ANTAGONISTIC ACTION OF OESTRADIOL AND TESTOSTERONE ON THE GROWTH OF HYPOPHYSECTOMIZED IMMATURE RATS

By

F. J. A. Paesi, G. Hellinga, M. J. Hoogstra and G. P. van Rees

In a preceding paper (Paesi & De Jongh, 1954) we described a growth-inhibiting action of oestradiol in the young hypophysectomized rat: the ahormonal body-weight and taillength-increases were significantly diminished by 0.5 µg. of oestradiol benzoate daily. We concluded that the well-known growth-inhibiting action of oestrogen in the intact rat might in part be due to this effect and not exclusively to a decrease of the production or action of pituitary growth hormone.

Since testosterone and oestrone have been found to act antagonistically on body growth (Claussen & Freudenberger, 1939) and on bone structure (Gardner & Pfeiffer, 1938; Halvorsen, 1949) in immature animals we were interested to know whether body growth-antagonism would also occur in hypophysectomized animals. The results of experiments carried out with young rats are reported in this paper.

MATERIAL AND METHODS

Immature female rats (30–35 gm.) were hypophysectomized and treated with 0.5 µg. or with 2.5 µg. of oestradiol benzoate daily for 12 days. Other animals received testosterone propionate (40 µg. daily) or both hormones. Control animals were injected with solvent (oil) only. The animals were sacrificed on the 13th day after hypophysectomy. At the start and at the end of the experiment bodyweight and taillength were recorded. The right M. gastrocnemius and the liver were weighed on a torsion

1. Supported by a grant from the foundation »Die Drie Lichten«.

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balance. In animals in which it was suspected that a pituitary remnant had been left the cranial base was examined histologically in the usual way. Animals with remnants were discarded.

RESULTS

1) Bodyweight

The tables show clearly that the marked depressant action of 0.5 μg. of oestradiol benzoate is not increased by a higher dose (2.5 μg.); this is in keeping with data published previously (Paesi & De Jongh, 1954).

The values for Student's 't' given in the tables indicate that testosterone in both series significantly diminished the oestradiol effect. However, the decrease is only partial, as is illustrated by the fact that significant differences are found between the bodyweight increases in the oestrogen + testosterone treated rats and those in the oil-treated control animals: t = 2.6 (series 1) and t = 4.7 (series 2). It is not surprising that 40 μg. testosterone had a greater effect against 0.5 μg. than against 2.5 μg. of oestradiol benzoate.

Testosterone alone had no influence on bodyweight (Table 1).

2) Tail length

The decrease of tailgrowth by 0.5 μg. of oestradiol is identical with that caused by 2.5 μg. In both cases, 40 μg. of testosterone propionate completely inhibited the oestradiol effect.

Testosterone alone appears to have caused a significant additional taillength increase (p < 0.05).

3) M. gastrocnemius and liver

With 0.5 μg. of oestradiol benzoate a significant reduction of the muscular weight was obtained which was prevented by testosterone. With 2.5 μg. (2nd series), however, no influence on the average muscular weight was found in spite of a marked overall depressant action on body weight increase. We cannot explain this discrepancy at present.

The values for the liver weights in both series are remarkable since they show that testosterone had no effect whatever against the marked weight decrease caused by oestradiol. Significant differences exist in both series between the average liver weights in the oestrogen treated rats and those of the untreated control animals (ser. 1: t = 3.9; ser. 2: t = 4.1). The differences between the liver weights in the rats treated with both oestradiol and testosterone and the control animals are also significant (ser. 1: t = 5.4; ser. 2: t = 4.3). Treatment with testosterone only did not influence the liver weight.

4) Mortality

In accordance with the results published previously the death rate was enhanced with oestrogen: 44 per cent of the rats receiving 0.5 μg. daily in the
Table 1.
Decrease of the growth rate of hypophysectomized immature rats by 0.5 µg. of oestradiol benzoate and its prevention by testosterone.

<table>
<thead>
<tr>
<th>Number of surviving animals</th>
<th>Treatment (daily dose)</th>
<th>Bodyweight (init.) (gm.)</th>
<th>Bodyweight increase (gm.)</th>
<th>Taillength increase (mm.)</th>
<th>M. Gastrocnemius (mg.)</th>
<th>Liver (mg.)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>52) Solvent (oil)</td>
<td>34</td>
<td>12.5 ± 0.61(^2)</td>
<td>12.5 ± 0.51</td>
<td>174 ± 3.1</td>
<td>1920 ± 46</td>
<td>27 % (19 of 71)</td>
<td></td>
</tr>
<tr>
<td>37 Oestradiol benz. 0.5 µg.</td>
<td>32.5</td>
<td>7.6 ± 0.57</td>
<td>9.5 ± 0.38</td>
<td>147 ± 3.8</td>
<td>1630 ± 52</td>
<td>44 % (29 of 66)</td>
<td></td>
</tr>
<tr>
<td>52 Oestradiol benz. 0.5 µg. + test. prop. 40 µg.</td>
<td>33.5</td>
<td>10.0 ± 0.65</td>
<td>12.1 ± 0.49</td>
<td>167 ± 4.5</td>
<td>1645 ± 44</td>
<td>30 % (22 of 74)</td>
<td></td>
</tr>
<tr>
<td>48 Test. prop. 40 µg.</td>
<td>34</td>
<td>12.8 ± 0.77</td>
<td>14.0 ± 0.55</td>
<td>177 ± 4.1</td>
<td>1865 ± 55</td>
<td>32 % (23 of 71)</td>
<td></td>
</tr>
</tbody>
</table>

