THE EFFECT OF ESTROGEN ON WATER AND ELECTROLYTE METABOLISM. I. THE NORMAL

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The possibility that estrogens might under certain circumstances influence water and electrolyte metabolism is suggested by several observations. For instance, estrogens are related chemically to the strongly sodium-retaining steroid desoxycorticosterone (1); the recurrent ankle edema observed in some women before menstruation was considered by Fluhmann (2) to be associated with an increase in blood estrogen levels; and the edema frequently found in late pregnancy occurs at a time when estrogen production is at its height (3).

These considerations led several workers to attempt to establish a direct relationship between water and salt retention on the one hand, and estrogenic activity on the other. Thorn and Engel (4) found sodium, chloride and water retention after a single injection of estrogen into male dogs. Later, the same group of workers (5), in a study of premenstrual fluid retention, found that decreased urinary excretion of salt and water was in two patients associated with an increased output of estogenic material in the urine. Knowlton, Kenyon, Sandiford, Lotwin, and Fricker (6) found diminished urinary excretion of sodium following the daily administration of estradiol in one normal woman, but Sharpey-Schafer and Schrire (7), using twice the amount of estradiol in ten women, found no alteration in urinary volume.

As part of an investigation into the possible role of estrogens in the fluid retention of liver disease it became necessary to establish the effect of continued estrogen administration on water and electrolyte metabolism in the normal. Water and electrolyte balance studies were therefore carried out in eleven normal subjects, and the effect of daily administration of estrogen observed.

PROCEDURE

Eleven subjects without evidence of cardiac, hepatic, renal or other disease likely to affect water and electrolyte metabolism, were selected from the wards of The London Hospital. Five subjects were under treatment for peptic ulcer, during which time no medication other than magnesium trisilicate or aluminum hydroxide was given. Two subjects had sciatic nerve pain, one osteoarthritis of the hip, one torn medial meniscus of the knee, one hallux valgus, and in one a tuberculous pleural effusion had subsided three weeks previously.

Each subject underwent a water and electrolyte balance study, lasting approximately two weeks and divided into two periods—the first a "control period," and the second an "estrogen period," during which daily injections of estrogen were given. The subjects were given a normal diet, gauged in amount and composition to individual appetite, and remaining constant throughout the study in respect of Cl, Na, K, PO₄, water and calorie content. Diet composition was calculated from appropriate tables (8). Sample dietary analyses were not carried out. The balance diet was commenced 2 to 3 days before any observations were made. Since this diet corresponded closely in composition to previous intake in each case, no longer period for metabolic adjustment was considered necessary. In all subjects daily urinary output of water, Na and Cl was estimated. In eight subjects daily urinary K output and in six subjects daily urinary PO₄ output were measured. Fecal output of electrolytes and water were not determined. "Water balance" (Table I) was calculated by subtracting urinary volume from total water intake. Eight subjects were weighed daily in the fasting state, and in the same clothes. Cases 7, 20 and 21 were confined to bed, and weighing was not possible.

Blood analyses. Plasma concentrations of Cl and Na, the total serum protein and the venous hematocrit were determined on two separate days during the control period, and again on two separate days at the end of the estrogen period.

Extracellular fluid volume. In eight subjects an estimate of changes in extracellular fluid volume was obtained by determining the volume of distribution of a known quantity of thiocyanate. Exactly 20 ml. 4 per cent w/v NaCNS was injected intravenously, no food or drink having been taken for the previous twelve hours. Plasma samples were collected four and six hours later, and their thiocyanate concentration estimated. "Thiocyanate space" was calculated by linear extrapolation of these values to zero time. The space was estimated twice on separate days both during the control period and again at the end of the estrogen period. (Na injected for these determinations, about 10 mEq., was in addition to dietary Na intake).

Water tolerance test. After emptying the bladder, one liter of tap water was drunk in twenty minutes, no food
or drink having been taken for the previous twelve hours. The bladder was then emptied hourly for four hours, and the volume of urine measured. Two tests were carried out in the control period, and two at the end of the estrogen period, on separate days.

