Assessment of urine-concentrating ability in man: effect of fludrocortisone and urea in enhancing response to vasopressin

C. DE F. W. GOONARATNA(1) AND O. M. WRONG
Department of Medicine, Dundee University, and Medical Unit, University College Hospital Medical School, London

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Summary

1. Healthy subjects, given a long-acting preparation of vasopressin intramuscularly, excreted a significantly less concentrated urine than when subjected to fluid deprivation for 28 h.

2. When fludrocortisone, a potent mineralocorticoid, was given in addition to vasopressin the urine was not significantly less concentrated than after fluid deprivation.

3. Oral urea-loading also enhanced the urine-concentrating power of vasopressin but its effect was less marked than that of fludrocortisone. Oral urea did not increase further the urine concentration achieved by combined fludrocortisone and vasopressin.

4. Renal concentrating power was assessed in fourteen patients with renal disease and impaired concentrating ability. Fludrocortisone significantly enhanced the urine concentration achieved by vasopressin alone and the resultant urine was not significantly less concentrated than that achieved by fluid deprivation.

5. The action of fludrocortisone in enhancing the urine-concentrating effect of vasopressin is similar to that of aldosterone and is probably due to the increased sequestration of solute in the renal medulla, caused by increased reabsorption of sodium chloride in the ascending limb of the loop of Henle.

6. In the clinical assessment of renal concentrating power, the combined use of fludrocortisone and vasopressin has potential advantages over established methods.

Key words: fludrocortisone, urea, urine-concentrating ability, vasopressin.

Introduction

Renal concentrating ability can be assessed by prolonged water deprivation, or alternatively by the use of a long-acting preparation of antidiuretic hormone without water deprivation. The latter procedure is less uncomfortable for the subject but is known to produce urine of significantly lower concentration than that obtained by water deprivation (Sodeman & Engelhardt, 1942; Taylor, Peirce & Page, 1945; Little, Wallace, Whatley & Anderson, 1947; Miles, Paton & de Wardener, 1954; Jones & de Wardener, 1956); a similar difference has been noted in studies of the dog (West, Traeger & Kaplan, 1955).

It has been suggested that this discrepancy arises from differences in aldosterone secretion in the two situations (Crabbé, 1962). Water deprivation leads to some contraction of extracellular fluid, with stimulation of aldosterone secretion, increased sodium reabsorption in the loop of Henle and hence an increased renal medullary osmolality (Kessler, Allen, Kirman & Strauss, 1964), with which the urine in the collecting ducts equilibrates in the presence of antidiuretic hormone. However, vaso-
pressin without fluid restriction may lead to overhydration, with consequent suppression of aldosterone release (Beck, Dyrenfurth, Giroud & Venning, 1955; Bartter, Liddle, Duncan, Barber & Delea, 1956), reduced sequestration of sodium in the renal medulla and hence a reduced urinary concentration even in the presence of vasopressin. In support of this suggestion, Crabbé (1962) showed that aldosterone significantly enhanced the urine-concentrating power of vasopressin and that the aldosterone antagonist spironolactone had the opposite effect on the urine concentration achieved during hydropenia.

Urea is another substance known to enhance the urine-concentrating power of vasopressin. Epstein, Kleeman, Pursel & Hendrikx (1957) showed that the maximum urine to plasma osmolal ratios attained by healthy subjects receiving vasopressin were significantly higher when they were on a high protein diet and that a similar effect could be produced by oral urea supplements. This effect is likely to be the result of sequestration of urea in the renal medulla, which is known to be increased in animals by dietary urea supplementation.

The present study was undertaken to ascertain whether administration of a mineralocorticoid or urea, or both, to subjects given vasopressin would produce urine concentrations as high as achieved by fluid deprivation. We hoped thereby to introduce a method of assessing renal concentrating power in patients with renal disease that would not involve fluid deprivation but would yet yield a urine as concentrated as can be achieved by the latter procedure.

**Material and methods**

**Subjects**

The normal subjects comprised twenty-eight healthy volunteers aged 18–46 years (mean 28, sd 7; eleven females). Each subject performed in random order at least three of the six experiments described below. The interval between consecutive experiments in the same subject was usually more than 5 days and never less than 3 days. During experiments, subjects performed their normal duties but refrained from strenuous exercise likely to produce excessive sweating. Except in experiments 1 and 6, which entailed fluid deprivation, subjects consumed a diet and fluids according to choice.

