Relationship between Renal Prostaglandin E and Renal Sodium Handling during Water Immersion in Normal Man

MURRAY EPSTEIN, MEYER D. LIFSCHITZ, DAVID S. HOFFMAN, AND JAY H. STEIN

SUMMARY Previous studies from this laboratory have demonstrated that the central hypervolemia induced by water immersion to the neck (NI) constitutes a suitable model for assessing the hormonal response to volume expansion without concomitant alterations in plasma composition. The NI model was used to assess in a kinetic fashion the relationship between renal prostaglandin E (PGE) and renal sodium handling. Nine normal subjects were studied twice in the sodium-replete state during NI: with indomethacin (Ind) pretreatment (50 mg q6h x 5)(NI + Ind) and without indomethacin (NI). Urinary sodium, potassium, and PGE excretion (UPGEV) were measured hourly. NI was associated with marked increases in U\text{Na}\text{V} from 67 ± 20 (8h) to 219 ± 25 μEq/min (P < 0.05) and UPGEV from 6.4 ± 1.4 to 12.9 ± 2.5 ng/min (P < 0.05). Although indomethacin administration lowered the basal rate of UPGEV prior to immersion, it neither prevented the subsequent augmentation of UPGEV during NI + Ind nor affected the magnitude of the natriuresis during NI + Ind. Subsequently, six of the subjects were restudied following dietary sodium restriction (10 mEq/day). The changes in UPGEV during NI and NI + Ind were qualitatively similar to those observed in the sodium-replete state. In contrast to the sodium-replete studies, however, the natriuresis of immersion was attenuated markedly by indomethacin pretreatment. In summary, the data demonstrate that immersion-induced central volume expansion is associated with a striking increase in renal PGE excretion which is attenuated but not prevented by indomethacin. In addition, indomethacin administration attenuates markedly the natriuretic response of immersion in sodium-depleted, but not in sodium-replete, normal subjects. These observations are consistent with the suggestion that renal PGE may constitute a determinant of the renal response to volume expansion in sodium-depleted man.

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DESpite extensive study, the relationship of the renal synthesis of prostaglandins to renal sodium excretion remains controversial (Dunn and Hood, 1977). Although it has been proposed that alterations in prostaglandin release may constitute a determinant of the natriuretic release to extracellular fluid volume expansion (ECVE) (Gross and Bartter, 1973; Verberckmoes et al., 1976; Donker et al., 1976; Patak et al., 1975; Berg, 1977; Dusing et al., 1976), the effect of volume expansion on endogenous prostaglandin release and the contribution of endogenous renal prostaglandins to the natriuresis of volume expansion remain unsettled (Kirschenbaum and Stein, 1977; Mountokalakis et al., 1978; Bohan and Wesson, 1976). The contradictory nature of the available data may relate in part to species differences and to attempts to extrapolate from studies in anesthetized or acutely operated animals to man.

The present studies were carried out to examine in a kinetic fashion the effect(s) of a "volume stimulus" on renal prostaglandin synthesis and the relationship of renal prostaglandins to the resultant natriuretic response. Since previous studies from our laboratory have demonstrated that water immersion to the neck (NI) constitutes a potent "central volume stimulus" without the necessity of infusing exogenous volume expanders, and thus without altering plasma composition (Epstein, 1976; Epstein, 1978; Begin et al., 1976; Epstein et al., 1976), the immersion model was used to assess the role of renal prostaglandins in mediating the natriuretic response to ECVE. In an effort to evaluate the relative contribution of renal prostaglandins to the natriuresis of immersion, immersion studies were performed before and after partial inhibition of prostaglandin synthesis by indomethacin (Ind).

Methods

Nine male subjects between the ages of 23 and 50 years with a mean of 28 years were studied. None had a history of hypertension, cardiovascular disease, or diabetes. Significant renal disease was excluded in all subjects by documenting a normal urine sediment and creatinine clearance. Nine subjects underwent water immersion to the neck on
two occasions separated by a 5 to 7-day interval. Prior to the second immersion study, the subjects were pretreated with indomethacin 50 mg per os every 6 hours for the 24-hour period preceding study (NI + Ind). Subsequently, six of the subjects underwent additional immersion studies following dietary sodium restriction. During the latter studies, the subjects were housed in an environmentally controlled metabolic ward at a constant temperature. Each consumed a diet, the composition of which remained unchanged throughout the study, containing 10 mEq of sodium, 100 mEq of potassium, and 2500 ml of water per day. Daily 24-hour urine collections were made for the determination of sodium, potassium, and creatinine. After dietary equilibration, each subject underwent an immersion study without indomethacin administration (NI), followed 5–7 days later by a repeat immersion study preceded by 24 hours of indomethacin administration (NI + Ind).

The experimental protocols on the 2 immersion study days were similar and were carried out as follows: after 11 hours of overnight water restriction, the subject was awakened at 7:00 a.m., voided, and sat quietly for 1 hour. At 7:15 a.m., an oral water load of 400 ml was administered, and at 8 a.m. the subject voided again and assumed the seated position for 6 hours (8:00 a.m. to 2 p.m.). The subject sat in the tank immersed in water to the neck for 4 hours (9:00 a.m. to 1:00 p.m.), preceded and followed by 1 hour of quiet sitting outside the tank (prestudy and recovery hours, respectively). Each subject stood briefly to void spontaneously at hourly intervals during the study. To maintain an adequate urine flow, 200 ml of water was administered orally every hour during the study. Sodium, potassium, creatinine, and osmolality were measured in samples of the hourly urine collections, and samples of each hourly urine specimen were frozen promptly for prostaglandin E determinations. In addition, urine pH was measured during the sodium-depleted studies in five of the subjects.

