THE REDISTRIBUTION OF BODY SODIUM IN WOMEN ON LONG-TERM OESTROGEN THERAPY

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SUMMARY

1. The effects on the serum electrolytes of long-term treatment with either mestranol or a placebo were determined in 175 healthy middle-aged oophorectomized women. In forty of these women the packed cell volume (PCV), serum albumin, serum and urinary osmolality, inulin space and total exchangeable sodium ($Na_e$) were also measured.

2. The mean serum sodium and chloride concentrations were significantly lower in the mestranol-treated women, and this was associated with a significant reduction in the mean PCV and the mean serum albumin concentration by comparison with the placebo-treated group.

3. The mean urinary osmolality was higher and the mean serum osmolality lower in the mestranol group such that there was a significant increase in the mean urine/serum osmolality ratio as compared with the placebo group.

4. The mean inulin space was significantly higher in the mestranol group as compared with the controls, but there was no significant difference in $Na_e$.

5. These findings support the hypothesis that oestrogen-induced fluid retention is the result of primary water retention with secondary redistribution of body sodium.

Key words: chloride, mestranol, oestrogen, sodium.

It has been known for many years that women retain fluid during pregnancy (Miller, Keith & Rowntree, 1915), pre-menstrually (Eufinger & Spiegler, 1928), and after the administration of oestrogen (Thorn & Engel, 1938). This phenomenon has been characterized by weight gain, a reduction in urine volume and a fall in packed cell volume (PCV) (Okey & Stewart, 1933; Sweeney, 1934; Witten & Bradbury, 1951). Thorn, Nelson & Thorn (1938) observed a pre-menstrual fall in urinary sodium output in normal women and from this they inferred
that the female sex hormones cause the retention of salt in a similar way to that seen after the administration of adrenocortical steroids. This hypothesis was, however, challenged by Klein & Carey (1957), who were unable to demonstrate a significant change in total body sodium during the menstrual cycle. Furthermore fluid retention during pregnancy is associated with relative hyponatraemia and not hypernatraemia as would be expected if the female sex hormones mimicked the action of the adrenocorticoids (Newman, 1957). These findings suggest that the fluid retention caused by the female sex hormones is associated with the redistribution of body sodium, rather than sodium retention.

In order to test this hypothesis we investigated salt and water homeostasis by measuring the extracellular fluid volume and total exchangeable sodium in oophorectomized women on long-term oestrogen therapy, and compared these values with those obtained from a comparable group of oestrogen-deficient women.

**MATERIALS AND METHODS**

One hundred and seventy-five healthy postmenopausal women aged 37–59 years, who had undergone hysterectomy and bilateral salpingo-oophorectomy for non-malignant disease within the previous 8 years, were reviewed. Fifty-eight of these women had been prescribed 129 nmol (40 μg) of mestranol (17α-ethynyl-3-methoxyoestra-1,3,5(10)-trien-17β-ol) daily and sixty-one women were given placebo tablets of identical composition but without the active ingredient. The women formed part of a controlled trial of oestrogen therapy for the prevention of postmenopausal osteoporosis (Aitken, Hart & Lindsay, 1973), and 1–3 years of treatment had been completed at the time of investigation. The actual dose of mestranol taken by individual women did, however, vary between 32 and 129 nmol (10 and 40 μg) daily. Informed consent was obtained from the women studied for all the various aspects of this investigation, which had been approved by the Ethical Committee of the Hospital.

Venous blood was obtained with minimal venostasis after an overnight fast from all the women and allowed to clot. Measurements of serum albumin, creatinine, osmolality and PCV were made in twenty mestranol-treated and twenty age- and weight-matched placebo-treated women, who also provided a urine sample passed between 07.00 and 09.00 hours for the estimation of urinary osmolality and creatinine. The triceps fat-fold thickness was measured with skin callipers. These women subsequently reattended on a separate occasion for the measurement of the extracellular fluid volume and total exchangeable sodium.

Serum electrolytes were determined by an AutoAnalyzer four-channel electrolyte procedure N-21, with sodium and potassium measured with a Technicon flame photometer mark III, chloride by method N-5a and bicarbonate by method N-8a. The PCV was measured with a Coulter S counter. Small changes in plasma osmolality do not affect the PCV when measured in this way. Serum albumin was determined by a dye-binding technique using Bromocresol Green (Northam & Widdowson, 1967). Serum and urinary osmolality was measured by freezing-point depression with a Knauer Halbmikro osmometer. Serum and urinary creatinine were determined by an AutoAnalyzer using method N-11.