The patients initially investigated comprised twenty-two subjects with renal disease who were thought likely to have mild to moderate impairment of renal concentrating power. Eight of them, when given vasopressin alone, produced a maximum urine concentration within the 95% confidence limits of the mean value produced by our normal subjects and were therefore excluded from further study. Details of the remaining fourteen subjects, aged 15–65 years (mean 43, sd 17; nine females), are shown in Table 1. Patients with severe renal failure were not studied as they commonly have a fixed isosthenuria, which would be unlikely to show differences with minor changes in experimental protocol. None of the patients was on protein restriction or diuretic drugs. All fourteen patients performed experiments 2 and 4, but only eight of them were able to perform experiment 1 (fluid deprivation); patients were not given urea (experiments 3 and 5) as our studies on normal subjects had convinced us that oral urea-loading is less pleasant than taking fludrocortisone and does not further increase the urine concentration achieved by vasopressin and fludrocortisone. Electrocardiograms were performed on all patients and no one suspected on clinical or electrocardiographic grounds of ischaemic heart disease was included in the study. Informed consent was obtained from every adult studied and from the parents of the one child. The research project had the approval of the Ethical Committee of University College Hospital and Medical School.

**Experimental protocols**

*Experiment 1 (fluid deprivation).* Subjects underwent a 28 h period of fluid deprivation commencing after a normal breakfast and ending around noon the following day. During the test, they abstained from all liquids including ice-cream, soups and custards. Apart from these restrictions, they ate a diet of their choice. On the evening of the first day of fluid deprivation, subjects emptied their bladders as completely as possible before going to bed, and noted the time. On the second day, three timed urine collections were made: (1) the early morning specimen passed on rising, (2) and (3) specimens passed 2 and 4 h respectively after the first. The periods of fluid deprivation corresponding to these specimens were 24+, 26+ and 28+ h respectively.

*Experiment 2 (vasopressin alone).* Subjects received 5 units of vasopressin tannate in oil intramuscularly.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Clearance (ml/min)</th>
<th>Uo.m.max</th>
<th>Uo.m.mean</th>
<th>Uo.m.max</th>
<th>Uo.m.mean</th>
<th>Uo.m.max</th>
<th>Uo.m.mean</th>
<th>Uo.m.max</th>
<th>Uo.m.mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>M</td>
<td>Thalassaemia with bilateral renal enlargement</td>
<td>8.0</td>
<td>0.09</td>
<td>88</td>
<td>620</td>
<td>622</td>
<td>608</td>
<td>604</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>20</td>
<td>M</td>
<td>Chronic pyelonephritis</td>
<td>6.7</td>
<td>0.12</td>
<td>94</td>
<td>667</td>
<td>720</td>
<td>697</td>
<td>681</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>3</td>
<td>24</td>
<td>F</td>
<td>Medullary sponge kidney</td>
<td>5.0</td>
<td>0.11</td>
<td>107</td>
<td>707</td>
<td>707</td>
<td>661</td>
<td>661</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>31</td>
<td>F</td>
<td>Chronic interstitial nephropathy of unknown cause</td>
<td>13.5</td>
<td>0.30</td>
<td>30</td>
<td>475</td>
<td>510</td>
<td>460</td>
<td>494</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>5</td>
<td>34</td>
<td>F</td>
<td>Perguative induced hypokalaemia, renal calculi</td>
<td>10.5</td>
<td>0.23</td>
<td>23</td>
<td>64</td>
<td>70</td>
<td>60</td>
<td>72</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td>42</td>
<td>F</td>
<td>Analgesic nephropathy, chronic pyelonephritis</td>
<td>8.7</td>
<td>0.11</td>
<td>71</td>
<td>475</td>
<td>453</td>
<td>366</td>
<td>420</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>F</td>
<td>Analgesic nephropathy, mild hypertension</td>
<td>6.3</td>
<td>0.15</td>
<td>95</td>
<td>908</td>
<td>849</td>
<td>745</td>
<td>721</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>8</td>
<td>43</td>
<td>M</td>
<td>Chronic bilateral ureteric obstruction</td>
<td>15.3</td>
<td>0.34</td>
<td>34</td>
<td>27</td>
<td>572</td>
<td>496</td>
<td>527</td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>48</td>
<td>F</td>
<td>Diuretic induced hypokalaemia, mild hypertension</td>
<td>8.3</td>
<td>0.14</td>
<td>14</td>
<td>80</td>
<td>873</td>
<td>719</td>
<td>789</td>
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</tr>
<tr>
<td>10</td>
<td>48</td>
<td>M</td>
<td>Bladder-neck obstruction with bilateral pyelonephritis</td>
<td>7.8</td>
<td>0.15</td>
<td>15</td>
<td>56</td>
<td>399</td>
<td>298</td>
<td>335</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>11</td>
<td>63</td>
<td>F</td>
<td>Neoplastic diabetes insipidus of unknown cause</td>
<td>4.0</td>
<td>0.12</td>
<td>12</td>
<td>110</td>
<td>108</td>
<td>85</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>64</td>
<td>F</td>
<td>Mild hypertension, primary hyperparathyroidism</td>
<td>5.3</td>
<td>0.14</td>
<td>14</td>
<td>85</td>
<td>839</td>
<td>726</td>
<td>686</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>65</td>
<td>M</td>
<td>Mild hypertension, primary hyperparathyroidism and pyelonephritis</td>
<td>7.0</td>
<td>0.11</td>
<td>11</td>
<td>84</td>
<td>604</td>
<td>533</td>
<td>678</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
at 21.00 hours on the first day of the experiment, and collected urine samples on the following day, as in experiment 1. The preparation of vasopressin used was pitressin tannate in oil (Parke, Davis) and all ampoules were drawn from two batch numbers.

