Increased Plasma Aldosterone Concentration in Response to Hemodialysis in Nephrectomized Man

By Robert E. McCoo, Connie S. McCoo, Dale G. Read, John D. Bower, and Arthur C. Guyton

ABSTRACT

The nephrectomized patient maintained on intermittent hemodialysis while awaiting renal transplantation provides an excellent model for studying the influence of factors other than the renal renin-angiotensin system that play a role in the regulation of plasma aldosterone concentration. Peripheral plasma aldosterone concentrations were determined using a sensitive radioimmunoassay method in nine anephric patients in the recumbent position immediately before and after hemodialysis. Average patient weight fell 1.5 kg following 12 hours of dialysis on the Kii dialyzer. Plasma sodium concentration decreased from 137 ± 1 (SE) to 132 ± 1 mEq/liter, plasma potassium concentration decreased from 4.8 ± 0.3 to 3.2 ± 0.1 mEq/liter, and the ratio of sodium to potassium increased from 31.3 ± 1.9 to 41.7 ± 0.9. Plasma aldosterone concentration increased from 9.7 ± 1.6 ng/100 ml plasma before dialysis to 17.9 ± 2.1 ng/100 ml plasma after dialysis despite the lack of kidneys to produce renin. Plasma cortisol concentration did not change significantly (17.5 ± 2.0 μg/100 ml plasma to 14.8 ± 1.9 μg/100 ml plasma). These results show that plasma aldosterone concentration increased in anephric patients in response to hemodialysis. This increase occurred without a concomitant increase in plasma cortisol levels, suggesting absence of an adrenocorticotropic hormone response, and was independent of the renal renin-angiotensin system.

KEY WORDS adrenal cortex kidney cortisol angiotensin renin radioimmunoassay sodium potassium

Since the observation in 1960 of the aldosterone-stimulating effect of exogenous angiotensin in man (1, 2), considerable experimental evidence has accumulated indicating that the renal renin-angiotensin system plays a major role in the regulation of aldosterone secretion. Plasma sodium and potassium concentrations and pituitary secretion of adrenocorticotropic hormone (ACTH) are thought to play important secondary roles in controlling aldosterone secretion.

Our knowledge of the regulation of aldosterone secretion would be enhanced by determining whether the concentration of aldosterone in the plasma can be maintained within normal physiological limits and whether the plasma aldosterone concentration can change in response to acute stimuli independently of
the renal renin-angiotensin system. The nephrectomized human being who must be maintained on chronic hemodialysis while awaiting renal transplantation provides an excellent model for studying the influence of humoral factors other than the renal renin-angiotensin system that play important roles in controlling plasma aldosterone concentration.

The purpose of this study was to determine the effect of hemodialysis, with its removal of electrolytes, fluids, and metabolites, on the plasma aldosterone concentration in the nephrectomized patient. Furthermore, modification of a sensitive radioimmunoassay procedure (McCaa, R. E., and McCaa, C. S., unpublished results) for determining aldosterone concentration in peripheral plasma allowed us to measure this steroid hormone with a high degree of precision and sensitivity; the method for this procedure is presented in detail in this paper.

Methods

Studies in Normal Subjects.—Six healthy volunteer subjects were used to obtain a normal range of values. The subjects were required to stand for 2 hours after having been recumbent for at least 2 hours. Venous blood samples were drawn before and after the period of standing. Five of the volunteers were placed on a low-sodium diet for 5 days. On the fifth day, they were again required to stand for 2 hours after having been recumbent for at least 2 hours, and venous samples were drawn immediately before and after the period of standing. The same five subjects were then placed on a high-sodium diet for 5 days. On the fifth day, blood samples were drawn from these subjects. All blood samples were collected in tubes treated with ethylenediaminetetraacetic acid (EDTA) and immediately centrifuged. The plasma samples were frozen until they could be analyzed for plasma aldosterone concentration.