Immersion was carried out in a waterproof tank described in detail in previous communications (Epstein et al., 1973). A constant water temperature of 34.5 ± 0.5°C was maintained by two heat exchangers, controlled by an adjustable temperature-calibrated control meter with input derived from two thermistors immersed at different water levels.

Urine PGE was assayed using extraction and assay procedures described previously (Rosenblatt et al., 1978) similar to those of Smigel et al. (1974). A 1- or 2-ml sample of urine was adjusted to pH 6.8 with 0.1 M formic acid. Neutral lipids were extracted from the urine with benzene-butyl chloride (1:1). The pH then was adjusted to 3.5 with formic acid and the fatty acids (including PGE) were extracted with 20 ml CHCl₃. The CHCl₃ was then flash evaporated and the residue applied to a Sephadex LH 20 column measuring 10 × 130 mm. The solvent system used was heptane-chloroform-ethanol-acetic acid (100:100:30:2, by volume). The samples were applied to the column. The appropriate fractions were collected from the column and, after concentration, were entered into the assay procedure. Recovery of labeled prostaglandin E (PGE) was consistently greater than 75% after these procedures. The percent recovery of labeled PGE was determined for each sample and the final result was corrected by this recovery factor.

Previously prepared rat liver membranes which contain a class of receptors which bind specifically to PGE were used in this assay. Male Sprague-Dawley rats, of the Holtzman strain, weighing between 160 and 200 g, were the source of the liver membranes. Each batch of membranes was tested for sensitivity and specificity, and the optimal conditions for conducting the assay were determined. Known and unknown amounts of PGE were added to separate tubes that contained a constant amount of ³H-PGE, and this mixture was incubated with a constant amount of rat liver membranes at 37°C for 60 minutes. Bound and free ³H-PGE were separated by centrifugation and a sample of the free (supernatant), and the bound (pellet) fractions were counted in a beta scintillation counter (Beckman). The standard curve for all batches of membranes used includes a range from 62 to 2000 pg, and the bound-over-free ratio ranges from 0.9 to 0.1.

Studies in this laboratory have confirmed previous findings concerning the specificity of this class of receptors. PGE cannot be differentiated from PGE₂ as there is almost identical binding to these two compounds, but prostaglandins of the B and F series including 6-keto-PGF₁₀ demonstrate no measurable binding, and PGA₁ binds with approximately one-sixteenth the affinity of PGE₁. Recovery of cold PGE₁ was determined over a range of 200 to 10,000 pg, and in nine separate samples determined on different days, the measured values averaged 99 ± 9% of the predicted values. All samples from a given patient were analyzed at the same time. All samples were run in duplicate and at two different dilutions. Since these receptors are relatively indifferent to PGE₁ and PGE₂, although the major urinary prostaglandins are in the PGE₂ series, the present results are expressed as PGE.

The radioreceptor assay was validated by analyzing urine samples by a gas chromatography-mass spectrometry system and by radioreceptor assay. There was good agreement between the two assays (r = 0.99).

Analytic methods for sodium, potassium, and creatinine determinations have been reported previously (Epstein et al., 1973). Urine and serum were analyzed for total solutes with a Fiske osmometer. Urine pH was measured within 3 minutes of collection on a Radiometer acid-base cart ABC-1. In the presentation of the data, mean values are followed by the standard error of the mean as an index of...
TABLE 1 Urinary Excretory Patterns during Immersion in Sodium-Replete Subjects

<table>
<thead>
<tr>
<th></th>
<th>Time (hours)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prestudy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>NI</td>
<td>3.0 ± 0.8</td>
<td>5.5 ± 0.9</td>
<td>7.8 ± 0.7</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>NI + Ind</td>
<td>2.1 ± 1.1</td>
<td>4.3 ± 0.9</td>
<td>6.4 ± 0.6</td>
<td>6.0 ± 0.7</td>
</tr>
<tr>
<td>UNSV (µEq/min)</td>
<td>NI</td>
<td>87 ± 20</td>
<td>111 ± 22</td>
<td>147 ± 31</td>
<td>179 ± 34</td>
</tr>
<tr>
<td></td>
<td>NI + Ind</td>
<td>73 ± 21</td>
<td>113 ± 30</td>
<td>136 ± 26</td>
<td>161 ± 29</td>
</tr>
<tr>
<td>UBV (µEq/min)</td>
<td>NI</td>
<td>40 ± 8</td>
<td>59 ± 9</td>
<td>75 ± 17</td>
<td>70 ± 12</td>
</tr>
<tr>
<td></td>
<td>NI + Ind</td>
<td>57 ± 10</td>
<td>79 ± 14</td>
<td>80 ± 12</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>Cc (ml/min)</td>
<td>NI</td>
<td>133 ± 9</td>
<td>141 ± 7</td>
<td>132 ± 7</td>
<td>128 ± 8</td>
</tr>
<tr>
<td></td>
<td>NI + Ind</td>
<td>134 ± 9</td>
<td>134 ± 6</td>
<td>131 ± 6</td>
<td>127 ± 7</td>
</tr>
<tr>
<td>CNa/Cc × 100</td>
<td>NI</td>
<td>0.44 ± 0.08</td>
<td>0.57 ± 0.11</td>
<td>0.82 ± 0.19</td>
<td>1.00 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>NI + Ind</td>
<td>0.41 ± 0.13</td>
<td>0.62 ± 0.18</td>
<td>0.78 ± 0.17</td>
<td>0.89 ± 0.14</td>
</tr>
<tr>
<td>CNaO (ml/min)</td>
<td>NI</td>
<td>0.5 ± 0.9</td>
<td>2.4 ± 0.8</td>
<td>4.6 ± 0.8</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>NI + Ind</td>
<td>0.0 ± 1.0</td>
<td>1.6 ± 0.8</td>
<td>3.5 ± 0.6</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>Uosm (mOsm/kg H2O)</td>
<td>NI</td>
<td>436 ± 122</td>
<td>184 ± 42</td>
<td>107 ± 11</td>
<td>160 ± 24</td>
</tr>
<tr>
<td></td>
<td>NI + Ind</td>
<td>622 ± 116</td>
<td>291 ± 82</td>
<td>146 ± 25</td>
<td>149 ± 17</td>
</tr>
</tbody>
</table>