The extracellular fluid volume was measured, in the fasting state after a period of 8–10 h overnight water deprivation, by the intravenous injection of a bolus of 2 g of inulin followed by regular blood sampling for 1 h. Serum inulin was measured by the method of Kulka (1956). The inulin space was determined by dividing the quantity of inulin given by the theoretical
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inulin concentration at zero time, the latter being calculated by extrapolating the 30-60 min exponential of the inulin disappearance curve to zero time. This method assumes that the mixing of inulin throughout the extravascular space is completed within 30 min of the time of injection.

Total exchangeable sodium (Na,) was measured, after the intravenous injection of 10 μCi of $^{24}$Na, by dividing the dose administered less that lost in the urine by the serum specific radioactivity at 24 h. The serum sodium radioactivity was also measured 10 min after injection. Since the activity of the $^{24}$Na given varied on different occasions, the actual quantity given was scaled up for ease of calculation to give a theoretical count rate of $10^7$ c.p.m., and the activity of the 10 min serum samples was scaled up proportionately.

Osmolar excretion was calculated from the formula:

$$\text{urinary osmolality} \times \frac{\text{serum creatinine}}{\text{urinary creatinine}} \text{ (mosmol/100 ml of glomerular filtrate).}$$

The statistical methods employed were Student's t-test and standard linear correlation.

RESULTS

The effect of long-term mestranol therapy on the serum electrolytes appears in Table 1. The mean serum sodium and chloride concentrations were very significantly lower in the mestranol-treated women than they were in the untreated controls ($P<0.001$). The mean serum potassium concentration was lower and the mean serum bicarbonate concentration was higher in the oestrogen-treated women than they were in the controls, but these differences were not significant.

The PCV, serum albumin and serum and urinary osmolalities are shown in Table 2. The mean PCV was 6.6% lower ($P<0.001$), and the mean serum albumin concentration was 9.6% less ($P<0.001$) in the mestranol-treated women as compared with the controls. The mean serum osmolality was significantly lower and the mean urinary osmolality was higher in the mestranol group as compared with the controls, thus giving a urine/serum ratio which was about 12% higher in the oestrogen-treated women. The mean osmolar excretion was $0.541 \pm 0.029$ mosmol/
100 ml of glomerular filtrate in the controls, and 0.438 ± 0.021 mosmol/100 ml of glomerular filtrate in the mestranol-treated women. This difference was statistically significant \((P < 0.02)\).

The mean inulin disappearance curves for the mestranol and placebo groups are shown in Fig. 1. By 30 min from the time of injection the rate of inulin disappearance had become constant in both groups and formed exponentials the straightness of which were very close to unity \((r = 0.9999\) and 0.9997). The calculated mean inulin space and body build of the women

### Table 2. Effects of long-term treatment with mestranol or a placebo in postmenopausal women

Results shown are means ± SEM. N.S., not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Packed cell volume</th>
<th>Serum albumin (g/100 ml)</th>
<th>Osmolality (mosmol/kg)</th>
<th>Osmolality ratio urine/serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no. of subjects)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (20)</td>
<td>50.4 ± 1.2</td>
<td>0.422 ± 0.006</td>
<td>4.46 ± 0.06</td>
<td>295 ± 0.7</td>
<td>624 ± 26</td>
</tr>
<tr>
<td>Mestranol (20)</td>
<td>51.2 ± 1.0</td>
<td>0.395 ± 0.005</td>
<td>4.05 ± 0.05</td>
<td>292 ± 0.8</td>
<td>703 ± 25</td>
</tr>
</tbody>
</table>

**Comparison between groups**

\[ t = 3.91 \]

\[ t = 5.34 \]

\[ t = 3.27 \]

\[ t = 2.18 \]

\[ t = 2.43 \]

**Results**

Placebo: 0.001 < \( P < 0.005 \)

Mestranol: 0.05 < \( P < 0.02 \)

### Table 3. Effect of long-term treatment with mestranol or a placebo on inulin space in postmenopausal women

Results shown are means ± SEM. N.S., not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Triceps fat-fold thickness (mm)</th>
<th>Inulin space (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>64.7 ± 2.2</td>
<td>159 ± 1.2</td>
<td>18.3 ± 1.1</td>
<td>8.01 ± 0.29</td>
</tr>
<tr>
<td>Mestranol</td>
<td>63.4 ± 1.7</td>
<td>160 ± 1.0</td>
<td>18.5 ± 0.9</td>
<td>9.54 ± 0.53</td>
</tr>
</tbody>
</table>

**Comparison between groups**

N.S.

N.S.

N.S.

\[ t = 2.54 \]

**Results**

Placebo: N.S.

