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The effect of androgens on tissue regeneration

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Androgens, male sex hormones, have been shown to increase the overall growth rate of mammals (Brody, 1945; Gaunt, 1954), though there is some doubt as to their influence on reparative growth. Promitotic activity has been demonstrated (Montagna & Kenyon, 1949), and Barbera, Pollice & Mazzarella (1962) have reported that certain doses of androgens stimulate the production of granulation tissue in wounds. It has also been shown (Joseph & Dyson, 1966*a*) that the administration of synthetic anabolic androgens to females stimulates regeneration. In contrast Taubenhaus & Amromin (1949) have demonstrated that large doses of testosterone have an inhibitory effect on granulation tissue formation, while Cavallero, Maurizio, Baroni & Lami (1963) have reported that anabolic androgens administered alone are unable to influence the growth of granulation tissue.

During a quantitative study of tissue regeneration in the rabbit's ear it was observed that growth of the regenerate was significantly faster in males than in females (Joseph & Dyson, 1965). It was suggested that the higher androgen level in males might be concerned in producing this effect, since androgens possess considerable anabolic activity. The present paper describes a series of experiments performed to test this possibility.

MATERIALS AND METHODS

Mature rabbits, all over 6 months old, of mixed stock and of mean initial weight 2·975 kg (S.D. $\pm$ 0·549) were used.

Operating technique

The rabbits were anaesthetized with intravenous Nembutal, supplemented with anaesthetic ether as required, and the surfaces of the ears were cleansed with a 1 % solution of Cetrimide. With a specially designed punch a square piece of tissue of area 1 cm^2 was excised through the whole thickness of each ear, in a region free from major blood vessels. Tattoo marks were made in the ventral (inner) skin about 1–2 mm from each corner and the mid-point of each side of the square with a no. 15 straight triangular suture needle dipped in indian ink. No dressing was applied. The wounded ears were photographed from the ventral side immediately after operation and subsequently at intervals of 7 d, so that a photographic record was obtained of the growth of the regenerate which formed at the margins of the excision zone (Figs. 1, 2). A centimetre rule was placed next to and in the same plane as the wound before photographing. The animals were weighed at least once a week.

Measurement of the regenerate

The extent of growth of the regenerate, in terms of surface area, was calculated from projections of photographic transparencies on to graph paper, using the method described in an earlier paper (Joseph & Dyson, 1966*b*). The area of the regenerate was arrived at by subtracting the area of the hole (see Fig. 2) from the total area enclosed by mature tissue. Only the contribution of the regenerate towards the closure of the hole was considered since it has been shown elsewhere (Joseph & Dyson, 1966*b*) that though the rate of regeneration varies with the sex of the animal the extent of contraction does not. All areas were recorded as percentages of the area of the initial lesion to aid comparison.

Experimental treatment

Groups of animals were treated as follows:

A. *Androgen implantation*

(1) In four females four pellets of fused testosterone, each weighing 5 mg, were inserted around the margins of the excision zone of one ear, between the dorsal skin and the cartilage as shown in Fig. 3. They were held in place by stitches. The other ear had tissue excised in the usual way but was left as an untreated control.

(2) A further group of four females was treated in a similar manner to the group above but dummy pellets, consisting of 80% calcium pyrophosphate and 20% acacia gum, were used instead of the pellets of fused testosterone.

B. *Androgen injection*

The androgens used were androst-4-en-17β-3-one phenyl propionate (testosterone phenyl propionate or TPP), and 19-nor-androst-4-en-17β-3-one phenyl propionate ('Durabolin', nandrolone phenyl propionate or NPP). They were dissolved in sesame oil to which 0·5% phenol had been added as a bacteriostat and were administered by subcutaneous injection.

(1) Four groups, each of six females, received the following injections: (*a*) Low-dose TPP, 5 mg/ml, 1 mg/3 kg body weight 5 times weekly. (*b*) High-dose TPP, 50 mg/ml, 25 mg/3 kg body weight twice weekly. (*c*) Low-dose NPP, 5 mg/ml, 1 mg/3 kg body weight 5 times weekly. (*d*) High-dose NPP, 35 mg/ml, 17·5 mg/kg body weight twice weekly.

