THE INFLUENCE OF TESTOSTERONE PROPIONATE ON SKELETAL DEVELOPMENT IN THE IMMATURE RABBIT

BY

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The word growth is sometimes used in the sense of general development. It refers to the increase in size, which is the result of proliferation of cells, and is a part of development. The other part of development is the process of maturation represented by cell differentiation. If the term growth is used loosely to mean development, this leads to misunderstanding and confusion (Davenport et al., 1939).

Problems concerning the development of the body are often conveniently studied in the skeleton, as this gives, relatively easily, information concerning the growth as well as of the process of maturation. Growth in length in animal experiments may be estimated with greater accuracy by measuring selected bones on an X-ray film, than by direct measurement of the body length. The histology of the epiphyseal cartilage not only reflects the maturation of the skeleton, but may also give information concerning the growth in length.

The increase in weight during the period of growth is also an index of development, but it is more difficult to estimate,
as it is influenced by factors which have no direct relationship with development e.g. water retention.

It is well known that the gonads influence the development of the body. Castration of boys before puberty increases the duration of growth in length by delaying the closure of the epiphysis. By an increased production of male sex hormones, or other androgens, as in precocious puberty caused by an interstitial cell tumour of the testis or by tumours of the adrenal cortex, there is a generally accelerated development. In the skeleton, this development is reflected by an increased growth in length — an abnormally hastened development of the ossification centres, and a premature closure of the epiphysis. Growth is accelerated, but the duration of growth is shortened, by the even more rapid process of maturation. The end result is that the individual is of shorter stature than normal. This has been experimentally reproduced in monkeys by the administration of testosterone (van Wagenen, 1947).

Observations of this nature do not, of course, mean that the cessation of normal growth in length is necessarily associated only with the increased production of sex hormones around the period of puberty. Whether the sex hormones influence the skeleton directly or indirectly through other endocrine glands, especially the pituitary, is not very clear. Observations on hypophysectomized animals show, however, that testosterone may have a direct effect on the epiphyseal cartilage (Simpson et al., 1944, Reiss et al., 1946). It is likely that changes in growth and general development of the body associated with an increased, or a decreased, production of sex hormones result from a complicated mechanism.

The influence of androgens, mainly testosterone and its esters, on the body development has been repeatedly studied in animals. The results, however, vary considerably. It has been shown by different authors that administration of androgens to young castrated or normal animals inhibits the rate of weight increase (Korenchevsky et al., 1937, Bauer & Koch, 1937, Rubinstein et al., 1939, Shay et al., 1941, Rubinstein & Solomon, 1941 (a), Ettinger, 1942, Reiss et al., 1946, Seguin,
In other experiments androgens have caused an increase in weight (Korenchevsky et al., 1937, Bulliard & Ravina, 1938, Bottomley & Folley, 1938, Clausen & Freudenberg, 1939, Coffman & Koch, 1939, Rubinstein & Solomon, 1940 (a) and 1940 (b), 1941, (b), Shay et al., 1941, Seguin, 1946, van Wagenen, 1947 and 1949). In a third group, there has been no effect at all on the weight (McEuen et al., 1937, Bauer & Koch, 1937, Bottomley & Folley, 1938, Sainton et al., 1938, Rubinstein & Solomon, 1940 (b), Shay et al., 1941, Turner et al., 1941). The same holds true for the growth in length, although most authors have obtained a reduction of growth (Levie, 1938, Rubinstein et al., 1939, Rubinstein & Solomon, 1941, Reiss et al., 1946) or no effect (Sainton et al., 1938, Clausen & Freudenberg, 1939, Rubinstein & Solomon, 1940 (b), Turner et al., 1941, van Heuverswyn, 1945). Investigations demonstrating a stimulating effect of androgens on growth in length are few (Rubinstein & Solomon, 1940 (a) and 1940 (b), van Wagenen, 1947 and 1949).

These divergent results are possibly due to some extent to variation in the dose of the androgen. Rubinstein & Solomon (1940) are of the opinion that in rats small doses of testosterone propionate have a stimulating effect on growth, but that larger doses inhibit growth. Korenchevsky et al. (1937) reached the same conclusion in their investigations on weight increase in castrated rats. Factors other than the dose also influence the results. Among these are the type of androgen, the duration of the treatment, the age and sex of the animals, and castration before treatment, but these factors will not account for all the opposing results that have been reported (Gardner & Pfeiffer, 1943). It is remarkable that experiments which have been performed with the same dose and under similar conditions have not given the same results. It is difficult in some experiments to draw definite conclusions as the data are incomplete or there is no statistical analysis.

The effect of testosterone on the epiphyseal cartilage has mainly been investigated in rats and mice (Levie, 1938, Sainton et al., 1938, van Heuverswyn, 1945, Silberberg & Silberberg,
1946, Reiss et al., 1946). It might be expected that, considering the variations in the results on growth in length, the reports on the effect of testosterone on the epiphyseal cartilage would be conflicting. This is not the case. Rats and mice treated with testosterone have a narrower cartilage indicating a decreased cell proliferation and an intensified and early ossification. In mice in which both proliferation and maturation in the epiphyseal cartilage have been inhibited by castration, adequate doses of testosterone appear to restore the natural rate of these processes (Silberberg & Silberberg, 1946).