**Experiment 3 (urea and vasopressin).** Subjects took 333 mmol (20 g) of urea daily [83 mmol (5 g) four times a day in 100–150 ml of water, coffee or orange juice] for 2 consecutive days. On the second day they received 5 units of vasopressin intramuscularly at 21.00 hours. Three urine collections were made on the following day as in experiment 1.

**Experiment 4 (fludrocortisone and vasopressin).** Subjects were given 3 nmol (1 mg) of fludrocortisone (9α-fluorocortisol acetate) by mouth 12 hourly, and 5 units of vasopressin intramuscularly at 21.00 hours on the first day of the test. The next day, three timed urine collections were made as in experiment 1. Fludrocortisone is a potent mineralocorticoid, with approximately the same sodium-retaining potency as aldosterone (Nelson, 1962), but unlike aldosterone is effective when taken by mouth.

**Experiment 5 (urea, fludrocortisone and vasopressin).** Subjects took 333 mmol (20 g) of urea by mouth for 2 days as for experiment 3, and fludrocortisone and vasopressin on the second day as for experiment 4. Urine collections were made on the third day as in experiment 1.

**Experiment 6 (fludrocortisone and fluid deprivation).** This experiment differed from experiment 1 only in that subjects took 3 nmol (1 mg) of fludrocortisone at 12 h intervals on the first day of fluid deprivation.

**Analytical**

All urine and plasma samples were frozen immediately after collection. Osmolality was measured by cryoscopy, and creatinine by AutoAnalyser (Technicon Instruments Co. Ltd, Basingstoke). Osmolal clearances \( C_{\text{osm}} \) and free water absorption \( T_{\text{H}_2\text{O}} \) were calculated by conventional formulae (Wesson, Anslow & Smith, 1948). The significance of results was assessed by paired \( t \)-tests (Moroney, 1953).

**Results**

Blanching of the face, and mild intestinal colic, were noted by several subjects after injection of vasopressin but there were no adverse effects attributable to fludrocortisone or urea.

**Normal subjects**

Tables 2 and 3 show the effects of the different procedures on urinary osmolality, urine flow, solute excretion and free water absorption. Renal concentrating ability is expressed both as the maximum urinary concentration observed in any specimen \( U_{\text{osm. max.}} \), and the average urinary concentration of specimens 1, 2 and 3 \( U_{\text{osm. mean}} \).

