Adrenocortical Hormones in Human Hypertension and Their Relation to Angiotensin


Previous work from our laboratory has shown a significant mean increase in urinary aldosterone in groups of patients with essential, renal, and malignant hypertension and its excessive daily fluctuations in benign and severe hypertensive patients. These results have been confirmed by several groups of workers and by R. Romanelli (personal communication). More recently, Laragh et al. have found increased aldosterone secretion rates in most patients with severe hypertension and almost all patients with malignant hypertension, but not in so-called primary benign hypertension. This report was not confirmed in preliminary studies of patients with malignant hypertension by Muller.

In addition, we have demonstrated a significant mean decrease in urinary pregnanetriol in the same groups of hypertensive patients as compared to normal subjects. The urinary pregnanetriol/aldosterone ratio is below the lower limit of the normal range in 92 per cent of all hypertensive patients studied. As significant as these results appear in human hypertension, it is difficult, and indeed impossible, to overlook the role of the kidney if one considers the whole of the clinical and experimental evidence linking the kidney to the hypertensive process.

The strong experimental evidence in rats linking the juxtaglomerular cells of the kidney to the adrenal zona glomerulosa and sodium is outlined in great detail in a recent and most comprehensive review by Tobian.

It was therefore logical for our group to study the relationship between the renal pressor mechanism, specifically valine-5 angiotensin II, which had just been synthesized by Schwyer et al., angiotensin, and sodium regulation.

Three years ago, we reported the lack of any significant effect of epinephrine and nor-epinephrine infusion on urinary aldosterone and 17-hydroxycorticosteroids in three normal subjects and three patients with benign hypertension. We wish to present our findings on the effects of acute and long-term intravenous infusions of valine-5 angiotensin II on urinary volume, sodium and potassium excretion, and glomerular filtration rate—and on urinary aldosterone, cortisol, cortisone, and their tetrahydro derivatives—both in normal subjects and patients with benign hypertension.

Methods

Sodium and potassium were measured by flame photometry, using a Perkin-Elmer Model 52 and lithium as internal standard. Creatinine clearance was used as an approximate measure of the glomerular filtration rate. The procedures used for isolation in a high degree of purity and the determination of aldosterone, cortisone, cortisol, and their tetrahydro derivatives have been previously described.

Twelve volunteer medical students and two normotensive women with anxiety neurosis were used as normal controls. The hypertensive patients were in the early benign phase of essential hypertension. Six acute experiments with angiotensin II given for periods of 40 to 60 minutes were made in six normal subjects. Twelve long-term (5 to 14 hours) experiments were performed with intravenous valine-5 angiotensin II infusions, two with norepinephrine, and two with phenylephrine (Neo-synephrine, Winthrop). The pressor substances were diluted in 5 per cent glucose infused at a rate sufficient to maintain a constant increase of diastolic pressure of 25 to 35 mm Hg above control levels for the duration of the infusions. Control infusions of 5 per cent glucose were given to five subjects for periods of eight hours. In addition, three other experiments were per-
Table 1

Effects of Phenylephrine Infusions* on Urinary Sodium and Aldosterone in Normal Subjects

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Day</th>
<th>Na mEq./day</th>
<th>Aldosterone µg./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.T.</td>
<td>1. Control</td>
<td>82</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>2. Infusion period 8.3 µg./min. (8 hours)</td>
<td>235</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Postinfusion period</td>
<td>151</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>3. Control</td>
<td>50</td>
<td>34</td>
</tr>
<tr>
<td>P.M.</td>
<td>1. Control</td>
<td>64</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>2. Infusion period 10 µg./min. (8 hours)</td>
<td>274</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Postinfusion period</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3. Control</td>
<td>21</td>
<td>12</td>
</tr>
</tbody>
</table>

*Increase in diastolic pressure of 30 mm. Hg.
†Nowaczynski, Koiw, and Genest's procedure.
‡On fixed diet (102 mEq. Na and 90 mEq. K/day)

The results obtained with 5 per cent glucose, norepinephrine, and phenylephrine

Control infusions of 5 per cent glucose for eight hours did not produce any significant rise in blood pressure. All subjects and patients were maintained on a fixed sodium and potassium intake (102 and 90 mEq./day respectively) for a period of five to six days prior to and during the whole experimental period, under metabolic balance conditions. Urines were collected for the period of infusion and again separately for the rest of the 24-hour period. All results on urinary electrolytes and steroids are expressed on a daily basis.

Results Obtained with Valine-5 Angiotensin II

Short-Term Experiments

These experiments were designed to study the acute effects of valine-5 angiotensin II infusions given for 40 to 60 minutes, at a rate sufficient to produce an increase in diastolic pressure of 30 mm. Hg above control levels. The results show that a marked decrease in urinary volume and in sodium and potassium excretion occurred in all six normal subjects studied. In most, the glomerular filtration rate, as measured by creatinine clearance, fell significantly during the angiotensin infusions (fig. 1). These results confirm those of Peart reported in December, 1959.22, 23

Long-Term Infusions

These experiments were performed to determine the effect of valine-5 angiotensin II on urinary sodium, potassium, aldosterone, cortisol, cortisone, and their tetrahydro derivatives in normotensive individuals and those with benign hypertensive disease.