**Estrogen.** During the estrogen period, estradiol monobenzoate 10 mg. in oil was injected intramuscularly daily. This was equivalent to between 0.14 and 0.20 mg. per kg. body weight.

**Chemical methods.** Heparinized plasma was used for all determinations of plasma Na, Cl and CNS. Serum was used for total protein determinations. Plasma and urinary Na, and urinary K were estimated by the flame photometer of Hollliday and Preedy (9), except in case 4 where the plasma and urine Na were estimated chemically as described by Aitken and Preedy (10). Plasma and urinary Cl were estimated by the method of Schales and Schales (11), urinary P by the method of Youngburg and Youngburg (12), total serum protein by the Kjeldahl method (13) and plasma CNS by the method of Bowler (14). The venous hematocrit was obtained by spinning heparinized blood in 10-cm. Wintrobe tubes for 20 minutes at 3,000 r.p.m., radius 15 cm.

**Statistical analysis.** Where daily measurements were obtained (i.e., weight, water balance, urinary outputs of Cl, Na, K, PO₄), both individual cases and the group as a whole were statistically analyzed. Significant differences between mean values for control and estrogen periods were estimated by application of the t test, a value for P of 0.02 or less being taken as critical both here and in all succeeding tests. The significance of group mean differences between control and estrogen period means (d) was similarly estimated.

Where less frequent observations were made (i.e., plasma determinations of Cl, Na, serum protein, venous hematocrit, thiocyanate space) group analysis only was undertaken, the significance of group mean differences (d) being estimated by the t test as above.

Weight measurements in cases 43 and 44 could not be so analyzed due to a steady gain in weight during both control and estrogen periods. Regression lines for both periods were therefore constructed. The significance of differences between control and estrogen regression lines were estimated by the t test.

**RESULTS**

Measurements of weight, "water balance," and urinary outputs of Na, Cl, K, PO₄ were obtained daily. Mean values for control and estrogen periods derived from these daily observations are given in Table I. Figures in brackets under each pair of means indicate the probability (P) that the difference between these means could have been due to chance. As "water balance" denotes the figure obtained when daily urinary volume is subtracted from total daily water intake, an increase in this figure implies retention of water.

Measurements of serum protein, venous hematocrit and thiocyanate space appear only in Table I, the figures representing means of two, or occasionally three, observations on separate days.

At the foot of Table I are given the group mean differences (d) between control and estrogen periods for each class of observation (e.g., urinary output of Cl, K, etc.) for the whole group, together with the value for P.

**Weight.** Group mean difference (without cases 43 and 44) was + 0.28 kg., P = 0.1, indicating no significant variation following the administration of estrogen. However, analysis of individual cases shows that there was in fact a small but statistically significant mean rise in weight in Nos. 4, 13, and 45 during the estrogen period. In addition, there was a significant alteration of the regression line slope in No. 44, indicating an increased rate of weight gain.

**Water balance.** No significant difference between control and estrogen periods was observed. Neither the group means nor the means in any of the eleven cases showed any significant change.

**Urinary outputs. Chloride and sodium.** Group Cl and Na means did not differ significantly between periods. There was no significant difference between means in any individual case, with the exception of No. 13, where the mean daily output of Cl increased by 36 mEq. (P = 0.02), but without any significant change in mean Na output.

**Potassium.** There was no significant difference between group means. Differences between means in the eight individual cases in which this measurement was made were likewise non-significant.

**Phosphate.** The was no significant difference between group means. However, in two cases out of the six in which this estimation was made (Nos. 21 and 76), there was a mean daily decrease in phosphate output of 13 and 17 mEq. respectively. These differences were significant (P = 0.01). In the remaining four cases the differences were non-significant.

**Total serum protein level.** No significant decrease in the group mean serum protein value was observed following the administration of estrogen (d = − 0.26 g., per 100 ml., P = 0.1). This estimation was done in seven cases.