The variations in results of animal experiments make it difficult to draw definite conclusions from the literature. The opinion, that in male rats, testosterone can prevent the inhibitory effect of castration on growth, appears well founded and is supported by investigations on the histology of the epiphyseal cartilage. Whether large doses of testosterone really reduce the rate of both growth in length and gain in weight does not seem quite certain. Direct measurement of the length gives conflicting results, but histological investigation of the epiphyseal cartilage indicates that growth in length can be reduced by larger doses of testosterone.

MATERIAL AND METHODS

The material consists of immature rabbits which have been injected subcutaneously with testosterone propionate dissolved in arachis oil. The testosterone treated animals were compared with litter mates, which with a few exceptions received corresponding amounts of arachis oil. The injections were started before the eighth day of life when the average weight was about 100 grams (maximum 160 grams). In litters in which the initial weight had a wide range, the animals intended for testosterone treatment were chosen so that each had a control with the same initial weight. No differentiation was made between males and females as it was almost impossible to decide
the sex at the age of one week without laparatomy. The animals were weighed three times each week.

Seventeen animals from 6 different litters (with the exception of 3 who received a somewhat lower dose) were injected with 5 mg. testosterone propionate 3 times each week and were compared with 17 controls. The litters were killed by air embolism at an average age of 53 days (minimum 47 and maximum 67). The left tibia and the left femur were X-rayed after dissection of the soft tissues, and the length of these bones was measured on prints from the X-ray film. In two other litters (each of 2 controls and 2 testosterone treated animals) only the length of the femur was measured.

The average length of the tibias in the testosterone treated animals and in the controls in each litter is shown in Table 1. Table 2 gives the corresponding figures for the femur. Table 3 shows the average weights.

In five of the litters (12 testosterone treated animals and 12 controls) the distal epiphyseal cartilage of the femur was examined histologically. Special care was taken to ensure that

<table>
<thead>
<tr>
<th>Average length of tibias in mm. in each litter</th>
<th>Difference in length in mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Testosterone treated group</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>52.5</td>
<td>50.4</td>
</tr>
<tr>
<td>63.8</td>
<td>63.7</td>
</tr>
<tr>
<td>74.1</td>
<td>71.8</td>
</tr>
<tr>
<td>67.2</td>
<td>65.0</td>
</tr>
<tr>
<td>68.5</td>
<td>67.5</td>
</tr>
<tr>
<td>66.4</td>
<td>65.8</td>
</tr>
</tbody>
</table>

\[ z = 1.38 \]
\[ s = 1.13 \]
\[ t = 2.73 \] (5 degrees of freedom)
\[ 0.02 < p < 0.05 \] : almost significant

Table 1.

Difference in length of the tibia in the two groups following testosterone treatment.
### Table 2.

<table>
<thead>
<tr>
<th>Control group</th>
<th>Testosterone treated group</th>
<th>Difference in length in mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.6</td>
<td>54.7</td>
<td>1.7</td>
</tr>
<tr>
<td>54.5</td>
<td>53.6</td>
<td>0.9</td>
</tr>
<tr>
<td>45.8</td>
<td>44.6</td>
<td>1.2</td>
</tr>
<tr>
<td>56.0</td>
<td>55.8</td>
<td>0.2</td>
</tr>
<tr>
<td>64.3</td>
<td>63.0</td>
<td>1.3</td>
</tr>
<tr>
<td>58.3</td>
<td>57.5</td>
<td>0.8</td>
</tr>
<tr>
<td>60.1</td>
<td>59.8</td>
<td>0.3</td>
</tr>
<tr>
<td>58.2</td>
<td>56.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\[ z = 0.96 \]
\[ s = 0.49 \]
\[ t = 5.18 \]

(7 degrees of freedom)

0.001 < p < 0.01 :: significant

Difference in length of the femur in the two groups following testosterone treatment.

### Table 3.

<table>
<thead>
<tr>
<th>Control group</th>
<th>Testosterone treated group</th>
<th>Difference in weight in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>767</td>
<td>745</td>
<td>22</td>
</tr>
<tr>
<td>687</td>
<td>682</td>
<td>5</td>
</tr>
<tr>
<td>342</td>
<td>338</td>
<td>4</td>
</tr>
<tr>
<td>540</td>
<td>535</td>
<td>5</td>
</tr>
<tr>
<td>939</td>
<td>904</td>
<td>35</td>
</tr>
<tr>
<td>540</td>
<td>540</td>
<td>0</td>
</tr>
<tr>
<td>920</td>
<td>890</td>
<td>30</td>
</tr>
<tr>
<td>852</td>
<td>750</td>
<td>102</td>
</tr>
</tbody>
</table>

\[ z = 25.38 \]
\[ s = 33.65 \]
\[ t = 2.13 \]

(7 degrees of freedom)

0.05 < p < 0.1 :: not significant

Difference in the weight in the two groups following testosterone treatment.
the section through the cartilages was made from the same place, and in the same direction, in all the animals. The width of the cartilage was measured by microphotographs.