In Normal Subjects. In all normal subjects studied (table 2), infusions of valine-5 angiotensin II were always accompanied or followed by a 2.5- to 10-fold increase in urinary aldosterone excretion, and by a parallel rise in its reduced metabolite: pregnane-3-a,18,21-triol,11,20-dione. This increase persisted in some subjects for one to two days after the infusion. It was accompanied by a marked sodium retention and a decrease in the urinary Na/K ratio. In two of four patients extensively studied for steroid excretion (table 2) there has been a 2- to 3-fold increase in urinary cortisol and tetrahydrocortisone. These changes may be within the normal limits of variation in the excretion of these two steroids. Nevertheless, this order of magnitude of rise in some 17-hydroxycorticosteroids or in the sum of their total individual values appears small in terms of physiological significance in comparison to that caused by the

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GENEST, BIRON, KOIW, NOWACZYNSKI, CHRETIEN, BOUCHER
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J. L. B. 24 years NORMAL SUBJECT

*ON A CONSTANT DIET (Na: 102 ± K: 90 mEq/DAY)

Figure 1

Acute effects of angiotensin infusion (1.2 µg/min. for 50 min.; blood pressure changes +22/+35 mm. Hg) on urinary volume, sodium, Na/K ratio, potassium, and glomerular filtration rate (GFR) as measured by creatinine clearance. In most other subjects, GFR fell 20 to 40 per cent during the angiotensin infusion.

Increased urinary aldosterone during or after the angiotensin infusion.

The detailed results of urinary aldosterone of nine normal medical students receiving angiotensin infusions at hypertensive rates (increase in diastolic pressure of 25 to 35 mm Hg above control levels) are indicated in Table 3. In all cases, the increase in aldosterone excretion is significant.

In subject J. M. (fig. 2) the increase in aldosterone persisted for at least three to four hours after cessation of the angiotensin infusion. Despite the continued hyperaldosteronuria, urinary sodium and Na/K ratio rose toward preinfusion levels.

The comparative effects of angiotensin and norepinephrine are illustrated in figure 3. The 10-fold increase in urinary aldosterone with concomitant sodium retention and a fall in the Na/K ratio persisted after the angio-
Figure 2

Subject J. M. Effects of angiotensin infusion (2.4 μg./min. for 14 hours; diastolic pressure +30 mm. Hg above control levels) on pulse rate, urinary sodium, Na/K ratio, potassium, aldosterone, and 17-hydroxycorticosteroids (as the sum of cortisone [E], cortisol [F], tetrahydrocortisone [THE] and tetrahydrocortisol [THF] individually determined). Note the very marked and sustained increase of aldosterone and the slight and progressive rise of 17-hydroxycorticosteroids during and after the angiotensin infusion.

Angiotensin infusion. The 2-fold rise in the sum of the urinary 17-hydroxycorticosteroids was, by far, the most marked of all the patients studied. By contrast, the norepinephrine infusion produced a slight increase in aldosteronuria and a very marked natriuresis and a rise in the Na/K ratio.

Almost complete sodium retention, with depression of the Na/K ratio to an extremely low level, followed an angiotensin infusion during which urinary aldosterone rose to 138 μg./day (fig. 4).

A comparison of the effects of angiotensin infused at subhypertensive and at hypertensive levels in two subjects is shown in figures 5 and 6. In both subjects, angiotensin infusions given at subhypertensive rates produced a significant aldosteronuria of 57 and 74 μg./day respectively. These two experiments indicate the extreme sensitivity of aldosterone excretion in response to angiotensin. This response is greatly increased when angiotensin is infused at hypertensive rates in the two subjects.

In Patients with Benign Hypertension. In all three patients in whom angiotensin was

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Table 2

Effect of Intravenous Infusions of Angiotensin and Norepinephrine in Normal Male Subjects