Studies in Anephric Patients.—Nine patients who had undergone bilateral nephrectomies 2 to 38 months (average 12 months) before this study were used. A summary of the clinical data is given in Table 1. Also shown are the reasons for nephrectomy and the length of time each patient had been nephrectomized. Each patient was restricted to 34 mEq/24 hours of dietary sodium. Other restrictions in the diet are given in Table 1. The blood pressure of all the patients was under

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Summary of Clinical Data in Nine Anephric Patients before and after Hemodialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Sex</td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
</tr>
<tr>
<td>T.A.</td>
<td>M</td>
</tr>
<tr>
<td>J.C.</td>
<td>M</td>
</tr>
<tr>
<td>M.C.</td>
<td>F</td>
</tr>
<tr>
<td>R.K.</td>
<td>M</td>
</tr>
<tr>
<td>R.L.</td>
<td>M</td>
</tr>
<tr>
<td>M.J.</td>
<td>M</td>
</tr>
<tr>
<td>D.R.</td>
<td>M</td>
</tr>
<tr>
<td>S.W.</td>
<td>F</td>
</tr>
</tbody>
</table>

* Dietary sodium intake for each patient was restricted to 34 mEq/24 hours.
control, and none of the patients took any medication for 1 week prior to the study.

Blood samples were drawn from the venous side of each patient's indwelling arteriovenous shunt 15 minutes before he was connected to the Kil dialyzer. Each patient was then dialyzed for 12 hours at a dialyzer blood flow of approximately 500 ml/min. Immediately after hemodialysis, venous blood samples were again drawn. These two sets of blood samples, labeled predialysis and postdialysis, were then analyzed for plasma sodium, potassium, cortisol, and aldosterone.

**Plasma Electrolytes.**—Plasma sodium and potassium determinations were performed using an Instrumentation Laboratories flame photometer.

**Plasma Cortisol.**—Plasma cortisol determinations were performed by the competitive protein-binding procedure reported by Murphy (3). This procedure is commonly used by many laboratories and does not need to be discussed further here.

**Plasma Aldosterone.**—The radioimmunoassay method for determination of plasma aldosterone concentration used in this study was a modification of the procedure reported by Mayes et al. (4). This modified procedure increased both the accuracy and the sensitivity of the determination, and, therefore, the method and the tests of its validity are given in detail in the subsequent paragraphs.

Aldosterone carboxymethoxime was produced by the reaction of aldosterone and carboxymethoxylamine hemihydrochloride (Aldrich Chemical Company, Inc.) in the presence of sodium acetate and methanol. The aldosterone oxime derivative was coupled to bovine albumin by the carbodiimide method (5), and the steroid-protein complex was purified by dialysis at 4°C for 36 hours against 20 liters of distilled water.

The aldosterone oxime-albumin complex was emulsified with complete Freund adjuvant containing *Mycobacterium butyricum*, and an aliquot of the emulsion was injected subcutaneously into ten rabbits every 2 weeks for the first three injections and then monthly. Six of the rabbits developed a usable antibody following the fourth injection. Sera were stored at —10°C. An antialdosterone serum preparation at a dilution of 1 to 75,000 was used in this series of experiments.

One-tenth ml of $^3$H-$1,2$-aldosterone (10,000 counts/min), checked weekly for purity and isoadosterone formation, was added to 5-, 10-, or 20-ml plasma samples, depending on the expected aldosterone concentration. Samples were extracted for 6 minutes with eight volumes of redistilled dichloromethane. The dichloromethane layer was separated from the plasma layer by centrifugation, and the plasma layer was removed by aspiration. The dichloromethane extract was evaporated to dryness in a water bath at 37°C under a stream of nitrogen. Each sample was dissolved in 0.055 ml of a methanol-acetone (1:1) solution and applied to Whatman no. 1 chromatography paper which had been washed continuously for 48 hours with redistilled methanol in a modified Soxhlet extraction apparatus (Corning Glass Works). The paper was removed from the wash and dried immediately before samples were applied. The chromatogram was equilibrated for 2 hours and then was developed by descending chromatography in a benzene-hexane-methanol-water (9:1:5:2.5) solvent system for 12 hours. Aldosterone was located using cortisone and cortisol standards. Aldosterone was approximately 8 cm behind cortisone and 15 cm ahead of cortisol in this system after 12 hours of development.