\(^t\)bodyweight\_{\text{oestr.}} / \text{oestr. + test.} = 2.4

\(^t\)bodyweight\_{\text{oil}} / \text{oestr.} = 5.6

\(^t\)taillength\_{\text{oestr.}} / \text{oestr. + test.} = 4.3

\(^t\)taillength\_{\text{oil}} / \text{oestr.} = 4.3

1. By application of the Peirce-criterion one or two animals may have been discarded in some calculations.
2. Standard error of the mean.
Table 2.
Decrease of the growth rate of hypophysectomized immature rats by 2.5 µg. of oestradiol benzoate and its prevention by testosterone.

<table>
<thead>
<tr>
<th>Number of surviving animals</th>
<th>Treatment (daily dose)</th>
<th>Bodyweight (init.) (gm.)</th>
<th>Bodyweight increase (gm.)</th>
<th>Taillength increase (mm.)</th>
<th>M. Gastrocnemius (mg.)</th>
<th>Liver (mg.)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>57¹</td>
<td>Solvent (oil)</td>
<td>32.5</td>
<td>10.4 ± 0.50²</td>
<td>12.7 ± 0.49</td>
<td>153</td>
<td>1715 ± 36</td>
<td>34 % (30 of 87)</td>
</tr>
<tr>
<td>37</td>
<td>Oestradiol benz. 2.5 µg.</td>
<td>32.5</td>
<td>6.0 ± 0.38</td>
<td>10.2 ± 0.51</td>
<td>153</td>
<td>1510 ± 35</td>
<td>64 % (66 of 103)</td>
</tr>
<tr>
<td>54</td>
<td>Oestradiol benz. 2.5 µg. + test. prop. 40 µg.</td>
<td>32.5</td>
<td>7.3 ± 0.39</td>
<td>13.5 ± 0.45</td>
<td>154</td>
<td>1485 ± 23</td>
<td>44 % (42 of 96)</td>
</tr>
</tbody>
</table>

¹ bodyweight $\frac{\text{oestr.}}{\text{oestr. + test.}} = 2.8$

¹ bodyweight $\frac{\text{oil}}{\text{oestr.}} = 5.6$

¹ taillength $\frac{\text{oestr.}}{\text{oestr. + test.}} = 4.2$

¹ taillength $\frac{\text{oil}}{\text{oestr.}} = 4.3$

1. By application of the Peirce-criterion one or two animals may have been discarded in some calculations.
2. Standard error of the mean.
course of the experiment, whereas 27% of the control animals died. The $\chi^2$ test reveals a significant difference ($p < 0.05 > 0.01$). With 2.5 $\mu$g. daily, the death rate was much higher than with 0.5 $\mu$g.; 64 per cent (against 34% in the control rats). Again, the difference with the control-value was significant ($p < 0.001$).

If 40 $\mu$g. of testosterone propionate was added to 0.5 $\mu$g. of oestradiol benzoate, the increased mortality was reduced to normal; the difference (oestr. benz.-combin.) is almost significant ($p > 0.05$). The same dose of testosterone propionate only partly inhibited the toxic effects of 2.5 $\mu$g. of oestradiol benzoate (difference significant: $p < 0.05 > 0.01$).

**DISCUSSION**

Though testosterone is well known to be active as a growth promoting substance in the human subject, attempts to demonstrate a similar action in rats have led to contradictory results (reviewed by Gardner & Pfeiffer, 1943).

_Stimulation_ of growth was found in young rats by Clausen & Freudenberger, 1939 (1 mg. of testosterone propionate daily, for 9 days), Rubinstein & Solomon, 1940 and 1941 (50 $\mu$g. testosterone propionate daily, 6–7 weeks) and Kochakian, 1950 a (0.5 mg., ca. 30 days) and in adult rats by Korenchevsky et al., 1937 (167 $\mu$g., 23 days), and Gordon et al., 1947 (0.5 mg., 5 days).

_No effect_ was noted by Mc Euen et al. (1937) (200 $\mu$g., 30–60 days), Turner et al. (1941) (250 $\mu$g., 87 days) and Shay et al. (1941) (1–5 mg. $3 \times$ weekly, 25 weeks).