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Day</th>
<th>K†</th>
<th>Na†</th>
<th>ADT</th>
<th>THF</th>
<th>CORT</th>
<th>Cortiso</th>
<th>Zone</th>
<th>Sum of &quot;F&quot; + &quot;E&quot;THF + THE mg./day</th>
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</thead>
<tbody>
<tr>
<td>R.A., 22 yr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>84</td>
<td>55</td>
<td>11</td>
<td>76</td>
<td>70</td>
<td>133</td>
<td>3017</td>
<td>1407</td>
<td>4.6</td>
</tr>
<tr>
<td>2. Angiotensin inf.</td>
<td>78</td>
<td>35</td>
<td>105</td>
<td>505</td>
<td>218</td>
<td>227</td>
<td>2382</td>
<td>6792</td>
<td>9.6</td>
</tr>
<tr>
<td>Postinf. period</td>
<td>46</td>
<td>35</td>
<td>98</td>
<td>383</td>
<td>444</td>
<td>131</td>
<td>307</td>
<td>907</td>
<td>3.6</td>
</tr>
<tr>
<td>3. Control</td>
<td>90</td>
<td>108</td>
<td>28</td>
<td>138</td>
<td>125</td>
<td>153</td>
<td>3744</td>
<td>1815</td>
<td>5.8</td>
</tr>
<tr>
<td>4. Control</td>
<td>75</td>
<td>73</td>
<td>19</td>
<td>65</td>
<td>180</td>
<td>74</td>
<td>2699</td>
<td>803</td>
<td>3.2</td>
</tr>
<tr>
<td>5. Norepinephrine inf.</td>
<td>69</td>
<td>230</td>
<td>41</td>
<td>493</td>
<td>508</td>
<td>511</td>
<td>2348</td>
<td>3259</td>
<td>6.6</td>
</tr>
<tr>
<td>Postinf. period</td>
<td>65</td>
<td>26</td>
<td>26</td>
<td>73</td>
<td>106</td>
<td>246</td>
<td>990</td>
<td>1445</td>
<td>2.8</td>
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<tr>
<td>J.M. 26 yr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>39</td>
<td>39</td>
<td>14</td>
<td>195</td>
<td>210</td>
<td>89</td>
<td>1229</td>
<td>790</td>
<td>2.3</td>
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<tr>
<td>2. Angiotensin inf.</td>
<td>81</td>
<td>14</td>
<td>52</td>
<td>336</td>
<td>248</td>
<td>97</td>
<td>1916</td>
<td>1357</td>
<td>3.6</td>
</tr>
<tr>
<td>Postinf. period</td>
<td>73</td>
<td>12</td>
<td>69</td>
<td>595</td>
<td>102</td>
<td>137</td>
<td>3752</td>
<td>2358</td>
<td>6.3</td>
</tr>
<tr>
<td>3. Control</td>
<td>48</td>
<td>55</td>
<td>68</td>
<td>226</td>
<td>193</td>
<td>197</td>
<td>3989</td>
<td>3404</td>
<td>7.8</td>
</tr>
<tr>
<td>J.P.D. 21 yr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>67</td>
<td>58</td>
<td>5</td>
<td>71</td>
<td>107</td>
<td>140</td>
<td>1024</td>
<td>1768</td>
<td>3.0</td>
</tr>
<tr>
<td>2. Angiotensin inf.</td>
<td>50</td>
<td>6</td>
<td>56</td>
<td>389</td>
<td>218</td>
<td>249</td>
<td>931</td>
<td>1852</td>
<td>3.2</td>
</tr>
<tr>
<td>Postinf. period</td>
<td>69</td>
<td>32</td>
<td>27</td>
<td>249</td>
<td>90</td>
<td>85</td>
<td>609</td>
<td>966</td>
<td>1.8</td>
</tr>
<tr>
<td>3. Control</td>
<td>95</td>
<td>59</td>
<td>15</td>
<td>56</td>
<td>229</td>
<td>197</td>
<td>1954</td>
<td>1362</td>
<td>5.5</td>
</tr>
<tr>
<td>N.N. 23 yr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>75</td>
<td>53</td>
<td>23</td>
<td>66</td>
<td>85</td>
<td>183</td>
<td>2199</td>
<td>1806</td>
<td>4.3</td>
</tr>
<tr>
<td>2. Angiotensin inf.</td>
<td>70</td>
<td>16</td>
<td>84</td>
<td>113</td>
<td>137</td>
<td>311</td>
<td>876</td>
<td>3055</td>
<td>5.3</td>
</tr>
<tr>
<td>Postinf. period</td>
<td>44</td>
<td>6</td>
<td>29</td>
<td>458</td>
<td>117</td>
<td>291</td>
<td>585</td>
<td>1211</td>
<td>2.2</td>
</tr>
<tr>
<td>3. Control</td>
<td>74</td>
<td>88</td>
<td>25</td>
<td>138</td>
<td>141</td>
<td>249</td>
<td>3317</td>
<td>912</td>
<td>4.6</td>
</tr>
</tbody>
</table>

*On a fixed Na and K intake (102 and 90 mEq./day respectively)
†mEq./day
‡Micrograms/day
§Micrograms/day. The measurement of the "tetrahydrocortisone zone" by the blue tetrazolium reaction includes also that of tetrahydroaldosterone and of 3-allo-tetrahydrocortisol
||E = Cortisone; F = cortisol; THE = tetrahydrocortisone; THF = tetrahydrocortisol

administered at a rate sufficient to produce a significant and stable increase of 15 to 25 mm. Hg diastolic pressure above control levels, there was a very marked natriuresis with a simultaneous increase in urinary Na/K ratio. Although a considerable rise in urinary aldosterone excretion was observed as in normal subjects, this increase in sodium excretion and in the Na/K ratio constitutes a fundamental difference in the response of hypertensive patients to angiotensin infusions as compared to normal subjects. One patient with benign essential hypertension (fig. 7) received an angiotensin infusion at subhypertensive rate. During the experiment, urinary sodium increased 3-fold with a rise in the Na/K ratio, despite a 2-fold increase in aldosterone excretion. Five days later, a second angiotensin infusion was given at the hypertensive rate, with an average of 33 mm. Hg in diastolic pressure above control levels. This time, the patient showed a more marked natriuresis to 317 mEq./day, with a 3-fold rise in the Na/K ratio, despite a 3-fold increase in urinary aldosterone. This significant loss of sodium was followed during the night pe-
Figure 3