**Venous hematocrit.** There was a significant decrease in the volume of packed cells, mean difference between control and estrogen period − 2.8
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Days in Control Period</th>
<th>Weight (kg.)</th>
<th>Water Balance (ml.)</th>
<th>Daily Urinary Output</th>
<th>Total Serum Protein (g./100 ml.)</th>
<th>Venous Hematocrit (%)</th>
<th>&quot;Thioglycollate Space&quot; (l.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6</td>
<td>51.8 52.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43.5 42.5</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>49.0 46.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>44.5 43.0</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>919 1046</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.6 15.2</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>950 986</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>54.5 53.5</td>
<td>21.4 22.5</td>
</tr>
<tr>
<td>21</td>
<td>11</td>
<td>790 851</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.4 6.6</td>
<td>17.4 17.0</td>
</tr>
<tr>
<td>43</td>
<td>7</td>
<td>1640 1194</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.4 6.0</td>
<td>46.4 21.6</td>
</tr>
<tr>
<td>44</td>
<td>8</td>
<td>935 809</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.2 6.0</td>
<td>55.5 34.0</td>
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<tr>
<td>45</td>
<td>8</td>
<td>62.0 62.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.1 5.9</td>
<td>20.5 17.4</td>
</tr>
<tr>
<td>55</td>
<td>8</td>
<td>68.6 68.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.7 6.7</td>
<td>51.5 50.0</td>
</tr>
<tr>
<td>74</td>
<td>8</td>
<td>70.7 70.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20.8 20.8</td>
<td>-</td>
</tr>
<tr>
<td>76</td>
<td>6</td>
<td>56.4 56.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.3 7.7</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean Difference: 

- P: 0.1
- 0.28
- -19
- -2.8
- -2.8
- 0.9
- 0.6
- 0.7
- 0.3
- 0.5
- 0.7
- 0.6
- 0.1
- 0.01
- 0.06

† Figures in brackets denote values for P relating only to the two means immediately above. An asterisk (*) indicates that the P value was derived from comparison of regression lines, mean values being omitted; "incr" indicates an increased and "decr" a decreased rate of weight gain. Mean differences for the whole group, with P values, are given at the foot of the table. Water balance = total water intake less urinary volume.
TABLE II

Water tolerance tests

Hourly urinary volume in ml. in (a) control period, (b) estrogen period. Each figure represents the mean of two or three tests on alternate days. \( \bar{d} \) = group mean difference. \( P \) = probability derived from t test

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Subject</th>
<th>1st Hour (a)</th>
<th>1st Hour (b)</th>
<th>2nd Hour (a)</th>
<th>2nd Hour (b)</th>
<th>3rd Hour (a)</th>
<th>3rd Hour (b)</th>
<th>4th Hour (a)</th>
<th>4th Hour (b)</th>
<th>All hours (a)</th>
<th>All hours (b)</th>
<th>All cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>H. L.</td>
<td>126</td>
<td>290</td>
<td>605</td>
<td>488</td>
<td>327</td>
<td>278</td>
<td>114</td>
<td>150</td>
<td>114</td>
<td>150</td>
<td>-97</td>
</tr>
<tr>
<td>7</td>
<td>P. D.</td>
<td>303</td>
<td>328</td>
<td>298</td>
<td>188</td>
<td>630</td>
<td>615</td>
<td>115</td>
<td>120</td>
<td>115</td>
<td>120</td>
<td>-62</td>
</tr>
<tr>
<td>13</td>
<td>A. W.</td>
<td>85</td>
<td>55</td>
<td>75</td>
<td>75</td>
<td>47</td>
<td>775</td>
<td>650</td>
<td>615</td>
<td>120</td>
<td>95</td>
<td>-15</td>
</tr>
<tr>
<td>20</td>
<td>W. D.</td>
<td>145</td>
<td>288</td>
<td>460</td>
<td>450</td>
<td>175</td>
<td>235</td>
<td>60</td>
<td>63</td>
<td>60</td>
<td>63</td>
<td>-11</td>
</tr>
<tr>
<td>21</td>
<td>E. E.</td>
<td>95</td>
<td>200</td>
<td>280</td>
<td>210</td>
<td>175</td>
<td>95</td>
<td>135</td>
<td>65</td>
<td>135</td>
<td>65</td>
<td>-1</td>
</tr>
</tbody>
</table>

\( \bar{d} \) = 0.83 L., \( P = 0.06 \).