Mestranol: 0.02 < \( P < 0.05 \)

The mean sodium radioactivity at 10 min after injection was 968 ± 31 c.p.m. in the placebo-treated women, which was significantly higher than the mean value of 895 ± 19 c.p.m. in the

studied are shown in Table 3. It can be seen that although the mean weights and heights of the mestranol and placebo groups were similar the mean inulin space was significantly lower in the latter group \((P < 0.02)\). Fig. 2 shows the relationship between inulin space and the degree of obesity in the controls. A significant inverse correlation obtained \((P < 0.02)\) such that the most obese women had an inulin space which formed a smaller proportion of total body weight than was found in thin women.
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Fig. 1. Inulin disappearance curves in placebo (×) and mestranol (●) treated women. Results are means ± SEM. $P_0$ and $M_0$, mean inulin concentrations at zero time in the placebo and mestranol groups respectively. Equations of 30–60 min exponentials from the equation $\log_{10}y = a + bx$ were: $\log_{10}y = 3.30 - 0.0144x$ ($r = 0.9991$) in the placebo group; $\log_{10}y = 3.17 - 0.0157x$ ($r = 0.9999$) in the mestranol group.

Fig. 2. Relationship between inulin space as a percentage of body weight and the triceps fat-fold thickness in twenty oophorectomized oestrogen-deficient women. ($y = 16.7 - 0.22x$; $r = 0.51$, $t = 2.52$, $P < 0.02$.)
mestranol-treated women \((P<0.05)\). The mean values for total exchangeable sodium are shown in Table 4. Although the mean serum sodium concentration was higher in the placebo group, \(\text{Na}_e\) was almost identical in both groups. By calculating the quantity of sodium in the inulin space, it was possible to determine the size of the exchangeable sodium pool outside the extracellular fluid space. There was significantly less exchangeable sodium outside the inulin space in the mestranol-treated women as compared with the oestrogen-deficient placebo-treated women \((P<0.02)\).

### Table 4. Effect of long-term treatment with mestranol or a placebo on body sodium in postmenopausal women

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum sodium ((\text{mmol/l}))</th>
<th>(\text{Na}_e) ((\text{mmol}))</th>
<th>Exchangeable sodium outside inulin space ((\text{mmol}))</th>
<th>Comparison between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>141.7±0.5</td>
<td>2402±53</td>
<td>1242±42</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mestranol</td>
<td>140.6±0.5</td>
<td>2386±46</td>
<td>1025±74</td>
<td>(t = 2.56) (P&lt;0.02)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Oestrogen-induced fluid retention is a common phenomenon, but the mechanism whereby these effects are mediated is far from clear. Thorn *et al.* (1938) interpreted the pre-menstrual fall in urinary sodium output in women as a sign of oestrogen-induced sodium retention. In subsequent metabolic balance studies Thorn & Emerson (1940) documented the retention of 60 mmol and 25 mmol of sodium in two women, who gained 2 kg and 1 kg in weight respectively, but no comment was made on the fact that almost five times more fluid was retained than would have been expected for the obligatory water retention accompanying such a small sodium load. These findings also contrast with the observations of Thorn & Emerson (1940) on a normal subject given deoxycorticosterone acetate, where a weight gain of 2.5 kg was accompanied by the retention of about 240 mmol of sodium and a rise in the serum sodium concentration of 2.8 mmol/l.

In the present study, where a small dose of oestrogen was given for a prolonged period of time, the associated relative reduction in the PCV and serum albumin concentration was suggestive of haemodilution and supports the findings of Witten & Bradbury (1951), who gave relatively large doses of oestrogens for a short period of time. These oestrogen-induced effects were attended by a small but highly significant relative hyponaetraemia and hypochloraemia similar to that seen in pregnancy (Newman, 1957). The extracellular fluid volume as determined from the inulin disappearance curve was significantly greater in the oestrogen-treated women, and, in the absence of any change in total exchangeable sodium, supports the hypothesis that oestrogen-induced haemodilution is the result of water retention with secondary sodium redistribution rather than sodium retention with an obligatory gain in body water.
The classical method for measuring the inulin space (Gaudino, Schwartz & Levitt, 1948; Gaudino & Levitt, 1949; Schwartz, Schachter & Freinkel, 1949) was not used in this study because it was thought unreasonable to expect healthy volunteers to participate in what is essentially a lengthy procedure. Furthermore, in the classical method it is customary to keep the subjects sufficiently hydrated to maintain a high urinary output in order to minimize the errors implicit in the accurate collection of urine after the infusion has stopped. It is therefore not possible to make an accurate measurement of the inulin space by the classical method after a period of water deprivation, as it is likely that the increased hydration necessary to maintain a high urinary output would artificially increase extracellular fluid volume (ECFV). The method we employed is the standard procedure used for determining the volume of distribution of rapidly administered drugs and radioisotopes (Cardozo & Edelman, 1952). With inulin used in this way the apparent ECFV was about 15% of body weight in the mestranol-treated women as opposed to 12.5% in the placebo-treated group. The classical method gives values of 15-5% (Gaudino et al., 1948) and 16-2% of body weight (Schwartz et al., 1949) in healthy males. Little information is available for normal women, but in view of the fact that women tend to be more obese than men and obesity lowers the percentage of body weight taken up by extracellular fluid, one would expect ECFV to be proportionately lower in women than in men. Our values for ECFV are therefore probably close to what one might expect to find with other methods, although it is possible that slight underestimation has resulted. The difference found between the oestrogen-deficient and the oestrogen-replete women could not be accounted for by altered body composition since the former were if anything slightly less obese than the latter. We are not in a position to state whether the mestranol- or the placebo-treated women had the 'abnormal' ECFV, but, in view of the observation that pre-menopausal women tend to share the same haemodilutional features as women given a small dose of mestranol (Aitken, 1973), it is likely that the low ECFV found in the oestrogen-deficient women should be regarded as 'abnormal'.