Subject R. A. Effects of infusions of angiotensin (4.2 μg/min. for 13 hours) and of norepinephrine (15 μg/min. for 7 hours) at hypertensive levels (diastolic pressure +30 mm. Hg above control levels) on pulse rate, urinary sodium, Na/K ratio, potassium, aldosterone, and 17-hydroxycorticosteroids (see legend of fig. 2). Note the tremendous rise in aldosterone excretion, with marked sodium retention and fall in the Na/K ratio during angiotensin infusion, in contrast to the considerable natriuresis and rise in the Na/K ratio during the norepinephrine infusion.

Period by a still greater aldosteronuria to 136 μg/day, accompanied by severe sodium retention and a fall in the Na/K ratio.

In another patient with benign essential hypertension (fig. 8) angiotensin infused at hypertensive rate (16 mm. Hg increase in diastolic pressure above control levels) resulted in a significant increase of urinary aldosterone, but again, in contrast to the response of normal subjects, there was simultaneously a marked natriuresis and rise in Na/K ratio.

Discussion

In October, 1959, we reported our preliminary results in normal subjects. These findings have now been confirmed in twelve normal subjects and have been reported in detail in June and July, 1960. Laragh et al. reported similar increases in aldosterone secretion in four normal subjects in September, 1960.

These results give strong clinical support to the experimental evidence linking the granulations of the juxtaglomerular cells to the width of the adrenal zona glomerulosa and sodium regulation. This evidence can be summarized as follows: The width of the adrenal zona glomerulosa secreting aldosterone and the granularity of the juxtaglomerular cells probably secreting renin and the renin content of the kidney vary in parallel. They increase in response to low sodium intake and in all cases in which the renal artery pressure is low, as after total bilateral adren-
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Subject G. P. Normotensive patient with anxiety neurosis. Effects of angiotensin infusion (1.7 µg/min. for 5 hours; B. P. +29/+24 mm. Hg above control levels) on urinary sodium, Na/K ratio, potassium, and aldosterone. The period of most marked sodium retention and fall in Na/K ratio occurred after the infusion, when aldosterone had already returned almost to the control levels.

The effect of angiotensin administration in normal subjects appears to a 2-fold one: (a) There is a hemodynamic effect with pronounced fall in the glomerular filtration rate and in the filtered load of water, sodium, chloride, and potassium, with subsequent reduction in their excretion. This fall in filtration rate is seen only when angiotensin is infused at a rate producing a significant rise in blood pressure. This effect on filtration rate has been shown by Bock to be transitory, since the glomerular filtration rate often returns to its control level even during the period of angiotensin infusion. (b) There is a direct effect on tubular reabsorption of water and sodium either per se or through an increase in aldosterone secretion, as the present experiment strongly suggests. The fact that aldosterone increases markedly, even during infusions of angiotensin given at a subhypertensive level and without changes in the glomerular filtrate rate, gives greater support to the latter concept. The stimulatory effect of angiotensin on aldosterone is quite marked and specific and is also produced by subhypertensive infusions of angiotensin.
Figure 5

Subject J.-L. B. Effects of angiotensin infused at subhypertensive (0.27 μg./min. for 8 hours; B. P. +4/+7 mm. Hg above control levels) and at hypertensive levels (3.2 μg./min. for 5.5 hours; B. P. +29/+26 mm. Hg) on urinary sodium, Na/K ratio, potassium, and aldosterone. Even at a rate of angiotensin infusion insufficient to raise the blood pressure significantly, there is a marked increase in aldosterone with a fall in sodium excretion and in Na/K ratio. Unfortunately, results of control urine before the angiotensin infusion at subhypertensive levels are not available.

Increased aldosterone excretion was not encountered with infusions of phenylephrine and norepinephrine (with one exception), although significant increases in blood pressure were achieved in all instances.

Our findings agree with those of Deane and Masson29 recently confirmed by Hartroft et al.28 and by Gross30 who have demonstrated that renin administration produces a marked and rapid increase in the width of the zona glomerulosa in rats.

Another aspect of these studies is given emphasis by the recent experiments of Hilton.31 Using in vivo perfusion of adrenal glands in dogs, Hilton has demonstrated that vasopressin stimulates cortisol secretion but not that of aldosterone. Since vasopressin is closely related chemically to angiotensin, it is of interest to note that these two related polypeptides can specifically and differentially stimulate the secretion of the two main corticosteroid hormones in urine.