We found, as have others, that the mean urinary osmolality after vasopressin alone \( U_{\text{osm. max.}} \) 952, \( U_{\text{osm. mean}} \) 909 mosmol/kg) was significantly less than after fluid deprivation (1103 and 1035 mosmol/kg respectively). Fludrocortisone had a highly significant effect in enhancing the urine concentration achieved with vasopressin alone, and the urinary concentration after exposure to both hormones \( U_{\text{osm. max.}} \) 1053, \( U_{\text{osm. mean}} \) 1003) was not significantly different from that achieved by fluid deprivation. Urine flow during fluid deprivation (Table 3) was not significantly different from that seen in the fludrocortisone–vasopressin experiment and both values were significantly less than during stimulation with vasopressin alone. The rate of solute excretion was higher after vasopressin alone than after fluid deprivation and was intermediate with combined use of vasopressin and fludrocortisone, but the values in these three procedures were not significantly different.

Oral urea also significantly enhanced \( U_{\text{osm. max.}} \) 1004 mosmol/kg, \( U_{\text{osm. mean}} \) 938 mosmol/kg) the urine concentration produced by vasopressin, but the effect was not as marked as with fludrocortisone \( U_{\text{osm. max.}} P > 0.2, U_{\text{osm. mean}} P < 0.05 \). In the urea–vasopressin experiment both urinary volume and solute excretion were markedly and significantly increased. Urea failed to further enhance the effect of fludrocortisone on vasopressin. In fact, in the urea–fludrocortisone–vasopressin experiment both \( U_{\text{osm. max.}} \) (1011 mosmol/kg) and \( U_{\text{osm. mean}} \) (967 mosmol/kg) were slightly less than with fludrocortisone–vasopressin, though the differences were not statistically significant. Urea-loading, in both experiments 3 and 5, had the predictable effect of increasing the rate of solute excretion.

The combination of fluid deprivation and fludrocortisone (experiment 6) did not achieve urine concentrations significantly higher than those achieved by fluid deprivation alone or the combination of vasopressin and fludrocortisone.

Free water absorption (Table 3) was greatest during experiments involving vasopressin alone and
### TABLE 2. Urine concentrations in healthy subjects

N.S. = not significant.

<table>
<thead>
<tr>
<th>Expt. 1 (fluid deprivation)</th>
<th>Expt. 2 (vasopressin only)</th>
<th>Expt. 3 (urea and vasopressin)</th>
<th>Expt. 4 (fludrocortisone and vasopressin)</th>
<th>Expt. 5 (urea, fludrocortisone and vasopressin)</th>
<th>Expt. 6 (fluid deprivation and fludrocortisone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>28</td>
<td>27</td>
<td>19</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>U&lt;sub&gt;osm&lt;/sub&gt;. max. (mosmol/kg)</td>
<td>1103</td>
<td>952</td>
<td>1004</td>
<td>1053</td>
<td>1011</td>
</tr>
<tr>
<td>SD</td>
<td>91</td>
<td>106</td>
<td>75</td>
<td>97</td>
<td>87</td>
</tr>
</tbody>
</table>

Comparisons
(P values: paired t-tests)

- with Expt. 1: <0.001
- with Expt. 2: -
- with Expt. 4: N.S.

| U<sub>osm</sub>. mean (mosmol/kg) | 1035                        | 909                            | 938                                       | 1003                                          | 967                                           | 1100                                          |
| SD                        | 85                          | 98                             | 98                                       | 82                                            | 102                                           | 109                                           |

Comparisons
(P values: paired t-tests)

- with Expt. 1: <0.001
- with Expt. 2: -
- with Expt. 4: N.S.

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### TABLE 3. Urine flow, solute excretion and free water clearance (T<sub>cH<sub>2</sub>O) in healthy subjects

N.S. = not significant.

<table>
<thead>
<tr>
<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
<th>Expt. 4</th>
<th>Expt. 5</th>
<th>Expt. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean urine flow (ml/min)</td>
<td>0.47</td>
<td>0.61</td>
<td>0.72</td>
<td>0.52</td>
<td>0.67</td>
</tr>
<tr>
<td>SD</td>
<td>0.12</td>
<td>0.16</td>
<td>0.16</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>n</td>
<td>28</td>
<td>26</td>
<td>19</td>
<td>24</td>
<td>16</td>
</tr>
</tbody>
</table>

Comparisons (P values)

- with Expt. 1: -
- with Expt. 2: -
- with Expt. 4: N.S.

| Mean solute excretion (mosmol/min) | 0.48    | 0.55    | 0.67    | 0.51    | 0.62    | 0.39    |
| SD      | 0.13    | 0.13    | 0.14    | 0.13    | 0.15    | 0.08    |
| n       | 28      | 26      | 19      | 24      | 16      | 8       |