Results are mean ± SE of nine subjects during sodium-replete studies. 
* P < 0.05 for NI + Ind compared with NI.
† P < 0.005 for NI + Ind compared with NI.

Table 1: Immersion without indomethacin pretreatment (NI) resulted in a progressive increase in UNSV to levels which were almost 3-fold greater than the prestudy values during the 4th hour of immersion (P < 0.001). Cessation of immersion (recovery) was associated with a prompt decrement in UNSV (P < 0.001 for recovery compared to hour 4 of NI), although it continued to exceed prestudy levels (P < 0.005). When the subjects underwent a repeat immersion following indomethacin pretreatment (NI + Ind), the natriuretic pattern did not differ from that of NI, aside from a lessened mean UNSV level during hour 4 of immersion and the recovery hour. As shown in Table 2, the cumulative sodium excreted during the 4 hours of NI + Ind did not differ from NI alone (P > 0.2).

Results

Urinary Sodium and Potassium

Sodium-Replete Studies

The effects of 4 hours of water immersion on urinary sodium and potassium excretion are shown in Table 1. Immersion without indomethacin pretreatment (NI) resulted in a progressive increase in UNSV to levels which were almost 3-fold greater than the prestudy values during the 4th hour of immersion (P < 0.001). Cessation of immersion (recovery) was associated with a prompt decrement in UNSV (P < 0.001 for recovery compared to hour 4 of NI), although it continued to exceed prestudy levels (P < 0.005). When the subjects underwent a repeat immersion following indomethacin pretreatment (NI + Ind), the natriuretic pattern did not differ from that of NI, aside from a lessened mean UNSV level during hour 4 of immersion and the recovery hour. As shown in Table 2, the cumulative sodium excreted during the 4 hours of NI + Ind did not differ from NI alone (P > 0.2).

Fractional excretion of sodium (CNa/Cc × 100) increased progressively during NI from 0.44 ± 0.08% in the prestudy period to 1.21 ± 0.17 during hour 4.
of immersion ($P < 0.001$) (Table 1). Recovery was associated with a decrement in $C_{Na}/C_{Cr} \times 100$, although the levels continued to exceed prestudy levels ($P < 0.01$). The pattern of increase in $C_{Na}/C_{Cr} \times 100$ observed during NI + Ind was similar to $U_{Na} V$, with the levels observed during NI exceeding NI + Ind during hour 4 ($P < 0.025$) and the recovery hour ($P < 0.001$).

The rate of potassium excretion ($U_{K} V$) increased during NI from 40 ± 7 to levels of 82-106 μEq/min during hours 1-4 of immersion (P < 0.05). A comparable increase in $U_{K} V$ was observed during NI + Ind. Cumulative potassium excretion during NI did not differ from the values observed during NI + Ind ($P > 0.2$) (Table 2).

**Studies during Dietary Sodium Restriction**

The effects of 4 hours of NI on urinary sodium and potassium excretion are shown in Table 3. NI resulted in a progressive increase in $U_{Na} V$ from 2 ± 1 to 37 ± 9 μEq/min ($P < 0.01$). Recovery was associated with a decrement in $U_{Na} V$ to 8 ± 3 μEq/min ($P < 0.005$ for recovery compared to hour 4). When the same six subjects were restudied during NI + Ind, the natriuretic response was markedly attenuated, with peak $U_{Na} V$ of 5 ± 2 μEq/min during hour 4 of immersion comprising only one-eighth of peak $U_{Na} V$ during NI. Examination of the individual natriuretic responses of the subjects during NI + Ind disclosed that none of the subjects attained peak $U_{Na} V$'s exceeding 10 μEq/min, with four of the subjects manifesting peak $U_{Na} V$'s of <6 μEq/min. Cumulative sodium excretion during the 4 hours of NI + Ind was significantly less than the amount excreted during NI ($P < 0.05$) (Table 2), mirroring the changes of $U_{Na} V$.