The samples were eluted from the paper with redistilled methanol. An aliquot was removed for liquid scintillation counting to allow determination of percent recovery, and then two aliquots equal in volume to the one measured for recovery were evaporated to dryness in a vacuum oven at 35°C for radioimmunoassay. A standard curve was constructed for each series of samples analyzed. Standards containing 0, 10, 25, 50, 100, 200, 500, and 1,000 pg of aldosterone (Ciba) dissolved in ethanol were added to test tubes, 2,500 counts/min of $^3$H-aldosterone was added to each standard, and the solutions were mixed well and evaporated to dryness in a vacuum oven. In addition, standards of 100, 500, and 1,000 pg of aldosterone were added to pooled plasma samples and carried through the complete purification procedure with each group of samples extracted.

Dilute antiseraum was prepared immediately before use by diluting stock antiseraum with borate buffer (0.05x, pH 8.0) containing 0.1% gamma globulin (human immune serum globulin, Merck, Sharp and Dohme) and 2% methanol. Three-tenths ml of the dilute antiseraum was added to the samples and standards, and the solutions were thoroughly mixed without foaming. The tubes were covered with Paraflim, placed in an ice bath, and incubated in a cold room for 2 hours. Cold, saturated ammonium sulfate (enzyme grade) solution (0.3 ml) was added to each tube. The contents were mixed well, incubated for 15 minutes in an ice bath, and centrifuged at 2,500 g for 15 minutes at 1°C. Three-tenths ml of the supernatant solution was transferred to a scintillation vial.

Ten ml of phosphor solution was added to one vial containing 0.3 ml of dilute antiseraum-saturated ammonium sulfate (1:1, background), to three vials containing the amount of $^3$H-aldosterone added as a recovery standard, to
three vials containing the amount of \(^{3} \text{H}\)-aldosterone added to the aldosterone standards, and to the vials containing 0.3 ml of supernatant solution from the tubes of the standard curve, the pooled plasma samples, the unknown samples, and the blanks. All samples were counted in a Mark II liquid scintillation counter (Nuclear Chicago) with automatic standardization using a \(^{133}\text{Ba}\) source.

The standard curve was constructed by plotting the percent of unbound steroid against the amount of unlabelled aldosterone added. The concentration of aldosterone in the aliquots of the unknown plasma samples was determined directly from the standard curve. The percent of unbound aldosterone was calculated from the following equation.

\[
\text{Percent unbound aldosterone} = \left( \frac{\text{counts/min of 0.3 ml of supernatant solution}}{0.5 \times \text{counts/min of recovery sample}} \right) \times 100.
\]

The aldosterone content in the samples in nanograms per 100 milliliters was calculated from the formula

\[
\text{counts/min added} \times \left( \frac{S - B}{\text{counts/min recovered}} \right) \times \frac{100}{\text{volume plasma used}}/1000,
\]

where \(S\) is the amount of aldosterone (in picograms) in the aliquot taken for assay as read from the standard curve and \(B\) is the aldosterone equivalent (in picograms) as read from the curve for an equal aliquot of the water blank.

Correction for the mass of \(^{3} \text{H}\)-aldosterone added to assayed samples was not necessary if the counts/min in the sample tubes equaled the counts/min in the standard tubes. However, if the counts/min of recovery differed from the counts/min of the \(^{3} \text{H}\)-aldosterone added to the standard tubes by more than 100 counts/min, correction had to be made for differences in mass contributed by \(^{3} \text{H}\)-aldosterone.

The recovery of \(^{3} \text{H}\)-1,2-aldosterone in the analysis of 155 plasma samples was 72 \pm 2.1\% (SE). Samples with a recovery of less than 50\% were discarded and not subjected to radioimmunoassay. Such samples occurred infrequently and were attributed to losses during chromatography.