Comparisons (P values)

- with Expt. 1: -
- with Expt. 2: N.S.
- with Expt. 4: N.S.

| Mean T<sub>c</sub>H<sub>2</sub>O (ml/min) | 1.03    | 1.80    | 1.59    | 1.19    | 1.53    | 1.00    |
| SD      | 0.35    | 0.60    | 0.46    | 0.34    | 0.69    | 0.30    |
| n       | 10      | 15      | 9       | 12      | 9       | 8       |

Comparisons (P values)

- with Expt. 1: -
- with Expt. 2: <0.005
- with Expt. 4: N.S.
Patients (Tables 1 and 4)

Subjects with renal disease and impaired concentrating ability showed a similar pattern of response to those with normal renal function. Six patients were unable to go without fluids for the time requested for experiment 1, some of these being patients who were shown in the other experiments to have the most marked deficits in urinary concentration (e.g. subjects 5, 6 and 11). The patients who were able to perform experiment 1 tended to be those with the greater powers of urinary concentration, and this partly explains their more efficient response to that of all fourteen patients performing experiments 2 and 4, which is shown in Table 4. A more direct comparison is provided by the response of the eight patients who performed all three tests; their values of mean $U_{\text{osm}}$ max. were 680, 641 and 658 mosmol/kg in experiments 1, 2 and 4 respectively, the corresponding values of $U_{\text{osm}}$ mean being 641, 537 and 632 mosmol/kg. The paired $t$-test, which is not invalidated by different numbers in the groups being compared, showed no significant difference in the response to the three experiments except that the average $U_{\text{osm}}$ max. and $U_{\text{osm}}$ mean values were both significantly greater in experiment 4 than experiment 2.

The mean urine flows were $0.69 \pm 0.20$, $0.81 \pm 0.22$ and $0.76 \pm 0.22$ ml/min in experiments 1, 2 and 4 respectively, but paired $t$-tests showed no significant differences between these values. Mean rates of solute excretion were $0.48 \pm 0.12$, $0.45 \pm 0.15$ and $0.45 \pm 0.16$ mosmol/min in experiments 1, 2 and 4 respectively, and again these differences were not significant. However, these differences were in the direction to be expected from our results on normal subjects (Table 3).

Discussion

The discrepancy between urine concentrations obtained during hydropenia and after administration of vasopressin to normally or overhydrated healthy subjects suggests that the former stimulus has some other mode of action in addition to release of endogenous antidiuretic hormone. Jones & de Wardener (1956) found that this discrepancy could not be explained by differences in solute output, urine flow, or any intrinsic property of the injected material which might prevent the kidney from concentrating urine maximally. The available evidence suggests that the discrepancy is related to differences in extracellular fluid volume in the two situations. Because changes in extracellular volume caused reciprocal changes in release of endogenous aldosterone, the possibility emerges that this hormone...
might contribute to the elaboration of the more concentrated urine passed during sustained water deprivation.

Crabbé (1962) examined the theoretical basis by which aldosterone might be thought to play such a role in the urine-concentrating mechanism and has tested this hypothesis. He found no statistically significant difference between urine concentrations obtained by the combined use of aldosterone and vasopressin and that achieved after 48 h of water deprivation. Furthermore, the aldosterone antagonist spironolactone increased both urinary flow and solute output during water deprivation, and significantly decreased urine osmolality. Of the possible mechanisms by which aldosterone might enhance the urine-concentrating power of vasopressin, the most likely one would seem to be that it increases sequestration of sodium chloride in the renal medulla by promoting active reabsorption in the ascending limb of the loop of Henle.

In our own experiments we used 9α-fluorocortisol (fludrocortisone), a synthetic steroid which has a mineralocorticoid potency similar to that of aldosterone and the advantage that it is effective by mouth. The dosage used of both vasopressin and fludrocortisone is supramaximal in effect and several times greater than the postulated levels of equivalent endogenous hormone likely to be stimulated by a water deprivation. By use of vasopressin and fludrocortisone we have been able to reproduce the results that Crabbé (1962) produced with vasopressin and aldosterone.

