STUDIES ON THE ANDROGENIC ACTIVITY OF \(9\alpha\) -
FLUORO-11\(\beta\)-HYDROXY-17-METHYLTESTOSTERONE
(‘ULTANDREN’). II. A COMPARISON BETWEEN THE
ANDROGENIC PROPERTIES OF ‘ULTANDREN’ AND
TESTOSTERONE PROPIONATE

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Summary. 1. The relative potency of parenterally administered
‘Ultandren’ compared with testosterone propionate is approximately 1
when seminal vesicle weight is used as an index of androgenic activity.
2. Coagulating gland weight is not a suitable indicator of male sex
hormone activity for use in the bioassay of ‘Ultandren’.

INTRODUCTION

Lyster, Lund & Stafford (1956) compared the effects of orally administered
‘Ultandren’ with those of other androgens given by the same route, and
concluded that ‘Ultandren’ has approximately ten times the potency of
methylandrostosterone. However, a possible criticism of their results is that the oral
method of administration may involve errors of dosage and differences in
absorption which are difficult to measure, and it seemed desirable to repeat the
assay with parenteral administration of the hormones, allowing a suitable
time to elapse after a single injection for maximal effects to occur. An experi¬
mental design which fulfils these criteria is that of Mathieson & Hays (1945) and
in the present investigation this method was employed, using testosterone
propionate (TP) as the standard and ‘Ultandren’ as the unknown preparation.
The validity of such an assay depends upon the assumption that the log dose-
response curves of standard and unknown are linear (Bliss, 1944); examination
of Hays & Mathieson’s (1945) results indicates that over the dosage range used
in the present experiment the log dose-response curve of testosterone propionate
is approximately linear. Emmens (1950) has pointed out that the assumption of
a linear log dose-response relationship is a valid approximation, departures from
which will be revealed during analysis of the results. Similarly Burn, Finney &
Goodwin (1950) claim that, provided the slopes of the log dose-response
regression lines are not significantly different, the question of the linearity of the
true log dose-response curve is of little importance. A six point assay would be
necessary to distinguish between deviations from parallelism and differences in
the shapes of the actual curves (Emmens, 1950).

During this investigation the opportunity was taken to repeat the citric acid
and fructose estimations made previously (Clegg, 1959 unpublished work;
<table>
<thead>
<tr>
<th></th>
<th>Group $S_1$</th>
<th>Group $S_2$</th>
<th>Group $U_1$</th>
<th>Group $U_2$</th>
<th>Group 0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.V.</td>
<td>C.G.</td>
<td>S.V.</td>
<td>C.G.</td>
<td>S.V.</td>
</tr>
<tr>
<td>Mean</td>
<td>10.6</td>
<td>4.8</td>
<td>18.1</td>
<td>7.3</td>
<td>18.3</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>1.12</td>
<td>0.64</td>
<td>1.35</td>
<td>0.66</td>
<td>1.35</td>
</tr>
</tbody>
</table>

$S.V.$ = Seminal vesicle; $C.G.$ = Coagulating gland.
Androgenic activity of ‘Ultandren’. II

Clegg & Farley, 1962), and an attempt was made to conduct a parallel assay using coagulating gland weight as an indicator of androgenic activity.

MATERIALS AND METHODS

Forty-nine male rats contained in eight litters and aged between 26 and 29 days were randomly segregated into four groups of ten and one of nine animals. They were castrated and left for 3 weeks to ensure maximal regression of the accessory reproductive organs. Each animal was then weighed and given a single subcutaneous injection. The numbers of animals in each group, the substance injected, its amount and its vehicle were as follows: Group S₁ (nine animals) received 0·2 mg TP in 0·2 ml arachis oil; Groups S₂, U₁ and U₂ (ten animals each) received respectively 0·8 mg TP, 0·8 mg ‘Ultandren’ and 3·2 mg ‘Ultandren’ in 0·2 ml arachis oil; Group 0 (ten animals) received 0·2 ml arachis oil only.

The doses of TP are those used by Mathieson & Hays (1945). Previous work (Clegg & Farley, 1962) indicated that the doses of ‘Ultandren’ were of approximately equal potency to the lower and higher doses of TP.

Seventy-two hours after injection the animals were killed. The seminal vesicles and coagulating glands were removed and weighed. None of the organs contained expressible secretion. Citric acid and fructose were estimated in the pooled organs of each group by methods previously described (Clegg & Farley, 1962).

RESULTS

Seminal vesicle and coagulating gland weights

These are indicated in Table 1.

Citric acid and fructose levels

These are indicated in Table 2.

ESTIMATION OF RELATIVE POTENCY

Estimation based on seminal vesicle weight

M, the log relative potency = 0·6187

R, the relative potency = \frac{antilog M}{dose\ ratio}

\frac{4·157}{4} = 1·039

b, the slope of the log dose-response lines = 9·027

s^2, the combined estimate of variance = 11·899

and s = 3·450

λ, the s.d. in log dose units = 0·382

s_m, the s.e. of M = 0·1732

The limits of error of M are ± t s_m, where t has a value obtained from the table of Student’s t distribution at P = 0·05 and 35 d.f.
Thus the limits are $\pm 0.3516$.

The limits of $R$ are $\frac{\text{antilog } 0.6187 \pm 0.3576}{4}$.

Lower limit is 0.582.
Upper limit is 2.335.