The main features of early benign hypertension in humans are described in table 4. Basically, these features center around two main aspects: (a) that the hypertensive pro-
Subject A. L. Nervous and tense individual. Effects of angiotensin infusion at subhypertensive (0.39 μg./min. for 8 hours; B. P. changes —2/4-4 mm. Hg) and hypertensive levels (2.8 μg./min. for 5.5 hours; B. P. +22/+35 mm. Hg above control levels) on urinary sodium, Na/K ratio, potassium, and aldosterone. Despite the significant increase of aldosterone during the subhypertensive infusion, sodium excretion was in balance with the intake.

The process in the early phase is essentially a discontinuous one, and (b) that it is profoundly influenced by disturbances in sodium, aldosterone, and progesterone regulation or metabolism.

On the basis of our previous work and the present series of experiments, we should like to propose the following concept for the pathogenesis of human arterial hypertension (fig. 9). First, the marked and quite specific stimulation of aldosterone secretion and excretion by angiotensin is well established. However, there is a possibility, based on interpretation of experimental evidence in rats, that aldosterone may reciprocally stimulate the liberation of renin by the kidney. There is no direct proof of this as yet. There is one interesting point here. Since we have observed that angiotensin given at subhypertensive levels results in a similar increase in urinary aldosterone, this might indicate that it would be possible for angiotensin to be liberated by the kidney at levels insufficient to produce any increase in blood pressure, but sufficient to increase aldosterone secretion, which would lead to an increased rate of intracellular so-

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Patient H. B. with benign essential hypertension. Effects of angiotensin infusions at subhypertensive (0.08 µg./min. for 9.5 hours; B. P. +2/-6 mm. Hg) and hypertensive rates (0.28 µg./min. for 5 hours; B. P. +47/+33 mm. Hg) on urinary sodium, Na/K ratio, potassium, and aldosterone. Despite the increase in urinary aldosterone during both infusions, there is a profuse natriuresis with a rise in Na/K ratio in contrast to the response of normal subjects. This patient showed an extreme sensitivity to the pressor effect of angiotensin.

Table 3
Effects of Intravenous Valine-5 Angiotensin II Infusions* in Normal Male Volunteers†

<table>
<thead>
<tr>
<th>Patients</th>
<th>Dose of angiotensin</th>
<th>Day 1</th>
<th>Angiotensin infusion</th>
<th>Post-angiotensin infusion</th>
<th>Day 3</th>
</tr>
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<tbody>
<tr>
<td>J. M.</td>
<td>2 mg./14 hrs.</td>
<td>14</td>
<td>52</td>
<td>69</td>
<td>68</td>
</tr>
<tr>
<td>R. A. (1)</td>
<td>3.1 mg./7 hrs.</td>
<td>10</td>
<td>14</td>
<td>47</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>3.4 mg./13 hrs.</td>
<td>11</td>
<td>105</td>
<td>98</td>
</tr>
<tr>
<td>F. M.</td>
<td>1.2 mg./8 hrs.</td>
<td>19</td>
<td>32</td>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td>J.-P. D.</td>
<td>0.9 mg./7 hrs.</td>
<td>5</td>
<td>36</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>H. N.</td>
<td>0.9 mg./12 hrs.</td>
<td>23</td>
<td>84</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>G. P.</td>
<td>0.52 mg./5 hrs.</td>
<td>20</td>
<td>138</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>J.-L. B.</td>
<td>1.03 mg./8.4 hrs.</td>
<td>24</td>
<td>107</td>
<td>62</td>
<td>25</td>
</tr>
<tr>
<td>A. L.</td>
<td>1.01 mg./5.5 hrs.</td>
<td>28</td>
<td>120</td>
<td>57</td>
<td>33</td>
</tr>
</tbody>
</table>

*Increase in diastolic pressure of 30 mm. Hg
†On fixed Na and K intake (102 and 90 mEq./day respectively)
‡Nowaczynski, Koiw, and Genest's procedure
HFMAX HYPERTEXSIOX. AXGIOTEXS1X. AND ADREXAL HORMONES 785

M. H 9  37 yr5  28177

Essential Hypertension

B.P. minHg 180 -•

140 -

100 -

mEq/DAY 240

160

80

0

URINARY Na 163/05 tl75/i2l

255

145/100

266x272

121x574

URINARY K 0

mEq/DAY 80

202x531

ANGIOTENSIN .92 j / min. (8 HRS. I

200x561

(8 HRS.)

3 DAY 4 1

DAY 2

DAY 3

DAY 4

Figure 8

Patient M. H. with benign essential hypertension. Effects of angiotensin infusion (0.92 µg./min. for 8 hours; B. P. +12/+16 mm. Hg above control levels) on urinary sodium, Na/K ratio, potassium, and aldosterone. Although this patient—the only one in the present series—could not be placed on a fixed diet, the electrolyte response to angiotensin is identical to that of patient H. B., illustrated in Fig. 7, despite an even greater increase in urinary aldosterone.