(2) Two groups, each of six males, received the following injections: (*a*) Low-dose TPP, 5 mg/ml, 1 mg/3 kg body weight 5 times weekly. (*b*) Low-dose NPP, 5 mg/ml, 1 mg/3kg body weight 5 times weekly.

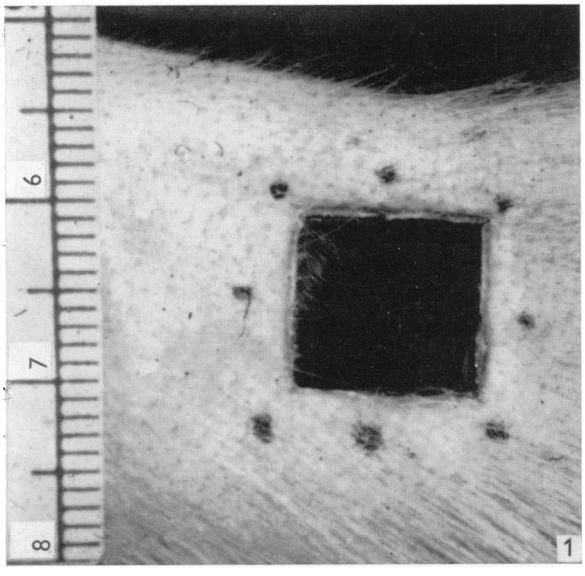
The total volume of fluid injected was thus 1 ml/3 kg body weight/week in all cases.

(3) Six females were injected with a total of 1 ml/week of the carrier, sesame oil containing 0·5% phenol, administered in five doses of 0·2 ml.

C. *Orchidectomy*

Orchidectomy was performed so that the effect of decreased androgen on regeneration in males could be studied.

(1) Six males were orchidectomized immediately before excision of ear tissue.



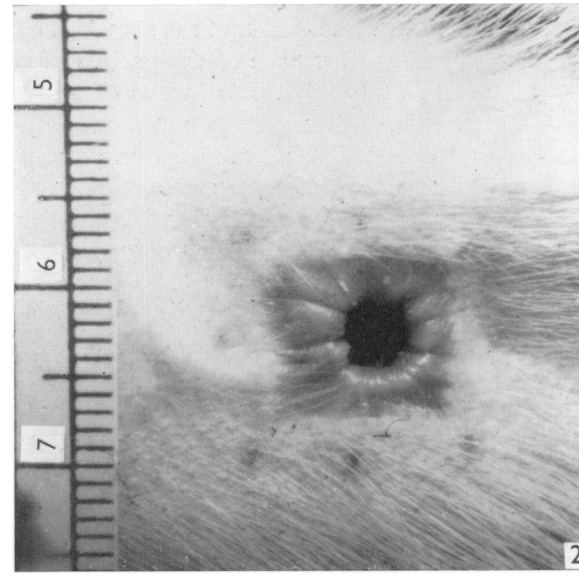


Fig. 1. The appearance of the ear immediately after excision.

Fig. 2. The appearance of the ear 35 d after excision. A blastema has developed at the cut edges of the mature ear tissue.

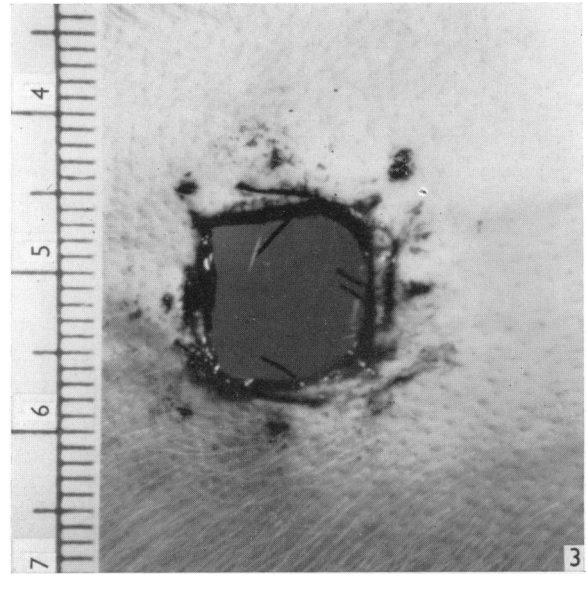


Fig. 3. The ventral view of the ear after excision and implantation of testosterone pellets. The pellets are held in place by the sutures.