It could be argued that oestrogen might give an apparent increase in ECFV by altering the permeability of one of the extracellular fluid compartments that does not normally allow free access to inulin. All these compartments, however, give free access to sodium and therefore such an hypothesis would only hold good if the mean serum sodium radioactivity after injection was similar in both groups of women. The mean serum sodium radioactivity, however, was significantly lower in the oestrogen-treated women 10 min after injection than it was in the placebo-treated women, as one would expect if the ECFV were indeed expanded. The fact that a similar significant difference was not found at later time-intervals after injection was not surprising in view of the almost identical values for Na⁺.

There was no evidence of significant weight change in either group of women during treatment, and hence it is improbable that a similar degree of expansion of intracellular fluid volume had occurred in the mestranol-treated subjects. However, it is possible that the increase in ECFV may in part have been due to the movement of water out of the intracellular space.

Our values for Na⁺ at roughly 38 mmol/kg were similar to those reported by Forbes & Perley (1951) in seven normal women. About 20% of the exchangeable sodium outside the extracellular space is found within the cells, and in a 65 kg woman this would amount to about 250 mmol. The low intracellular sodium content, which gives a concentration gradient of about 16:1 across the cell wall, is only maintained by efficient active sodium-pumping mechanisms. It is unlikely therefore that much of the additional sodium in the ECF could
have come from the intracellular compartment, without there being a considerable increase in sodium-pumping capacity. Most of the exchangeable sodium outside the extracellular pool is located in bone and this has been calculated to contribute to between 25% (Miller & Wilson, 1953) and 45% (Edelman, James, Baden & Moore, 1954) of the total skeletal sodium content. On the basis that bone constitutes about 16% of body weight (Edelman et al., 1954) and that this tissue contains between 200 and 340 mmol of sodium/kg of bone (Miller & Wilson, 1953) it can be calculated that a 65 kg woman should have between 2100 and 3500 mmol of sodium in her skeleton, of which between 500 and 1600 mmol would be exchangeable. In the oestrogen-treated women there was a mean reduction of 217 mmol in exchangeable sodium outside the extracellular fluid space, and this could well be accounted for by a reduction in exchangeable sodium in bone. Since most of the skeletal sodium is thought to be located in its crystalline components (Edelman et al., 1954) and since oestrogens are capable of increasing the radiographic density of bone (Edgren & Calhoun, 1956; Aitken et al., 1973), it is tempting to speculate whether or not some of the oestrogen effect on bone is mediated by an exchange of calcium for sodium on the bone crystal lattice, and likewise loss of oestrogenic activity may lead to an increase in the sodium/calcium ratio in bone.

The precise mode of action of oestrogen in effecting these changes in body fluid composition is ill-defined. It was, however, clear that the higher urine osmolality in the mestranol-treated group was not the result of a relative solute diuresis, since osmolar excretion was lower in these women, and hence a true decrease in water excretion was the most likely explanation. In the light of this observation the higher urine/serum osmolality found in the oestrogen-treated women as compared with the oestrogen-deficient women is consistent with the view that oestrogens increase antidiuretic hormone (ADH) activity. Whether such an increase in ADH activity is the consequence of increased circulating concentrations of ADH or an increase in the sensitivity of the renal tubules to ADH must await further investigation. This study also shows the importance of making allowances for haemodilution in oestrogen-treated subjects when interpreting changes in the concentrations of certain constituents in the blood, especially where these substances are protein bound.

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REFERENCES

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