Clinically transfer or to a rise in sodium content and to changes in cell-membrane potential. If this should be so, it would mean the blood angiotensin levels were below the limit of the sensitivity of 5 nanograms per 100 ml. of blood by the method devised by Boucher, Biron, and Genest. 

Second, a disturbance in aldosterone secretion or excretion in hypertension is also well established. There is disagreement concerning the early benign phase of the disease. However, our findings of a marked mean decrease in urinary pregnanetriol and of a lower pregnanetriol/aldosterone ratio in 92 per cent of hypertensive patients, in all stages of the disease, point to an important index of the disease. Pregnanetriol is a major metabolite of progesterone (after 17-hydroxylation), which has been shown by Landau, Kagawa, and Gornall to inhibit the sodium-retaining activity of aldosterone, and by Armstrong in studies confirmed by our group to exert a definite hypotensive effect in experimental as well as in human hypertension. We could then

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Table 4

Main Features of Early, Benign Hypertension in Humans

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
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<tbody>
<tr>
<td>Asymptomatic</td>
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<tr>
<td>Discontinuous process</td>
<td></td>
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<tr>
<td>Excessive fluctuations in blood</td>
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<tr>
<td>pressure</td>
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<tr>
<td>Emotional factors</td>
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<td>Environmental factors</td>
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<td>Maladjustment factors</td>
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<tr>
<td>High salt intake</td>
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<tr>
<td>Excessive fluctuations in daily</td>
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<tr>
<td>aldosterone excretion</td>
<td></td>
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<tr>
<td>Low urinary pregnanetriol/aldosterone ratio</td>
<td></td>
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<tr>
<td>Lowering of blood pressure by:</td>
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<tr>
<td>Natriuretic drugs</td>
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<tr>
<td>Aldosterone antagonists</td>
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<tr>
<td>Increased tubular rejection of</td>
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<tr>
<td>filtered sodium following salt loads</td>
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<tr>
<td>Natriuretic response to angiotensin</td>
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postulate that either an absolute increase of aldosterone or a relative excess of aldosterone over a decreased progesterone may be the major adrenocortical disturbance in arterial hypertension.

This absolute increase or relative excess of aldosterone over progesterone would lead to an increased rate of sodium transfer into the intracellular space, which appears to be the key phenomenon in the rise of arterial pressure, according to Friedman's elegant studies in rats and dogs, and to an increase in intracellular sodium content as Tobian had demonstrated in various types of experimental hypertension as well as in human hypertension. A marked increase in intracellular sodium content in muscle biopsies has been reported in patients with primary aldosteronism. These cationic disturbances in the arteriolar muscle cells would bring an important change in the cell-membrane potential leading to either an increased tonicity of the smooth muscle fiber or to its hyperreactivity to norepinephrine liberated in normal amounts at the myoneural junction.

This is our concept of the mechanism of the hypertensive process, which appears to us to unify most of the data known concerning experimental and human hypertension and that serves as the basis of our work. It leaves room for a hypotensive substance secreted by the kidney and opposing the effect of angiotensin. Preliminary studies on fractionation of blood polypeptides in our laboratory are consistent with this concept. On the other hand, if the concept that the disturbances in aldosterone secretion and angiotensin are late phenomena encountered only in the very advanced phase of the disease, we would still be in complete darkness concerning the mechanism of the hypertensive process in its early phase.
HUMAN HYPERTENSION, ANGIOTENSIN, AND ADRENAL HORMONES

Summary

In summary, we have demonstrated that:
(a) Infusions of epinephrine, norepinephrine, and phenylephrine in 5 per cent glucose for 7 to 10 hours have little effect on, or decrease, urinary aldosterone excretion. (b) Angiotensin II infusions markedly decrease sodium excretion and the Na/K ratio and increase the excretion of aldosterone; of its ring-A reduced metabolite, pregnane-3α,18,21-triol, 11,20-dione; and, to a much lesser degree, of cortisol and tetrahydrocortisone in all normal subjects studied. (c) In patients with benign essential hypertension, infusions of angiotensin also stimulate urinary aldosterone excretion but have a completely opposite effect on electrolytes—that of increasing sodium output and the Na/K ratio. This basic difference in response to angiotensin points to a fundamental problem to be solved for a better understanding of the disease. It is felt that the relative or absolute excess of aldosterone over progesterone secretion may be the important adrenal disturbance among the basic factors involved in the pathogenesis of arterial hypertension. This disturbance is definitely linked with angiotensin and sodium regulation.