(2) A further 12 males were orchidectomized, but excision of ear tissue was delayed for: (*a*) 14 d in 3 males; (*b*) 21 d in 3 males; (*c*) 28 d in 6 males.

The mean areas and the standard errors of the means of the ear regenerates were calculated for each of the groups of rabbits in Expts. A, B and C, at 7 d intervals from 14 to 49 d. The means of the experimental groups were compared with the means of the untreated controls, comprising 12 females and 18 males, and Student's *t* calculated using the method described by Simpson, Roe & Lewontin (1960) for testing the differences between the means of two samples. Experimental females were compared with control females (Table 2), and experimental males with control males (Table 3). In most cases, where the aim of the experiment was to determine whether androgens increased the rate of regeneration, one-sided tests were applied; where the aim was simply to see if there was a significant difference between the experimental and control groups, as in Expts. B2 and B3, two-sided tests were applied.

D. *17-oxosteroid determinations*

Quantitative estimations of the amount of urinary 17-oxosteroids excreted during 24 h periods were made for a group of rabbits (8 male and 6 female) by a modification of the Zimmerman technique (Zarrow, Yochim & McCarthy, 1964). The average urinary 17-oxosteroid level over a period of 6 weeks was correlated with the area of the regenerate at 49 d.

E. *Histological examination*

At the end of the experiments the animals were killed and samples of the following tissues were removed and fixed in Bouin's fixative: the regenerate, liver, kidney, adrenal, ovary, uterus and testis. Fixed tissue was dehydrated, embedded in paraffin wax (m.p. 56 °C) and sectioned at 10 μm. The sections were stained with haematoxylin and eosin and examined microscopically. In selected cases where abnormalities were found standard histochemical tests were applied for the following substances: DNA, RNA, glycogen, lipids, proteins and mucopolysaccharides (Pearse, 1961).

RESULTS

A. *The effect of androgen implantation*

The effect of implanting testosterone pellets around the margin of one wound was to increase the rate of growth in both ear regenerates (Table 1 and Fig. 4). By 49 d the mean areas of the regenerates of both the experimental and the opposite ears of the testosterone treated rabbits were significantly greater than the mean area of the regenerates of the untreated females, the results of which are given in Table 2 ($t = 2{\cdot}638$, D.F. = 14, $P = 0{\cdot}010$–$0{\cdot}005$ for the former, and $t = 2{\cdot}200$, D.F. = 14, $P = 0{\cdot}025$–$0{\cdot}010$ for the latter). The mean areas of both the experimental and the opposite ear regenerates of the rabbits receiving dummy pellets, however, were not significantly greater than the mean area of the regenerates of the untreated females at 49 d ($t = 0{\cdot}02$, D.F. = 14, $P > 0{\cdot}45$ for the former, and $t = 0{\cdot}285$, D.F. = 14, $P = 0{\cdot}40$–$0{\cdot}35$ for the latter).

Testosterone implants of the type used in this experiment do not appear, at the

light microscope level, to cause damage to the ovary, uterus, adrenal or kidney. In one animal slight necrosis was observed in a localized zone of the kidney but the rest of the tissue examined was normal in appearance and it is possible that the damage was due to a processing artefact.