$C_{Na}/C_{Cr} \times 100$ increased during NI from 0.01 ± 0.00 to 0.19 ± 0.04 during hour 4 of NI ($P < 0.005$). In contrast, NI + Ind was associated with a markedly attenuated increase in $C_{Na}/C_{Cr} \times 100$ during all 4 hours of immersion compared to NI.

$U_{K} V$ increased during NI from 40 ± 7 to levels of 82-106 μEq/min during hours 1-4 of immersion. Cessation of immersion was associated with prompt return of $U_{K} V$ to prestudy levels ($P < 0.001$ for hour 4 vs. recovery). Despite an identical sodium and potassium balance, indomethacin pretreatment resulted in a decrease in basal $U_{K} V$ during the prestudy hour. During the subsequent 4 hours of immersion, $U_{K} V$ during NI + Ind was less than the kaliuresis observed during NI ($P < 0.05$ compared to NI). Similarly, the cumulative potassium excreted during the 4 hours of NI + Ind was significantly less than the sum excreted during NI alone ($P < 0.05$) (Table 2).

**Urinary Volume, $C_{H_{2}O}$ and $C_{Cr}$**

**Sodium-Replete Studies**

NI was associated with a marked diuresis throughout immersion with peak urine flow (V) occurring during the 2nd hour of immersion ($P < 0.001$ compared to prestudy). The diuretic response during NI + Ind was unaltered compared to NI. An increase in free water clearance ($C_{H_{2}O}$) was observed during hour 2 of NI ($P < 0.005$ compared to prestudy), coinciding with peak V. A similar increase in $C_{H_{2}O}$ occurred during NI + Ind ($P < 0.05$ compared to prestudy), which was not different from that observed during NI.

Mean total urinary solute concentration ($U_{OSM}$) during the prestudy hour of NI + Ind tended to

### Table 3: Urinary Excretory Patterns during Immersion in Sodium-Depleted Subjects

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Prestudy</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$ (ml/min) NI</td>
<td>2.2 ± 0.4</td>
<td>5.6 ± 0.3</td>
<td>5.9 ± 0.6</td>
<td>5.2 ± 0.6</td>
<td>5.5 ± 0.3</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>(ml/min) NI + Ind</td>
<td>1.2 ± 0.5*</td>
<td>3.6 ± 0.6*</td>
<td>5.1 ± 0.8</td>
<td>4.8 ± 0.7</td>
<td>5.1 ± 0.7</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>$U_{Na} V$ (μEq/min) NI</td>
<td>2 ± 1</td>
<td>10 ± 3</td>
<td>17 ± 5</td>
<td>25 ± 7</td>
<td>37 ± 9</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>(μEq/min) NI + Ind</td>
<td>0 ± 0</td>
<td>1 ± 0*</td>
<td>2 ± 1*</td>
<td>3 ± 1*</td>
<td>5 ± 2*</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>$U_{K} V$ (μEq/min) NI</td>
<td>40 ± 7</td>
<td>82 ± 12</td>
<td>106 ± 10</td>
<td>106 ± 7</td>
<td>103 ± 9</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>(μEq/min) NI + Ind</td>
<td>23 ± 2*</td>
<td>48 ± 6*</td>
<td>74 ± 12*</td>
<td>73 ± 14*</td>
<td>75 ± 7</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>$C_{Na}$ (mOsm/kg H2O) NI</td>
<td>132 ± 6</td>
<td>144 ± 8</td>
<td>135 ± 5</td>
<td>130 ± 7</td>
<td>138 ± 3</td>
<td>113 ± 5</td>
</tr>
<tr>
<td>(mOsm/kg H2O) NI + Ind</td>
<td>116 ± 7</td>
<td>138 ± 11</td>
<td>128 ± 5</td>
<td>126 ± 6</td>
<td>127 ± 8</td>
<td>103 ± 8</td>
</tr>
<tr>
<td>$C_{K}$ (mOsm/kg H2O) NI</td>
<td>0.7 ± 0.4</td>
<td>3.6 ± 0.5</td>
<td>3.8 ± 0.6</td>
<td>3.1 ± 0.6</td>
<td>3.5 ± 0.5</td>
<td>0.01 ± 0.1</td>
</tr>
<tr>
<td>(mOsm/kg H2O) NI + Ind</td>
<td>0.1 ± 0.4</td>
<td>2.0 ± 0.7†</td>
<td>3.4 ± 0.9</td>
<td>2.9 ± 0.5</td>
<td>3.2 ± 0.4</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>$C_{Na}/C_{Cr} \times 100$ NI</td>
<td>0.01 ± 0.00</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>0.19 ± 0.04</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>× 100</td>
<td>0.00 ± 0.00*</td>
<td>0.01 ± 0.00*</td>
<td>0.01 ± 0.00*</td>
<td>0.02 ± 0.01*</td>
<td>0.03 ± 0.01*</td>
<td>0.01 ± 0.00*</td>
</tr>
<tr>
<td>$U_{OSM}$ (mOsm/kg H2O) NI</td>
<td>219 ± 35</td>
<td>96 ± 17</td>
<td>102 ± 12</td>
<td>112 ± 15</td>
<td>106 ± 19</td>
<td>287 ± 28</td>
</tr>
<tr>
<td>(mOsm/kg H2O) NI + Ind</td>
<td>472 ± 128</td>
<td>161 ± 40</td>
<td>110 ± 27</td>
<td>112 ± 17</td>
<td>102 ± 17</td>
<td>207 ± 22</td>
</tr>
</tbody>
</table>

Results are mean ± SE of six subjects.  
* $P < 0.05$ for NI + Ind compared with NI.  
† $P < 0.005$ for NI + Ind compared with NI.
exceed that during the corresponding NI study, although the difference did not attain statistical significance. Subsequently, mean U_{OSM} declined during both NI and NI + Ind to similar levels.