_Inhibition_ of growth was obtained with a relatively high dose by investigators who observed stimulation with lower doses: Korenchevsky et al. (1937) (1.4 mg., 23 days) and Rubinstein et al. (1939 and 1941). The latter authors obtained depression of body growth with 1 mg. of testosterone propionate administered daily for 80 days to intact and castrate male rats, the depression only becoming evident after 24 days of treatment. The bodyweight- and taillength-increases which had already been diminished by castration alone were still further decreased by testosterone.

These divergent results suggest an interplay of various effects. It is known, for instance, that in the human subject testosterone treatment, though temporarily accelerating growth, may result in a precocious closure of the epi-physyal disk and the final body-length or -weight may then be even lower than normal. The possible influence of testosterone on »bone age« in rats has not yet been definitely settled.

Several attempts were made to unravel the pattern of action of testosterone by the use of hypophysectomized animals. Gordon, Evans & Simpson treated hypophysectomized adult female (plateau-) rats with 0.5 or 2.5 mg. of tes-
testosterone propionate daily for 10 days, beginning 1 week following hypophysectomy. All rats received the same amount of food as taken by untreated hypophysectomized rats (10 gm. daily). Only nitrogen retention was obtained, however, and no bodygrowth. In previous experiments (Simpson et al., 1944) the authors met with no more success (1–4 mg. for 15–20 days, to hypophysectomized males, 29–91 days old; 0.1 or 1 mg. for 10 days to hypophysectomized females; 0.25 mg. for 20 days to hypophysectomized young males). They were, however, able to show that testosterone (0.25 mg. for 10 days), though in itself inactive, significantly enhances the bodyweight increase obtainable with pituitary growth hormone. The histological picture of the epiphyseal union showed no effects particular of testosterone and the authors think that the increase of the growth-hormone effect might well be part of the mechanism of the growth-promoting action of testosterone in the intact rat.

Kochakian (1950 b) was the first to obtain growth with testosterone in hypophysectomized rats. He used hypophysectomized castrated adult males and started treatment with 1.25 mg. daily, 6 months after castration. Before treatment, food supply was adjusted in such a way that the rats neither gained nor lost weight: 5–7 gm. of food daily. Testosterone administration then resulted in growth, accompanied by N-retention.

In comparing these experiments the impression is gained that the drastic restriction of food practised by Kochakian might perhaps have favoured the appearance of a growth effect. Testosterone may only show its activity if growth is curtailed first in some way. This consideration, added to results on oestrogen-androgen antagonism on growth in intact animals (Clausen & Freudenberger) prompted us to attempt to demonstrate the body-weight increasing effect of testosterone in hypophysectomized young rats whose growth had been checked with oestrogen. The attempt was successful and it may be concluded from this and from Kochakian’s result that the growth-promoting potency of testosterone is certainly not limited to an enhancement of the effect of growth-hormone, since it can be demonstrated in the absence of somatotrophin. We were able to supply data about tail length increase and to show that the body weight gain obtained cannot merely have been due to for instance increased uptake of water or increase in the amount of fat, since it was accompanied by extra bone growth.

Two questions, which among others arise whenever growth effects are recorded, remain to be answered: 1. Do all tissues benefit alike from the anabolic action or are some favoured more than others? 2. How is the weight gain achieved?

Ad 1: In both of our series of oestrogen-treated hypophysectomized rats the depression of tailgrowth was totally prevented by testosterone. Bodygrowth, however, was only partly restored and liver growth, though markedly inhibited by oestrogen, not influenced at all. Some tissues have, therefore, been able
to take greater advantage of testosterone than others. What tissues are these? It is clear that the bones have enjoyed some priority. In considering the results of the first series one is inclined to assume that skeletal muscles were also involved. Testosterone restored the weight loss of the M. gastrocnemius caused by 0.5 μg. of oestradiol benzoate. But since for some reason unknown to us the high dose of 2.5 μg. caused no weight loss in this particular muscle, conclusions about the muscular system as a whole must await further information.

Ad 2: We have no idea, at present, about the character or the localization of the reported oestradiol-testosterone antagonism. The results obtained in hypophysectomized rats by Kochakian suggest that food restriction might favour the anabolic effect of testosterone. Since oestrogen-treated hypophysectomized rats presumably eat less than their untreated companions, the growth-promoting effect of testosterone might perhaps be partly due to a return of pre-oestrogen appetite.

In this connection it would be interesting to know, to what extent the oestrogen-effect (the increased death rate included) might be prevented by forced feeding. We hope to be able to investigate this question in subsequent experiments.

**SUMMARY**

The inhibition of the body growth of hypophysectomized immature female rats by 0.5 or 2.5 μg. of oestradiol benzoate was partly prevented by the simultaneous administration of 40 μg. of testosterone propionate. The reduction of tail growth by oestrogen was completely inhibited by testosterone, that of the liver weight increase, however, remained unaffected. All differences are significant. Addition of testosterone caused a significant decrease in the death rate which had been increased by oestrogen treatment alone.

**REFERENCES**