Specificity of the aldosterone antisera was determined by studying the percent cross-reactivity of other steroids compared with aldosterone. Calculations of percent cross-reactivity were carried out as described by Abraham (8). If \(x = \text{picograms of aldosterone required to displace 50\% of the}^{3} \text{H}\)-aldosterone bound to the aldosterone antisera and \(y = \text{picograms of steroid S required to displace 50\% of the}^{3} \text{H}\)-aldosterone bound to the aldosterone antisera, then the percent cross-reactivity of steroid \(S = (x/y) \times 100.\) Corticosterone was the only steroid which appreciably cross-reacted, 3.5\%, with the aldosterone antisera. Other steroids which showed a cross-reaction were cortisol, 0.05\%, cortisone, 0.05\%, progesterone, 0.30\%, and deoxycorticosterone, 0.25\%. Because of the relatively large concentrations of the other steroids such as cortisol in plasma in relation to the concentration of aldosterone, chromatographic separation of contaminating steroids was essential. In the paper chromatographic system used, a benzene-hexane-methanol-water (9:1:5:2.5) solvent system, excellent separation of aldosterone and cortisol was achieved with the rate of migration of cortisol being 0.5 when aldosterone was used as a reference compound. The separation of aldosterone and cortisone was not as satisfactory. However, by using cortisone as a standard on each chromatogram and only cutting out the area well behind the cortisone to assay for aldosterone, contamination by cortisone was eliminated. To determine whether this procedure separated aldosterone and cortisone adequately, 5 ng of aldosterone, 200 ng of cortisone, and \(2 \times 10^{4}\) counts/min of \(^{3} \text{H} \text{-1,2-aldosterone} were added to five plasma samples. The specific activity of aldosterone was determined after chromatography in the routinely used system previously described and after two subsequent stages of chromatography in a toluene-ethyl acetate-methanol-water (95:5:50:50) solvent system and in an isooctane-isobutanol-water (10:5:9) solvent system. Although the last two systems provided excellent separation of aldosterone and cortisone, no significant alteration in specific activity was observed after the initial chromatography, indicating that adequate separation from cortisone was obtained with only the one chromatographic system. There was no interference from deoxycorticosterone or corticosterone, since both migrated well ahead of aldosterone in this system and, at the end of 12 hours of development, were completely off the paper.

To examine further the specificity of the procedure, the results obtained by radioimmunoassay were compared with those obtained on the same plasma samples by double-isotope dilution (7). As shown in Table 2, results using the two methods were comparable.

For estimation of the blank value, samples of...
Comparison of Aldosterone Values Obtained by Radioimmunoassay and Double-Isotope Dilution

<table>
<thead>
<tr>
<th>Subject</th>
<th>Radioimmunoassay (ng aldosterone/100 ml plasma)</th>
<th>Double-isotope dilution (ng aldosterone/100 ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>2.6</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>43.6</td>
<td>45.0</td>
</tr>
<tr>
<td>6</td>
<td>57.0</td>
<td>60.5</td>
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<tr>
<td>7</td>
<td>39.2</td>
<td>35.1</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Subjects 1-4 were normal young adult volunteers who were on an ad libitum sodium diet and who remained supine at 7:00 am after at least 6 hours of recumbency. Subjects 5-7 had taken hydrochlorothiazide (100 mg/24 hour) for 3 days and were standing at the time the sample was taken. Subject 8 was a four-year-old Addisonian patient.

distilled water equal to the volumes of plasma routinely used were treated as plasma samples and taken through the extraction and purification processes. The mean aldosterone equivalent content found in 20 samples of 5 ml of distilled water was 12 ± 3 (SE) pg/sample after correction for losses through the procedure. This blank apparently was not a result of material extracted from water, since increasing the volume of water extracted did not appreciably influence the blank value. Meticulous cleaning of the glassware, washing of the paper immediately before use with methanol, and purification of the solvents kept the blank in a range that could be tolerated. A water blank was processed with each series of samples. When sensitivity was calculated by determining the lowest value which could be distinguished from zero with a certainty of \( P = 0.01 \), it was found to be about 25 pg/5 ml plasma or 0.5 ng/100 ml plasma.