Creatinine clearance (C_{Ccr}) was relatively constant throughout the initial 5 hours of NI. Recovery was associated with a decrease in mean C_{Ccr} of 17 ml/min (P < 0.05 for recovery compared to hour 4). Mean C_{Ccr} during NI + Ind was unaltered compared to NI.

Studies during Dietary Sodium Restriction

NI was associated with a marked diuresis with V doubling during hours 1-4 of immersion compared to the prestudy hour. Despite identical hydration protocols, NI + Ind was associated with a lower mean V during both the prestudy hour and hour 1 of immersion as compared to NI (P < 0.05). V was similar during the subsequent 4 hours of study.

The increased V of NI was associated with a concomitant increase in C_{H,O}, attaining maximal levels of 3.1-3.8 ml/min during hours 1-4 of immersion. Likewise, the pattern of C_{H,O} increase during NI + Ind paralleled the response of V, with a lower mean C_{H,O} during the initial hour of immersion compared to NI. Subsequently, mean C_{H,O} values during hours 2-4 of NI + Ind did not differ from those observed during NI.

Mean U_{OSM} during the prestudy hour of NI + Ind tended to exceed that during the corresponding NI study, although the difference did not attain statistical significance. A period of 4 hours of immersion was associated with a significant decline in mean U_{OSM} during both NI and NI + Ind to similar levels of (98-112 mOsm/kg H_{2}O).

Mean C_{Ccr} remained relatively constant during NI. Indomethacin pretreatment did not alter mean C_{Ccr} during NI + Ind, as compared with NI (Table 3).

Changes in Urinary pH

In view of the possibility that alterations in urinary pH may have contributed to the observed changes in U_{PGE} during the present studies, urine pH was determined hourly in five of the six subjects studied during dietary sodium restriction during both NI and NI + Ind. As shown in Table 4, NI was associated with an increase in urine pH throughout the 4 hours of immersion. A similar increase in urinary pH occurred during NI + Ind which did not differ from that observed during NI.

An examination of the relationship between urinary pH and urinary PGE concentration failed to disclose a correlation (r = 0.03; P > 0.50). Indeed, an examination of this relationship in the seven subjects undergoing immersion without indomethacin pretreatment (NI) disclosed a negative correlation of urinary pH with urinary PGE concentration (r = -0.30; P > 0.10).

Urinary Prostaglandin Excretion

Sodium-Replete Studies

Urinary PGE determinations were carried out in seven of the nine subjects. As shown in Figure 1, NI was associated with a progressive increase in PGE excretion from 6.4 ± 1.4 ng/min to peak levels of 12.9 ± 2.5 and 11.3 ± 2.7 ng/min during hours 2 and 3, respectively. Recovery was associated with a prompt decrement in U_{PGE} from 9.2 ± 1.8 to 4.2 ± 0.7 ng/min (P < 0.01). Indomethacin pretreatment resulted in a 56% decrease in basal U_{PGE} during the prestudy hour. Despite the indomethacin-induced suppression of basal U_{PGE}, NI + Ind was still associated with a progressive increment in U_{PGE} which mirrored the pattern of U_{PGE} enhancement observed during the NI studies. Peak U_{PGE} levels were observed during hour 2 of NI + Ind, in analogy with the pattern observed during NI, although the absolute PGE excretory rates observed during NI + Ind were significantly less than those observed during NI during 3 of the 4 hours of immersion. Similarly, U_{PGE} decreased promptly and markedly during the recovery hour of NI + Ind from 5.7 ± 1.3 to 1.7 ± 0.4 ng/min (P < 0.025), as in the NI studies.

In addition to examining in a kinetic fashion the pattern of U_{PGE} during immersion, we compared the cumulative quantities of PGE excretion over the 4-hour immersion period of NI and NI + Ind. Mean PGE excretion during NI was 2547 ± 463 ng/4 hours, exceeding the 1455 ± 254 ng/4 hours excreted by the same subjects during NI + Ind (P < 0.025).

Studies during Dietary Sodium Restriction

The changes in U_{PGE} observed in the six subjects who underwent immersion studies following dietary sodium restriction are depicted in Figure 2. NI was associated with a progressive increase in U_{PGE} and attained peak levels of 9.3 ± 2.5 and 9.8 ± 4.3 ng/min during hours 3 and 4, respectively.