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sition of the norta in renal and adrenal hyper-


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Dr. Mendloivitz: I should like to congratulate Dr. Genest on a beautiful demonstration of these new phenomena and to express the hope that the theoretical exposition that he presented at the end of his talk is not one to which he is irrevocably committed. I say this because there are some stubborn facts that may interfere with that concept. We have studied about ten cases that we consider to be pure renal hypertension, based on chronic glomerulonephritis and chronic pyelonephritis, and found that their vascular reactivity to norepinephrine was normal. This is very upsetting in terms of this theory. Also, the turnover rate of infused angiotensin is decreased in the patient with essential hypertension. Thus, the increased angiotensin levels in the blood of essential hypertensives may have something to do with decreased destruction of vasoactive substances, such as angiotensin, rather than with its increased production.

Dr. Bradley: I want to say a few words in support of one of Dr. Genest's comments. It should be stressed that sodium and potassium excretion by the kidney must be interpreted not only in terms of tubular cellular function but also in terms of filtrate formation and renal blood flow. Drs. Cheves Smythe and James Nickel joined me in a study of this problem during 1950 to 1952, and the results of the investigation appeared in the Journal of Clinical Investigation in 1952 and in 1954. We were interested at the outset in determining whether epinephrine and norepinephrine would increase sodium output in the urine as was claimed to be the case in the rabbit. We were rather surprised to find that both agents reduced urinary output of sodium and potassium. Both also tended to maintain urine flow in accord with earlier claims that these agents tended to suppress vasopressin release. Since the glomerular filtration rate did not change in the face of the elevated arterial pressure and the intrarenal vasoconstriction that produced a fall in blood flow, the change in electrolyte output was ascribed, initially, entirely to a change in tubular reabsorption. In subsequent studies, however, we were forced to modify this conclusion, since it was found that a fall in renal blood flow appeared to be a constant concomitant that might also be instrumental in causing the change in tubular activity. In any case, it was evident that altered tubular reabsorption was a factor. Pressor agents like angiotensin, S-methyl isothiourea, and ephedrine all produced rises in blood pressure, but disparate effects on sodium and potassium excretion. The first two always induced a marked reduction in sodium and potassium output, invariably in association with a marked fall in filtration rate. For some reason as yet unknown, the latter was unique in producing a rise in sodium output in association with a fall in potassium excretion that was not dependent upon any change in renal blood flow or glomerular filtration rate. Of particular importance to the discussion today was the discovery that norepinephrine produces effects independently of adrenal cortical activity. In three subjects with adrenal cortical insufficiency (two with Addison's disease and one with bilateral adrenalectomy) infusion of l-norepinephrine was associated with the usual prompt reduction in sodium output seen in normal subjects. It seems reasonable to conclude, therefore, that, the immediate effects, at least of the pressor agents, must depend upon direct action on the renal tubules and/or hemodynamics independent of medication by the release of aldosterone. It may be added also that studies of patients with diabetes insipidus and after induction of high spinal anesthesia indicated that neither the posterior pituitary nor the central nervous system played a role in the action of the medullary amines upon renal function.

Dr. Haas: Dr. Genest, could you tell us briefly your experimental evidence for the existence of a hypotensive agent in the kidneys? In our laboratories we have processed thousands of pounds of kidneys by different methods. I think we should have come across such an agent accidentally, but we have not.

Dr. Hoobler: I am particularly interested in the increased sodium elimination in the
hypertensive patient as compared to the normotensive individual exposed to angiotensin infusion. I should like to ask whether you have given other pressor agents such as norepinephrine to normotensives and to hypertensives in order to see the effect on sodium elimination.

Dr. Freis: Dr. Genest, I seem to recall that Dr. Peart has presented similar observations on the effect of angiotensin on sodium excretion in hypertensive and normotensive subjects. I wonder if you would discuss your results in relation to his.

Dr. Wakerlin: Dr. Genest indicated there was no direct evidence for an effect of aldosterone on angiotensin. I might point out that there is indirect evidence, which goes back a good many years. Twelve years ago a student of mine, Dr. H. F. Bessinger, found that large doses of desoxycorticosterone in dogs produced a marked decrease in renin content of the kidney. Presumably this would have some effect on angiotensin formation.

Dr. Dustan: Dr. Genest, I am fascinated, but, as always, I am a little bit confused. One of the most potent antihypertensive treatments is sodium restriction. Since sodium restriction enhances aldosterone production, I wish you could tell me how such diets exert an antihypertensive effect.

Dr. Genest: In response to Dr. Mendlowitz, I should like to point out that the patient in whom we found one of the highest concentrations of blood "angiotensin material" by the procedure devised in our laboratory (Boucher, R., Biron, P., and Genest, J.: Procedure for isolation and determination of human blood angiotensin. Canad. J. Biochem. & Physiol. 39: 581, 1961), was a 15-year-old boy with a history of angina and typical manifestations of acute glomerulonephritis with hypertension. This patient had 100 nanograms (or 0.1 μg.) of "angiotensin material" per 100 ml. of blood. This amount was enough to check the mobility of this "angiotensin material" on another paper chromatographic system and on one electrophoretic system against the standard substance valine-5 angiotensin II. The blood pressure response curve obtained in the rat was identical with that of standard valine-5 angiotensin II. This patient was the only renal hypertensive patient we have studied for angiotensinemia. We are sure that the material isolated is not arginine vasopressin or isoleucine-5 angiotensin II.