To check the accuracy of the method, variable amounts of aldosterone were added to 5-ml aliquots of pooled plasma obtained from supine subjects on a high-sodium diet. A linear relationship was obtained over the range of 1.5 to 50 ng/100 ml plasma when the aldosterone concentration assayed was plotted as a function of the amounts added. To verify routinely the accuracy, standards of 100, 500, and 1,000 pg of aldosterone were added to previously assayed pooled plasma aliquots and assayed with each series of samples.

The precision, or repeatability, of the method was investigated by carrying out duplicate assays for aldosterone on a series of plasma samples. The standard deviation was calculated from the formula \( \sigma = \sqrt{\frac{2d^2}{2N}} \), where \( d \) is the difference between duplicates and \( N \) is the number of duplicates. The sp of a group of 20 duplicate samples was ± 0.2 ng/100 ml plasma.

Results

Studies in Normal Subjects—Plasma aldosterone concentration in response to postural changes was studied in six healthy volunteers while they were recumbent before arising in the morning and again after 2 hours of ambulation during periods of low-sodium intake and periods of high-sodium intake. The values for the plasma aldosterone concentrations under these conditions are shown in Table 3. In the six healthy subjects on an unrestricted sodium diet, there was a fourfold increase in plasma aldosterone concentration in response to changes in posture. In the supine position, the plasma aldosterone concentration averaged 5.8 ± 0.57 ng/100 ml plasma.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Normal diet</th>
<th>Low-sodium diet</th>
<th>High-sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td>Supine</td>
</tr>
<tr>
<td>L.H.</td>
<td>F</td>
<td>6.6</td>
<td>26.3</td>
</tr>
<tr>
<td>F.L.</td>
<td>F</td>
<td>4.8</td>
<td>19.3</td>
</tr>
<tr>
<td>J.L.</td>
<td>M</td>
<td>3.9</td>
<td>17.4</td>
</tr>
<tr>
<td>R.C.</td>
<td>M</td>
<td>5.8</td>
<td>22.0</td>
</tr>
<tr>
<td>R.H.</td>
<td>M</td>
<td>5.4</td>
<td>19.5</td>
</tr>
<tr>
<td>D.L.</td>
<td>M</td>
<td>8.3</td>
<td>32.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5.8</td>
<td>22.9</td>
</tr>
<tr>
<td>± SE</td>
<td></td>
<td>0.57</td>
<td>2.15</td>
</tr>
</tbody>
</table>

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plasma and increased to 22.9 ± 2.15 ng/100 ml plasma after 2 hours of ambulation. Following a period of low-sodium intake, the plasma aldosterone concentration averaged 18.3 ± 1.70 ng/100 ml plasma in the supine position and increased to 37.1 ± 2.42 ng/100 ml plasma after 2 hours of ambulation. Following a period of high-sodium intake, plasma aldosterone concentration averaged 1.9 ± 0.26 ng/100 ml plasma and increased to 3.7 ± 0.29 ng/100 ml plasma after 2 hours of ambulation. Although there was only a twofold increase in plasma aldosterone concentration in response to postural changes following periods of low- and high-sodium intake, these values were significant in both instances (P < 0.001).

Studies in Anephric Patients.—Mean values for plasma sodium, potassium, aldosterone, and cortisol concentrations and for the ratio of sodium to potassium obtained for nine nephrectomized patients immediately before and after hemodialysis are shown in Figures 1 and 2. When the patients were dialyzed against a dialysate with the composition of 130 mEq sodium/liter and 2.0 mEq potassium/liter, there was an average patient weight loss of 1.5 kg. Normally, plasma sodium concentration changes only slightly in response to hemodialysis. In this study, plasma sodium concentration decreased an average of 5 mEq/liter from 137 ± 1 (SE) mEq/liter to 132 ± 1 mEq/liter (P < 0.04). However, by collecting the used dialysate from the dialyzer and measuring the sodium concentration in the dialysate as well...
as the sodium concentration in a portion of the dialysate before its passage through the dialyzer, it was found that an approximate average of 450 mEq of sodium was removed by the process of hemodialysis. Also, an approximate average of 150 mEq of potassium was removed, resulting in a decrease of plasma potassium from 4.8 ± 0.3 mEq/liter to 3.2 ± 0.1 mEq/liter (P < 0.001). The removal of sodium and potassium from the body by the process of hemodialysis resulted in an increase in the ratio of sodium to potassium from 31.3 ± 1.9 to 41.7 ± 0.9 (P < 0.001).