The validity of these limits is indicated by the fact that $g = 0.083$, which is less than the 0.1 required for rejection. The whole calculation depends on the assumption that the log dose-response lines are parallel. Using the $t$-test, $t = 1.947$ with 35 d.f. Thus $P > 0.05$ indicating that the difference between the slopes of the two lines is not significant.

*Estimation based on coagulating gland weights*

Table 1 indicates that the log dose-response lines have obviously different slopes. Using the $t$-test to determine the significance of this difference, $t = 2.412$, with 35 d.f.

### Table 2

**CITRIC ACID AND FRUCTOSE LEVELS IN THE SEMINAL VESICLES AND COAGULATING GLANDS**

<table>
<thead>
<tr>
<th>Citric acid in seminal vesicle</th>
<th>Group $S_1$</th>
<th>Group $S_2$</th>
<th>Group $U_1$</th>
<th>Group $U_2$</th>
<th>Group $O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (ug)</td>
<td>3.5</td>
<td>3.7</td>
<td>2.6</td>
<td>3.8</td>
<td>0.0</td>
</tr>
<tr>
<td>(ug per 100 g body weight)</td>
<td>0.21</td>
<td>0.24</td>
<td>0.17</td>
<td>0.24</td>
<td>0.0</td>
</tr>
<tr>
<td>Fructose in coagulating gland</td>
<td>Group $S_1$</td>
<td>Group $S_2$</td>
<td>Group $U_1$</td>
<td>Group $U_2$</td>
<td>Group $O$</td>
</tr>
<tr>
<td>Total (ug)</td>
<td>2.4</td>
<td>4.3</td>
<td>2.6</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>(ug per 100 g body weight)</td>
<td>0.15</td>
<td>0.27</td>
<td>0.17</td>
<td>0.14</td>
<td>0.18</td>
</tr>
</tbody>
</table>

$0.05 > P > 0.02$, thus indicating that the difference between the slopes is significant and that any four point assay of 'Ultandren' with coagulating gland weight would be invalid.

**DISCUSSION**

The investigation indicates that if changes in seminal vesicle weight are used as a parameter of male sex hormone activity, 'Ultandren' has approximately the same potency (within fairly wide limits of error) as testosterone propionate when both compounds are given by subcutaneous injection. This result differs from that obtained in the previous investigation (Clegg & Farley, 1962), but is more reliable since cumulative effects of the hormones do not occur in this type of experiment. Thus previous claims of the high activity of 'Ultandren' (Lyster, Lund & Stafford, 1956; Buckle, 1959) are confirmed and extended.

The effect of 'Ultandren' on the coagulating gland seems to be small. Although the low dose of the hormone in Group $U_1$ animals is capable of raising the weight of the organs above castrate control levels, there is in fact a decrease with the much higher dose in Group $U_2$ (differences between the two groups are not significant ($0.5 > P > 0.4$)). It would appear that 'Ultandren'
exerts its maximal effect in these animals at a dosage of about 0.8 mg. This finding does not confirm that of the previous investigation (Clegg & Farley, 1962) in which a linear log dose-response curve in respect of the coagulating gland was demonstrated. It may be that the different designs of the two experiments, together with the different ages of the animals employed, are responsible for this discrepancy.

Although, for reasons considered previously (Clegg & Farley, 1962) citric acid production in the seminal vesicles does not lend itself to use in the bioassay of androgens unless much larger numbers of animals are employed, it is apparent from Table 2 that in the doses used ‘Ultandren’ and testosterone propionate are able to produce broadly similar amounts of citric acid, and it would appear that the potency of ‘Ultandren’ is rather less than that of testosterone propionate in this respect. However, the importance of citric acid as an ‘indicator’ of male sex hormone activity (Humphrey & Mann, 1949) is confirmed, since it is absent in the seminal vesicles of castrate control rats in Group 0.

The investigation confirms the inability of ‘Ultandren’ to affect fructose production in the coagulating gland (Clegg, 1959, unpublished work; Clegg & Farley, 1962). Indeed the levels recorded in both ‘Ultandren’-treated groups of animals are closely similar to the amount found in castrate control animals. This failure of an otherwise active androgen to influence fructose production does not appear to have been hitherto reported other than by the workers cited above.

An incidental point of some interest is that even 3 weeks after the castration of a group of sexually immature animals fructose was still found to be present in the coagulating glands. It may well be that this time was too short for total disappearance of the fructose and if so it is confirmatory evidence that fructose may be produced in sexually immature animals (Mann, Lutwak-Mann & Price, 1948). The presence of fructose and absence of citric acid confirm the findings of Mann, Davies & Humphrey (1949) who in a 14-week-old buck rabbit found no citric acid and small amounts of fructose in the accessory reproductive organs.

It is known that the substitution of halogen atoms in the Carbon-9 positions of various adrenal steroids can greatly modify their effects (Fried & Sabo, 1954; Chester Jones, 1957; Llaurado, 1961), and it seems possible that such a substitution in the methyltestosterone molecule is related in some way to the marked difference between the effects of ‘Ultandren’ on the seminal vesicle and on the coagulating gland.

Dorfman & Shipley (1956) have demonstrated that quantitative differences between different androgens may occur in respect of their actions on the seminal vesicle and ventral prostate. Some may exert a more pronounced effect on the vesicle, while the reverse may be true in other hormones. It may be concluded that ‘Ultandren’ exhibits an extreme example of the former case.

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REFERENCES

Mann, T., Davies, D. V. & Humphrey, G. F. (1949) Fructose and citric acid in the secretions of the accessory glands as indicators of male sex hormone activity. J. Endocrin. 6, 75.