Indomethacin pretreatment resulted in a 74% decrement in basal U_{PGE} during the prestudy hour

### Table 4: Effects of Immersion on Urine pH in Sodium-Depleted Subjects

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH NI</td>
<td>5.96 ± 0.25</td>
<td>6.73 ± 0.18</td>
<td>6.97 ± 0.06</td>
<td>6.97 ± 0.06</td>
<td>6.99 ± 0.08</td>
</tr>
<tr>
<td>pH NI + Ind</td>
<td>5.69 ± 0.06</td>
<td>6.13 ± 0.14</td>
<td>6.61 ± 0.11</td>
<td>6.71 ± 0.08</td>
<td>6.77 ± 0.02</td>
</tr>
</tbody>
</table>

Results are mean ± SE of five subjects.
of NI + Ind. During the subsequent 4 hours of immersion, UPGEV levels were 3-fold greater than the prestudy levels. Recovery was associated with a prompt decrement to 2.3 ± 0.3 ng/min (P < 0.01). Although indomethacin pretreatment was associated with a baseline UPGEV which was 74% lower than that observed during NI, it did not prevent a subsequent augmentation of UPGEV during immersion. Results are mean ± SE of six subjects. (Comparison with indomethacin with P < 0.05 designated by *.)

**Figure 2** Effect of water immersion on renal PGE in six normal sodium-depleted subjects, before (NI) and after (NI + Ind) indomethacin administration. Immersion was associated with a progressive increase in PGE excretion (UPGEV) from 4.0 ± 0.4 ng/min to peak levels of 9.3 ± 2.5 and 9.8 ± 4.3 ng/min during hours 3 and 4, respectively. Recovery was associated with a prompt decrement to 2.3 ± 0.3 ng/min (P < 0.01). Although indomethacin pretreatment was associated with a baseline UPGEV which was 74% lower than that observed during NI, it did not prevent a subsequent augmentation of UPGEV during NI + Ind. Results are mean ± SE of six subjects. (Comparison with indomethacin with P < 0.05 designated by *)

**Table 5** Changes in Serum Electrolytes and Osmolality during Water Immersion

<table>
<thead>
<tr>
<th></th>
<th>Prestudy</th>
<th>Poststudy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium-replete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>140 ± 1</td>
<td>139 ± 1</td>
</tr>
<tr>
<td>NI + Ind</td>
<td>139 ± 1</td>
<td>139 ± 1</td>
</tr>
<tr>
<td>Sodium-depleted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>138 ± 2</td>
<td>137 ± 1</td>
</tr>
<tr>
<td>NI + Ind</td>
<td>134 ± 1</td>
<td>135 ± 1</td>
</tr>
<tr>
<td>Potassium (mEq/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium-replete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>NI + Ind</td>
<td>4.3 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Sodium-depleted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>3.9 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>NI + Ind</td>
<td>4.0 ± 0.1</td>
<td>4.2 ± 0.1*</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg H₂O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium-replete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>280 ± 3</td>
<td>278 ± 3</td>
</tr>
<tr>
<td>NI + Ind</td>
<td>279 ± 4</td>
<td>279 ± 2</td>
</tr>
<tr>
<td>Sodium-depleted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>282 ± 2</td>
<td>282 ± 5</td>
</tr>
<tr>
<td>NI + Ind</td>
<td>287 ± 4</td>
<td>286 ± 4</td>
</tr>
</tbody>
</table>

Results are mean ± SE of nine subjects during sodium-replete studies and five subjects during sodium-depleted studies.

* P < 0.05 for poststudy compared with prestudy.
hanced prostaglandin production. Furthermore, it has been demonstrated that the injection into the renal artery of prostaglandins of either the A or E series increases renal blood flow and sodium excretion in the dog (Gross and Bartter, 1973; Vander, 1968).

The interpretation of such studies on the effects of prostaglandin administration on renal sodium and water handling is difficult. The demonstration that PGE is almost completely inactivated in each passage across the lung (McGiff and Itakovic, 1973) suggests that any action of this lipid on the kidney must be as a local tissue hormone. Thus, any evaluation of the physiological role of prostaglandins on urinary sodium excretion necessitates an experimental design in which the endogenous production of the lipid is altered. Indeed, recent investigations of the role of prostaglandins in renal sodium and water handling have focused on comparisons before and after the administration of inhibitors of prostaglandin synthetase.

Attempts to define the effect of prostaglandin release on the natriuresis of ECVE by inhibition of prostaglandin synthesis have been contradictory, with reports suggesting that inhibition of prostaglandin synthesis may be either anti-natriuretic (Verberckmoes et al., 1976; Donker et al., 1976; Patak et al., 1975; Berg, 1977; Dusing et al., 1976), natriuretic (Kirschenbaum and Stein, 1977; Mountokalakis et al., 1978; Kirschenbaum and Stein, 1976), or fail to affect renal sodium handling (Bohan and Wesson, 1976; Bailie et al., 1976). In several studies, inferences regarding the role of indomethacin on renal sodium excretion were drawn by assessing the natriuretic response to furosemide before and after indomethacin administration (Patak et al., 1975; Bailie et al., 1976). Additional studies have assessed renal sodium excretion during steady state conditions before and after the administration of either indomethacin (Verberckmoes et al., 1976; Donker et al., 1976; Mountokalakis et al., 1978) or acetylsalicylic acid (Berg, 1977; Bowden et al., 1978).