In order to study the development of the ossification centres, 12 rabbits from six different litters, some litters other than the above, were injected with various doses of testosterone propionate (minimum 18 mg. over 16 days, maximum 125 mg. over 14 days). Eighteen were kept as controls. When the rabbits weighed 150 grams to 250 grams (10—20 days) the left hindleg was X-rayed. The appearance and the number of ossification centres were compared with those of the corresponding leg in the untreated litter mates.

RESULTS

Table 1 and 2 show that there is a difference in the length of the tibia and femur, between the testosterone treated group and the controls. Both bones in the testosterone treated group tend to be shorter than the corresponding bones in the controls. A statistical analysis shows that the difference is very slight; nevertheless the femur results are significant and the tibia results almost significant.

Table 3 shows that there is no significant difference in the weight between the testosterone treated group and the controls.

The histological examination of the epiphyseal cartilage did not show any difference between the testosterone treated group and the controls. The width and the histological appearance are the same in both groups. The line of ossification is normal in the testosterone treated animals. No histological changes were found in the metaphysis.

The roentgenological investigation of the left hindleg shows that the heavier the animal (in the same litter i.e. the same age) the more developed are the ossification centres. The difference in appearance is, however, only obvious when the dif-
ference in weight is fairly great. Between the testosterone treated group and the controls, there is no difference in the appearance or number of the ossification centres, even when very large doses of testosterone propionate were injected during the first few weeks of life.

DISCUSSION

During the normal development of the skeleton, the processes of proliferation and of differentiation in the epiphyseal cartilage are parallel and balance each other. A disturbance of the internal balance of these processes must necessarily cause changes in the growth in length. An inhibition of growth in length may theoretically be caused either by a slowing of the process of proliferation, or an acceleration of the differentiation, probably associated with an inhibition of proliferation. A reduced growth in length is also the result of an inhibition of both processes, without any disturbance of their internal relationship. In the latter case, there are no changes in the histology or in the width of the epiphyseal cartilage. An acceleration of differentiation, or an inhibition of proliferation must, on the other hand, be reflected in the epiphyseal cartilage by a decreased width and changes in the histological appearance. Other investigations have demonstrated that testosterone propionate has this effect on the epiphyseal cartilage of mice, rats and guinea pigs.

It would appear that in experiments in which androgens have caused a stunting of growth in length, this has probably been caused by an upset in the internal balance between proliferation and differentiation, i.e., proliferation is relatively slow or differentiation is relatively fast.

In the present investigation, this effect of the testosterone propionate has not been demonstrated in rabbits. The histological examination of the epiphyseal cartilage, and the roentgenological investigation of the ossification centres showed that these were normal.
During the first few weeks of life the ossification centres in the extremities of the rabbit develop rapidly. By the administration of large doses of thyroid hormone during this period of life, it is possible to increase the degree of maturity (differentiation) of the skeleton. It is possible to demonstrate this by roentgenological examination of a leg. The ossification centres increase in number and size, and appear more mature in the thyroid treated animals than in the control group (Bergstrand, 1945). It is not possible to accelerate maturation during the first few weeks of life by the administration of testosterone propionate to such an extent that this is detected by X-ray examinations. This may be explained by assuming that the maturation effect of the testosterone on the skeleton is slower than that of the thyroid hormone.

In the present investigation, on the other hand, no histological changes were observed in the epiphyseal cartilage even after a longer period of testosterone administration. In the light of earlier experiments on the effect of testosterone on the epiphyseal cartilage this is surprising, especially when inhibition of growth in length has been demonstrated. The most likely explanation is that the inhibitory action of the testosterone was so slight that the effect was not reflected in the histology of the epiphyseal cartilage. It is possible that a continuation of the experiment for several months would have given a more marked reduction in the length of the long bones and decreased width of the epiphyseal cartilage.

SUMMARY

Immature rabbits were injected with 5 mg. testosterone propionate three times a week from the beginning of the second week of life to an average age of 53 days. The lengths of the left femur and of the tibia were measured on X-ray films and the animals were compared with controls from the same litter.
The femur and the tibia of the testosterone treated animals were shorter, but the difference was slight and significant only for the femur and almost significant for the tibia.

The difference in weight between the testosterone treated animals and the controls was not significant.

Histological examination of the distal femoral epiphyseal cartilage did not show any changes, in contrast to earlier investigations in rats and mice.

Immature rabbits were injected with various doses of testosterone propionate (including very high doses) during the first few weeks of life. Roentgenological examination of the left hind leg did not show any changes in comparison with controls from the same litter.

The significance of these results is discussed. It is pointed out that an inhibition of growth in length may, or may not, be accompanied by histological changes in the epiphyseal cartilage. Earlier investigations show that, in rats and mice, testosterone propionate has an effect on the histology of the epiphyseal cartilage. The absence of such changes in the present investigation can be explained by assuming that the inhibition of growth in length was too slight to be reflected in the epiphyseal cartilage.

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REFERENCES