Plasma chloride and sodium levels. These observations are not included in Table I. No significant difference between control and estrogen periods was observed (\( \bar{d} = 0 \) mEq. per L., \( P = 1 \), \( \bar{d} = -0.3 \) mEq. per L., \( P = 0.9 \), respectively).

Water tolerance tests. Details of the four hourly urinary volumes are given in Table II. Each figure represents the mean of two tests carried out on separate days. The tests were performed in five subjects. Mean differences for each hourly output between control and estrogen periods varied between +87 and -62 ml., none of which was significant. Mean difference for all hours and all cases was likewise non-significant.

For each subject daily urinary output figures for Cl and Na, together with those for water balance, were expressed as a percentage of the control pe-

per cent, \( P = 0.01 \). This estimation was done in eight cases.

"Thiocyanate space." The difference observed between the control and estrogen period group means of the eight cases in which this estimation was made was of borderline significance (\( \bar{d} = +0.83 \) L., \( P = 0.06 \)).

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For each subject daily urinary output figures for Cl and Na, together with those for water balance, were expressed as a percentage of the control pe-
period mean for that subject. The percentages for
corresponding days were then averaged for all
subjects and plotted graphically against days in
Figure 1. Daily weights (cases 4, 13, 45, 55, 74,
76) were similarly treated. For convenience fig-
ures for the last six days only of the control pe-
riod appear in the graphs.

Inspection of Figure 1 shows that following the
administration of estrogen there was a systematic
fall in Cl and Na output during the first four days,
followed by a rise in the control level or above
in the last four days. This was associated with
a similar but inverse variation in water balance.
That the water and electrolyte changes were
closely related is shown by a Na/water balance
correlation coefficient of 0.90, P = 0.001. Weight
showed a slight tendency to rise after estrogen
administration.

In Figure 2, the average daily urinary Na : Cl
ratios for the group were plotted against days.
There was a fall in the ratio, followed by a rise
to control levels or above, during the estrogen pe-
riod corresponding in time to the Na and Cl varia-
tions seen in Figure 1.
The statistical significance of these variations in
Cl and Na output is indicated by the presence of
at least four consecutive points below the 95 per
cent confidence limits in Figures 1 and 2. As re-
gards the weight observations, although the mean
value in any one day did not lie outside the 95 per
cent confidence limits, the fact that the last seven
points in the estrogen period were above the
control mean has a probability of occurring by
chance of only \(1/\frac{5}{4}\), or 1 : 128. The highly sig-
nificant negative correlation between Na output
and water balance already mentioned, indicates
that the variation observed in the latter was also
significant.

Calculation of the cumulative balance of Cl, Na
and water during the estrogen period (taking the
mean of the control period as indicating the aver-
age output of each substance at equilibrium on the
diet taken) showed that there was an average
positive balance of Cl at the end of the estrogen
period amounting to 20 mEq., and of Na amount-
ing to 33 mEq. The Na : Cl ratio of these figures
of 1 : 0.6 is that which would be expected, if all
Cl and Na apparently retained had been stored
as extracellular fluid (ECF). Calculated cumula-
tive balance of water, however, appeared to indi-
cate no change between periods, average balance
figure at the end of the estrogen period being + 43
ml. only.
The average weight gain at the end of the estro-
gen period was 0.6 kg. (cases 43 and 44 were
included by taking into account the difference be-
tween the regression lines). Of this, only 0.2 kg.
could be accounted for by increased volume of ex-
tracellular fluid, if the latter were calculated from
the average retention of Cl and Na given above.

DISCUSSION

Statistical analysis of the group as a whole
(Table I) failed to show that estradiol administra-
tion had any sustained effect on weight, or on the urinary output of water, Na or Cl. However, although no sustained alteration in urinary Na and Cl output was found, the presence of a systematic variation in the output of these substances during the estrogen period is clearly demonstrated in Figure 1. Since dietary NaCl intake remained constant, and assuming no significant change in fecal excretion of these ions, the findings indicate that estrogen caused a limited storage of Na and Cl followed by a diuresis while injections were still being continued.