In clinical practice assessment of renal concentrating power is usually made by measuring urine concentration after either prolonged fluid deprivation, or administration of vasopressin alone. The former procedure is unpleasant for many patients, and may indeed be dangerous when renal concentrating ability is markedly impaired. It is relevant that six of our fourteen patients with reduced concentrating power were unable or unwilling to complete the recommended period without fluids, presumably because they realized it would cause intolerable thirst. A further drawback is that some patients invalidate the procedure by drinking during the period of supposed fluid withdrawal, either deliberately or because of forgetfulness. Fluid deprivation has also the theoretical disadvantage that the longer the duration of the test, the stronger will the stimulus to urine concentration become.

The degree of water deficit developed during withdrawal of fluids depends on many uncontrolled factors, including insensible water loss, the rate of urinary solute excretion, and the extent of the underlying concentration defect. A survey of the literature reveals that authors are far from unanimous regarding the duration of the period of fluid abstinence they recommend, or the values of urine concentration which they regard as normal. Thus the length of fluid deprivation suggested has varied from 12-13 h (Kaitz, 1961; Jacobson, Levy, Kaufman, Gallinek & Donnelly, 1962) to 36-38 h (Lashmet & Newburgh, 1932; Jones & de Wardener, 1956) with many intermediate recommendations (Addis & Shevsky, 1922; McCance, 1945; Keitel, Thompson & Itano, 1956; Frank, Dreifus, Rarick & Bellet, 1957; Lindeman, Van Buren & Raisz, 1960; Isaacson, 1960; Hulet & Smith, 1961; Katz, Massry, Agmon & Toor, 1965). Similarly the mean urinary osmolality recorded during hypopenia has been as low as 773 mosmol/kg (Kaitz, 1961) and as high as 1220 mosmol/kg (McCance, 1945).

The use of vasopressin, although it overcomes some of the disadvantages, introduces other problems. As already shown, when used alone, it does not lead to the production of a maximally concentrated urine. The urine concentration achieved is known to be influenced by the state of hydration of the subject during the study (Sodeman & Engelhardt, 1942; Little et al., 1947), perhaps because the state of dehydration influences endogenous mineralocorticoid release.

The combination of fludrocortisone with vasopressin appears to be an improvement on these earlier methods of testing renal concentrating power. In this study, it produced in normal subjects urine concentrations which were not significantly different from those during 28 h of fluid deprivation. Because of the difficulty we had in persuading patients with impaired concentrating ability to go without fluids, we were able to compare the results of the tests with those of fluid deprivation in only eight of our fourteen patients, but this small group of subjects also showed no significant difference in response. One advantage of the test may lie in the fact that solute excretion is reduced (Crabbé, 1962), presumably because of the sodium-retaining effect of a mineralocorticoid, and approximates to that seen during water deprivation. This was seen in our own studies (Tables 3 and 4), though the mean rates of solute excretion during experiments 1, 2 and 4 were not significantly different. No adverse effects of fludro-
cortisone were observed either in healthy subjects or in patients with renal disease.

It could be argued that the use of vasopressin alone provides as good an assessment of urinary concentrating power as that provided by fluid deprivation. De Wardener (1956) found that the differences between the urine concentration achieved by fluid deprivation and vasopressin alone was less marked in those with renal disease and impaired concentrating ability, than in normal subjects, and suggested that the use of vasopressin was adequate for clinical purposes. He gave no clinical details of his subjects with renal disease, but it is possible that some of them had either severe hypertension or renal failure, both of which may cause secondary hyperaldosteronism (Laragh, Ulick, Januszewicz, Deming, Kelly & Lieberman, 1960; Bayard, Cooke, Tiller, Beitins, Kowarski, Walker & Migeon, 1971) and so might reduce the difference in urine osmolality observed between the two methods of assessment. Our own subjects did not include any with these complications yet even here the addition of fludrocortisone to vasopressin had less effect than in normal subjects, perhaps because some of these patients had diseases damaging the renal tubules, which might cause refractoriness to the tubular action of the mineralocorticoid. But although the addition of fludrocortisone to vasopressin had less effect than in normal subjects, the effect was significant. From these studies, and a priori, we suspect that an increase in the accepted normal value for maximum urine concentration, as a result of the use of combined vasopressin and fludrocortisone, will lead to the discovery of mild abnormalities of urine concentration which would not be revealed by the use of vasopressin alone.