Only two studies to date have assessed the effect of acute ECVE in man (Mountokalakis et al., 1978; Papanicolaou et al., 1975). Papanicolaou et al. (1975) assessed renal venous PGE levels without determining renal sodium excretion simultaneously. Although Mountokalakis et al. (1978) demonstrated that indomethacin administration augmented the natriuretic response to saline administration, renal PGE was not determined. Furthermore, in both studies, acute ECVE was induced by the administration of an intravenous saline load which presumably altered plasma composition. As pointed out by Levinsky (1974) in a critical review, acute saline loading induces large changes in blood composition which may confound the interpretation of studies purporting to assess changes in a number of hormonal systems, and perhaps prostaglandins as well.

The present studies, the first to describe alterations in PGE excretion during immersion, demonstrated conclusively that immersion is associated with a marked increase in renal PGE. The increase of PGE occurred whether the subjects were on a normal or low sodium intake, although the increase in PGE excretion was less when sodium intake was restricted. Although the prior administration of indomethacin (a potent inhibitor of prostaglandin formation) lowered the basal rates of PGE excretion, indomethacin administration did not prevent the subsequent immersion-induced increment in renal PGE excretion.

In addition to defining the effects of immersion on PGE excretion, the present studies demonstrate conclusively that indomethacin pretreatment attenuated markedly the natriuretic response to immersion in sodium-depleted normal subjects. In contrast, when the identical subjects were studied during the sodium-replete state using an identical regimen, indomethacin failed to affect renal sodium, potassium, or water handling.

Urinary PGE was measured because it presently is felt to constitute the best index of renal PGE production (Dunn and Hood, 1977). Studies in which both renal venous and urinary PGE were measured demonstrated parallel changes in both, but quantitatively more PGE was found in the urine (Dunn et al., 1978). The current demonstration of an increase in urine prostaglandin excretion suggests, therefore, that acute central blood volume expansion is associated with a rise in renal PGE synthesis. The possibility that other effects related to the experimental design might account for this increase in UPGEV must be considered. Although immersion was associated with an increase in urine flow rate, it is unlikely that this could explain our findings. Recent studies by Patak et al. (1977) have demonstrated that the increase in urine flow which follows administration of chlorothiazide or the carbonic anhydrase inhibitor benzolamide is not associated with an increase in UPGEV. Although immersion was associated with an increase in urine pH, several lines of evidence render it unlikely that such an effect facilitated ionic trapping with a resultant increase in UPGEV. First, the immersion-induced increment in urinary pH during NI + Ind did not differ from that observed during NI. Furthermore, studies by Patak et al. (1977) have demonstrated that, following benzolamide administration, UPGEV did not increase despite an increase in urinary pH.

Perhaps the most compelling evidence is derived from an assessment of the relationship between urinary pH and urinary PGE concentration. Such an examination failed to disclose a correlation ($r = 0.03; P > 0.50$). Indeed, there was a negative correlation of urinary pH with urinary PGE concentration in the seven subjects undergoing immersion without indomethacin pretreatment (NI). Taken together, these considerations mitigate against the possibility that ionic trapping plays a significant role in the increasing UPGEV associated with head-
out immersion. It is probable therefore, that the observed increment in $U_{PGE}$V reflects an increase in renal synthesis of PGE, a view consistent with a number of other experimental observations.

The change in PGE excretion with immersion was quite dramatic. In both the sodium-replete and sodium-depleted studies, NI was associated with marked increases in PGE excretion (Figs. 1 and 2). When many of these same subjects were studied during indomethacin administration, the basal rate of PGE excretion prior to immersion was significantly lower. This was expected since indomethacin is a potent inhibitor of cyclooxygenase, an early enzymatic step in the synthesis of prostaglandins (Vane, 1971). Nevertheless, during immersion there was still a marked increase in PGE excretion in the indomethacin-treated subjects. These results suggest that the indomethacin-induced inhibition of prostaglandin synthesis is only partial and when presented with an adequate stimulus, such as water immersion, the kidney still can augment markedly its rate of PGE production.

It is of interest to consider what factors might stimulate renal PGE synthesis during NI. It is unlikely that changes in the composition of the blood are important since previous studies have demonstrated no significant alterations of the blood with immersion. While both angiotensin II and vasopressin are potent stimuli to renal PGE synthesis in appropriate settings (Frölich et al., 1975; Lifschitz and Stein, 1977), previous studies have demonstrated a fall in plasma renin activity (Epstein et al., 1975b) and vasopressin levels (Epstein et al., 1975a) during immersion. There are considerable changes in systemic and possibly renal hemodynamics during neck immersion (Epstein, 1978), and it is conceivable that one of these factors, perhaps an increase in renal blood flow or papillary blood flow, could be important in this regard. As noted earlier, immersion is associated with a marked increase in sodium and potassium excretion and a fall in urine osmolality, and it is conceivable that one of these factors is important in stimulating PGE synthesis. Since the major portion of renal PGE is synthesized in the medulla (Larsson and Anggårds, 1973), and present concepts would suggest that alterations in transport in deep nephrons are critical to the increase in sodium excretion found with volume expansion (Stein et al., 1976), it is attractive to suggest that the increase in renal PGE production with immersion might in some way relate to the alterations in sodium transport which occur in the renal medulla with immersion. Although the attenuated natriuresis found with indomethacin in the low salt studies is consistent with this suggestion, additional studies clearly will be required to determine whether the parallel changes in renal PGE production and sodium excretion denote a physiological relationship or are only a chance association.