It appears that this NaCl storage occurred in association with an increase in extracellular fluid (ECF) volume, since there was no change in plasma Na and Cl concentrations, there was an increase in weight, and there were corresponding changes in water balance (Figure 1). There was in addition a significant decrease and increase in urinary Na:Cl ratio (Figure 2). The NaCl contents of a normal diet are roughly equivalent. This was reflected in the present series by an average urinary Na:Cl ratio of approximately 1:1 during the control period. Any increase in ECF volume under these conditions must be accompanied by a decrease in urinary Na:Cl ratio, such as is observed here.

Further evidence for an expansion of ECF is provided by the average increase in thiocyanate space, although this increase was of borderline significance only. The thiocyanate method for estimating ECF volume is open to various objections (15-17) (in common with many other methods), but is probably adequate when used to detect changes in the same subject under the conditions of the present study.

The diminution in serum protein concentration and venous hematocrit by almost the same percentage of the control mean (4.0 and 6.2, respectively) suggests a common influencing factor, that is, an increase in plasma volume of about 5 per cent. This is in agreement with the findings of other workers. Increased plasma volume has been found in both animals (18) and humans (19) after the injection of estradiol. It would appear, therefore, that the intravascular compartment takes part in the expansion of ECF following estradiol administration.

It is not certain that the observed increases in weight following estrogen can be entirely accounted for by an increase in ECF volume. The average cumulative balance figures, and those in some individual subjects, suggest that the weight increases may have been due in part to some other factor. This factor may be an increase in tissue formation, since estrogens have known anabolic properties (20).

The significant decrease in urinary PO_4 output after estrogen, observed in cases 21 and 76 might be accounted for by retention of PO_4 as part of the process of increased tissue formation. However, it seems more likely that PO_4 was retained in association with Ca, since Albright and Reifenstein (21) have shown that a positive calcium and phosphorus balance may be induced in patients with osteoporosis by estradiol administration. It is noteworthy that both our cases had a limb immobilized in plaster.

The absence of change in the result of water tolerance tests after estrogen is to be expected, since the tendency to store NaCl after estrogen administration in these subjects was both slight and transient, and daily urinary volume was not affected to any great degree.

Our results are in general agreement with those of Thorn and Engel (4) in dogs, and extend the observations of Knowlton, Kenyon, Sandiford, Lotwin, and Fricker (6) on one normal woman. The failure of Sharpey-Schafer and Schrire (7), using the same dosage of estradiol as ourselves, to find any alteration in urinary volume in their subjects was presumably due to the small changes involved. Urinary Na and Cl outputs (a more sensitive index) were not determined by them. However, Shapiro (22), using four to five times the dose of estradiol in normals, did observe a marked decrease in urinary volume followed by a diuresis on cessation of the estrogen. The difference between these results is presumably due to the dose employed.

The mode of action of estradiol in retaining salt and water is uncertain. The absence of any significant effect on urinary K excretion in dogs (4) or in man in the present series suggests that the action may not be via the adrenals. Moreover, Thorn and Engel (4) found that adrenalectomy did not affect the salt-retaining effect of estrogen in their dogs. The action of estradiol may therefore be directly in the kidney. Since it has been shown (23) that administered estradiol does not
affect the renal clearances of mannitol or of para-
aminohippuric acid, a direct action on the renal
 tubules may be involved.

SUMMARY

1. The effect of daily administration of estradiol
 on water and electrolyte metabolism has been studi-
ed in a group of 11 normal subjects.

2. A slight and transient decrease in urinary
 output of Cl, Na and water was observed, which
 was significant.

3. Plasma Cl and Na levels were not signifi-
cantly affected.

4. There was no significant change in urinary K
 output in any of eight cases.

5. Urinary PO₄ output was not significantly
 changed in 4 out of 6 cases. In the two remaining
 cases, there was a small but significant decrease.

6. There was a decrease in total serum protein
 and venous hematocrit by approximately the same
 percentage amount, suggesting an increase in
 plasma volume.

7. Evidence is presented that the transient de-
 crease in urinary Cl, Na and water output is as-
 sociated with an increase in extracellular fluid
 volume.

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