Peripheral plasma aldosterone concentration averaged 9.7 ± 1.6 ng/100 ml plasma in the predialysis samples and increased to 17.9 ± 2.1 ng/100 ml plasma in the postdialysis samples (P < 0.05). There was no significant change in plasma cortisol concentration in response to hemodialysis, although cortisol concentration actually decreased from 17.5 ± 2.0 μg/100 ml plasma in the predialysis samples to 14.8 ± 1.9 μg/100 ml plasma in the postdialysis samples.

Discussion

In the present study, plasma aldosterone concentration was in the upper normal physiological range in nephrectomized man despite the lack of kidneys to produce renin. Recent studies by Bayard et al. (8) also indicate that plasma aldosterone concentration is maintained within normal physiological limits in the absence of the renal renin-angiotensin system. These investigators reported a high positive correlation between plasma aldosterone concentration and serum potassium concentration in anephric man, and prevention of the rise in serum potassium by the administration of Kayexalate (sodium polystyrene sulfonate) between periods of dialysis caused the plasma aldosterone concentration to remain at low levels in these patients.

In the present study, we found a twofold increase in plasma aldosterone concentration in response to hemodialysis. Weidmann et al. (9) reported similar observations. Peart (10) and Mitra et al. (11) found no alteration in plasma aldosterone concentration in anephric patients in response to gradual dehydration induced by peritoneal dialysis. Furthermore, in our study, plasma cortisol concentration actually decreased during the period of dialysis, indicating that there was no increase in secretion of ACTH to account for the increase in plasma aldosterone concentration. Also, there was a decrease in plasma potassium concentration during hemodialysis. The removal of sodium and potassium by the process of dialysis resulted in an increase in the ratio of sodium to potassium. Both the decrease in plasma potassium and the increase in the ratio of sodium to potassium would normally be a negative stimulus for aldosterone secretion. Apparently, therefore, the increased plasma aldosterone concentration in response to hemodialysis was not mediated by angiotensin II, ACTH, or potassium ions. However, preliminary studies in our laboratory indicate that the sensitivity of the zona glomerulosa to ACTH is increased after hemodialysis (12).

A possible cause of the increased plasma aldosterone concentration which we observed in response to hemodialysis might be that the removal of sodium from the body, resulting in decreased plasma sodium concentration, is a more powerful direct stimulus to aldosterone secretion than most investigators have believed. As an alternate explanation, the increase in plasma aldosterone concentration could result from the removal of approximately 300 ml of blood from the cardiovascular system when the blood is diverted through the Kiil dialyzer. In effect, this represents acute hemorrhage. Another possible explanation could be that hemodialysis causes increased plasma aldosterone concentration by decreasing the metabolic clearance rate of aldosterone rather than by influencing the rate of secretion. In studies on dogs, Johnson et al. (13) reported a significant decrease in hepatic blood flow with hemorrhage and sodium depletion, and observations by Davis et al. (14) revealed a decline in the metabolic clearance rate of aldosterone. And yet another possible explanation, of course, is that there
might be some unidentified mechanism that responds to decreased sodium or fluid volume to control the secretion of aldosterone.

The significance of these data is that the nephrectomized patient can respond to acute stimuli with increased plasma aldosterone concentration in the absence of the renal renin-angiotensin system. Regardless of the precise mechanism(s) regulating aldosterone, these data indicate that powerful control mechanisms are still active after removal of the kidneys.

Acknowledgment
We wish to thank Miss Betty Preston, Mrs. Terry Cross, Mrs. Judy Blackburn, and Mrs. Sharon Kocher for their technical assistance in The Artificial Kidney Unit. We are also grateful to Dr. C. A. Nugent for his advice in development of the radioimmunoassay technique.

References
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