Neutral lipids from 5 ml of normal plasma (3.6 mg) caused a 13% (p < 0.025) reduction in renin activity with the partially purified homologous substrate; smaller quantities of synthetic triglycerides produced a similar effect. The degree of renin inhibition observed with plasma neutral lipids and the inability of neutral lipids to inhibit renin in whole plasma seem to indicate that these apparent lipid-renin interactions would have minimal impact on the renin-angiotensin system in vivo.
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