Urea supplementation has been shown to increase urine concentrating power both in man (Epstein et al., 1957) and the rat (Crawford, Doyle & Probst, 1959; Bray & Preston, 1961) and had a striking effect in our studies on healthy subjects, significantly enhancing the urinary concentration achieved by vasopressin, although the resultant urine was significantly less concentrated than that passed during fluid deprivation (Table 2). However, urea supplementation did not enhance urine concentration achieved by a combination of fludrocortisone and vasopressin; in fact, there was a slight (but not statistically significant) reduction in $U_{\text{sm max}}$ and $U_{\text{sm mean}}$. Probably the effect of urea depends partly on the basal excretion of urea, which was not controlled in our studies; quite possibly urea would have enhanced the urine-concentrating power of the vasopressin–fludrocortisone combination in subjects with lower basal rates of urea excretion. Urea supplementation is certainly less pleasant for subjects than fludrocortisone, and because of its failure to increase urine concentration in these studies beyond that achieved by vasopressin–fludrocortisone, and the remote possibility that it might cause harm to a patient with a raised plasma urea, we felt it unjustifiable to study its effect in patients with renal disease.

From the results of this study we recommend the combined use of vasopressin and fludrocortisone as providing the best clinical assessment of renal concentrating power. We have collected urine from the time of rising to 12.00 hours on the day after administration of the two hormones. During this period, in subjects with a marked circadian rhythm of sodium excretion the solute output may be on a rising curve, despite mineralocorticoid excess, and it therefore seems logical to disregard $U_{\text{sm max}}$, mean (the average urine concentration for three specimens) in favour of $U_{\text{sm max}}$, max. (the maximal osmolality of all three specimens) as an index of renal performance. The $U_{\text{sm max}}$, max. of our normal subjects was 1053 ±97 mosmol/kg and we would regard any value below 863 mosmol/kg (i.e. a figure below the 95% confidence limits of the normal mean) as indicating definite impairment of concentrating power.

There are two important contraindications to the use of vasopressin. Continued drinking despite water retention might cause water intoxication. In most adults there is no such risk, as fluid intake is closely related to need, and even mild water excess inhibits thirst. However, infants can become water-intoxicated when given vasopressin, because their feeds are fluid and hunger will lead to continued fluid intake even in the presence of water excess. Water intoxication may also occur in subjects with compulsive polydipsia (Barlow & De Wardener, 1959) where a psychic abnormality, rather than thirst, is the stimulus to drinking.

Subjects with ischaemic heart disease are also at risk from vasopressin. Physiological levels of vasopressin do not cause contraction of smooth muscle in normal subjects, but the rate of release of exogenous hormone from the depot preparation used in these studies is inconstant, and to be sure of a persistent circulating level capable of causing a maximum antidiuretic effect it is necessary to use the
dosage employed here (unpublished studies), which in some persons does cause a mild intestinal colic and blanching of the skin of the face. The effect of exogenous hormone on the coronary circulation and blanching of the skin of the face. The effect may be dangerous in those with coronary artery angina, and even sudden death have been reported after the use of aqueous vasopressin in patients with previously diagnosed myocardial ischaemia (Ruskin, 1947; Mills, Burchell, Parker & Kirklin, 1949). The more slowly liberated suspension of vasopressin tannate in oil used here (unpublished studies), which in some persons does cause a mild intestinal colic more slowly liberated suspension of vasopressin tannate in oil used here must inevitably be less likely to cause such complications, but even so we consider it inadvisable to use the preparation in patients with known ischaemic heart disease. Perhaps this problem will be overcome by the use of 1-desamino-8-D-arginine vasopressin (Vávra, Machova, Holeček, Cort, Zaoral & Šorm, 1968), as this form of anti-diuretic hormone has much less pressor effect than vasopressin, despite a similar antidiuretic potency; unfortunately no careful comparison has yet been made between the effect of this substance and vasopressin on maximum urine concentration. However, considerations of the possible cardiac effect of vasopressin should not seriously impair the usefulness of a vasopressin-fludrocortisone combination in assessing renal concentrating power, as its effect of vasopressin should not seriously impair the usefulness of a vasopressin–fludrocortisone combination in assessing renal concentrating power, as its main role is likely to be the diagnosis of tubular abnormalities in young patients with congenital or hereditary disease.

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