The mechanism(s) whereby indomethacin administration alters renal sodium handling merits consideration. In the present studies, the increase in sodium excretion usually found with immersion was attenuated when the subjects on the low sodium intake were pretreated with indomethacin. It is well recognized that indomethacin has the potential to inhibit a large number of enzymes (Flower, 1974). Since none of the products of any of these enzymes other than those relating to prostaglandin production were measured in the present studies, it is difficult to state whether the doses of indomethacin used here were effective on any of these systems. Nevertheless, similar observations on renal sodium handling using other agents that inhibit prostaglandin synthetase (Verberckmoes et al., 1976; Donker et al., 1976; Patak et al., 1975; Berg, 1977; Düsing et al., 1976) support the interpretation that the encountered alterations in sodium excretion are indeed attributable to the effect of indomethacin on prostaglandin synthetase. Although indomethacin has been reported to decrease glomerular filtration rate (GFR) in a number of clinical settings including decompensated cirrhosis (Zipser et al., 1977) and nephrotic syndrome (Arisz et al., 1976), the present demonstration that the effects of indomethacin occurred in the absence of concomitant alterations in GFR exclude a decrease in filtered sodium load as a mechanism for the decreased natriuresis in the sodium-depleted subjects. Taken together, these observations suggest that the decrease in prostaglandin excretion is causally related to the abolition of the natriuresis in sodium-depleted subjects.

Since indomethacin is known to inhibit prostaglandin synthesis, and in fact clearly did so in the present study, it is of interest to consider the possible mechanisms whereby an inhibition of PGE synthesis might impair the ability of the kidney to increase $U_{Na}$V during immersion. First, a decrease in renal PGE might affect sodium excretion by directly facilitating sodium reabsorption in some nephron segment. Although Stokes and Kokko (1977) found a direct inhibitory action of PGE on sodium reabsorption in the perfused cortical collecting tubule, subsequent studies by others using a number of systems including the isolated perfused rabbit nephron (Fine and Trizna, 1977), renal slices, and nephron suspensions (Dunn and Howe, 1977) all have failed to find a direct effect of PGE on sodium transport.

Alternatively, it is possible that the indomethacin-induced inhibition of PGE synthesis affects renal sodium handling by altering renal hemodynamics. The latter possibility could be formulated as follows: immersion may induce an increase in cortical and medullary flow with a resultant "medullary washout" and a consequent increase in sodium excretion. If the increase in medullary flow is attributable to an increase in renal PGE production,
then a decrease in the rate of PGE synthesis would diminish this "medullary washout" with a resultant decrease in sodium excretion. Since, in contrast to the sodium-replete state (Epstein et al., 1976), renal hemodynamics have not been determined in sodium-depleted man during immersion, such a possibility must remain speculative. A third possibility is that indomethacin might directly affect renal vascular resistance (or specifically medullary vascular resistance) and thus alter the natriuretic response to immersion independently of a change in prostaglandin synthesis.

The observation that an inhibitor of prostaglandin synthetase, indomethacin, affected renal sodium handling only in the sodium-depleted state is in accord with some previous reports (Berg, 1977; Zipser et al., 1977). Sodium depletion might potentiate the antinatriuretic effects of indomethacin in man in one of several ways. First, the unmasking of the indomethacin-induced effects on sodium excretion may be related to the activation of the renin-angiotensin system encountered in sodium-depleted man. If, as has been suggested, the prostaglandins counterbalance the vasconstrictor effects of hormones such as angiotensin II, then inhibition of prostaglandin synthesis would tend to lead to unopposed activity of the renin-angiotensin system with resultant sodium retention. The failure of indomethacin to decrease GFR in the present study is not in keeping with this formulation, but obviously does not exclude an effect on renal resistance with a preferential alteration of afferent arteriolar resistance. Alternatively, it is possible that the enhancement of mineralocorticoid action, present in sodium-depleted man, predisposes to the antinatriuretic effects of indomethacin.

The interrelationship of renal prostaglandins and renal water handling in the present study merits comment. As noted earlier, indomethacin administration was associated with a lower mean V and free water clearance during the prestudy hour and hour 1 of immersion in the sodium-restricted subjects. Concomitantly, the most striking decrement in basal PGE excretion was observed during the prestudy hour of NI + Ind in this group. Furthermore, in some, but not all instances, the peaking of PGE excretion tended to coincide with peak urine volume and free water clearance (Table 1 and Figure 1). This observation raises the possibility that increased PGE synthesis affects renal water handling primarily. Alternatively, it is conceivable that changes in urine flow or hypoosmolality or some determinant thereof may mediate the encountered changes in renal PGE. Additional studies during states of hydropenia and maximal water diuresis will be necessary to assess this possibility.

In summary, the present studies demonstrate that immersion-induced central volume expansion, in addition to being associated with a marked natriuretic and kaliuretic response, also is associated with a striking increase in renal PGE excretion. Although indomethacin lowers the basal rate of PGE excretion, it does not prevent the marked increase in PGE excretion with immersion. In addition, indomethacin administration attenuates markedly the natriuretic and kaliuretic response of immersion-induced central volume expansion in sodium-depleted but not in sodium-replete normal subjects. In concert, these observations are consistent with the possibility that renal PGE constitutes a determinant of the renal response to volume expansion in sodium-depleted man.

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