I wish to thank Dr. Bradley for his comments. I was aware of his previous studies. If I did not mention them, it was because we were more interested in the hormonal changes after administration of pressor agents. In regard to the evidence for a hypotensive substance from the kidney, I apologize for a slip of the tongue. This has been a presumption on our part. In the final paper chromatogram in which our "angiotensin material" is finally isolated in a high degree of purity, we have observed a slower-moving component (or components), which has a mobility similar to that of arginine vasopressin and which is well separated from the valine-5 angiotensin II. This depressor component has been found repeatedly. It is probable that it is a polypeptide in structure, possibly similar to the material isolated by Dr. Grollman from renal extracts.

In answer to Dr. Hoobler: I do not have the data on the sodium excretion during norepinephrine infusions, the results of which were reported in June, 1958, at the meeting of the Canadian Federation of Biological Societies, in Kingston. My recollection is that the change in sodium excretion was not significant in the three normotensive and three hypertensive patients studied. In answer to Dr. Freis's comments, I have said that our work on acute administration of angiotensin confirms completely that of Peart concerning its effect on electrolyte excretion and glomerular filtration rate both in normal subjects and in hypertensive patients. Our contribution has been to extend these observations, especially in regard to the response of aldosterone and other steroids and to establish, for the first time in human hypertension, a definite link between sodium, the adrenal cortex, and the renal pressor mechanism.

In answer to Dr. Wakerlin: I have mentioned that, after desoxycorticosterone administration, workers have observed a fall in the granulation index of the juxtaglomerular...
cells and in renal renin content. There is other evidence that can be interpreted the other way, although it is quite unlikely. We are, nevertheless, interested in measurements of "angiotensin material" in cases of primary aldosteronism in order to establish whether a hypersecretion of aldosterone might stimulate the release of angiotensin from the kidney.

Finally, Dr. Dustan has brought up an important question that I cannot answer. Aldosterone is increased in almost all instances of severe sodium restriction. Why is there not an increase in blood pressure and why, on the contrary, is there often a drop in blood pressure in many patients with benign essential hypertension? Is it possible that the severe restriction of sodium inhibits the effects of aldosterone at the arteriolar cell level? Your point is an excellent and obvious one; I regret being unable to say more about it.

Dr. Baldwin: I believe we have the data to answer the question raised by Dr. Hoobler. We have infused norepinephrine intravenously in a number of hypertensive patients and found decreased sodium excretion comparable to that observed in the normotensive subject. We are not so sure that this is related solely to a reduction in glomerular filtration because the decreased sodium excretion persisted in a number of patients after the infusion when the filtration rate had returned to normal.

Dr. Hawthorne: One thing that impressed me was the drop in systolic pressure in the hypertensive patients after the infusion of angiotensin was stopped. What, if any, interpretation have you placed on that? Do you think there might be a difference in cardiac output in these patients?

Dr. Genest: I cannot answer the question. All investigators who have given angiotensin infusions with significant increases in blood pressure have observed a very rapid drop in blood pressure below control levels as soon as the infusion was stopped. This happens within 60 to 90 seconds. We have not studied cardiac output.

Dr. Finney: If the infusion is continued for more than 4 to 5 hours, the arterial pressure frequently remains below control levels for 12 to 14 hours. Postural hypotension is severe during this period. This same reduction in arterial pressure is found after discontinuation of infusions of norepinephrine. We have been most interested in why this occurs. Cardiac-output determinations on five of these patients have shown a marked decrease.

Dr. Page: If it can be conclusively demonstrated that excess aldosterone is an important factor in the production of hypertension, another important advance will have been made. Even this advance has a history. Dr. Corcoran made the original observation on the renal hemodynamic results of angiotensin infusion and Dr. Georges Masson and I suggested a schema relating the adrenal cortex, sodium retention, angiotensin, and vascular disease. Substitute DCA for aldosterone and I am sure you will see what I mean.

We should keep in mind that we are all looking for a specific physiological or metabolic defect that will characterize essential hypertension. Angiotensin may have properties that are specific, and Dr. Genest may have come across one of these. Yesterday we were trying to find a specific action for the catecholamines that might fit into the mechanism of hypertension. So far we have had no singular success. This is one of the reasons why we must be doubly careful to supply proof of each step in our reasoning and not give in to the temptation of explaining all types of hypertension from one aspect.

I have tended to feel that perhaps norepinephrine, serotonin, angiotensin, and bradykinin are all highly potent in their vascular action and that they act in concert to aid the nervous system in the control of vascular tone. I suspect that the amounts we are dealing with and their equilibrium relationships are such that we are not likely to have any very adequate description within the near future. But the foundation has been laid and it is up to us to build exactly but with imagination, because nature has an eye for beauty. Polish your stone carefully so it will fit the more perfectly.
Adrenocortical Hormones in Human Hypertension and Their Relation to Angiotensin
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