Table 1. *Effect of testosterone implantation of regenerative growth in females*

Treatment	Days after excision	*N*	Mean area of regenerate as % of initial deficit	S.E.
(1) Testosterone pellets				
(*a*) Experimental ear	14	4	*	*
	21	4	54·4	±11·7
	28	4	64·4	± 7·9
	35	4	73·8	± 6·5
	42	4	80·9	± 3·8
	49	4	91·9	± 5·4
(*b*) Opposite ear	14	4	31·5	± 4·5
	21	4	53·3	±11·2
	28	4	55·2	± 9·9
	35	4	68·1	± 9·6
	42	4	81·5	± 5·9
	49	4	87·8	± 5·4
(2) Dummy pellets				
(*a*) Experimental ear	14	4	*	*
	21	4	42·9	± 2·3
	28	4	51·2	± 8·0
	35	4	57·7	± 1·2
	42	4	63·7	± 6·9
	49	4	67·0	± 7·2
(*b*) Opposite ear	14	4	16·5	± 4·9
	21	4	29·3	± 6·1
	28	4	46·5	±11·4
	35	4	57·4	± 8·0
	42	4	63·5	± 9·9
	49	4	70·3	±11·6

* Not measured, scab still attached.

B. *Androgen injection*

(1) *The effect of synthetic androgens on females*

(*a*) *TPP.* Both high and low doses of TPP increased the growth of the regenerate in females significantly in comparison with the growth in untreated females. The growth rate increased to the male level (Joseph & Dyson, 1966*a*). By 49 d the mean area of the regenerates from females treated with a high dose of TPP was 18·3 % higher than that of the untreated controls (t = 2·027, D.F. = 16, P = 0·05–0·025). The mean area of the regenerates of females given a low dose was 18·6 % higher than that of the controls at 49 d (t = 2·365, D.F. = 16, P = 0·025–0·010).

Some histological abnormalities were detected after prolonged treatment of females with TPP at the high dose level. Several ovaries were smaller and had fewer ovarian follicles and a denser stroma than ovaries from untreated animals. In one case where TPP had been given at the high dose level for 112 d the ovaries were reduced in size and contained atypical follicles with a vacuolated, basophilic

secretion containing neutral mucopolysaccharides and either a muco- or a glycoprotein (Fig. 5). Degenerate cells with deeply staining chromatin were present. Of the six rabbits treated with TPP at the high dose level, three had pseudopregnant uteri and in the remaining three the uteri were at the proliferative stage. One of the adrenals examined showed small lesions in the zona fasciculata but the others were normal in appearance. One rabbit had small oviducal cysts.

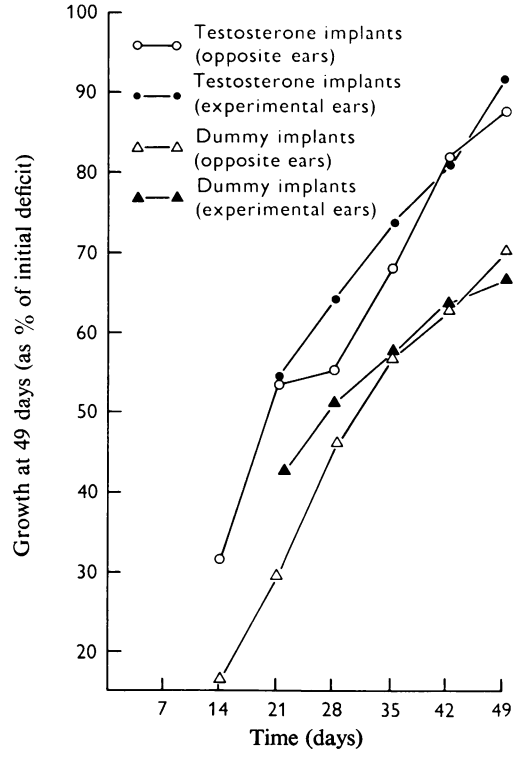


Fig. 4. Graph showing the growth rates of the regenerates in animals with testosterone implants and in animals with dummy implants.

No histologically detectable abnormalities were found in the tissues of the rabbits treated with TPP at a low dose level.

(*b*) *NPP*. Both high and low doses of NPP increased the growth of the regenerates of females significantly compared with the regenerates of untreated females. The growth rate exceeded that of normal males (Joseph & Dyson, 1966*a*). By 49 d the mean area of the regenerates of female rabbits treated with a high dose of NPP was 24·6 % higher than that of the untreated control females (t = 3·022, D.F. = 16, P = 0·005–0·0005). The mean area of the regenerates of females given a low dose of NPP was 20·0 % higher than that of the control females (t = 2·630, D.F. = 16, P = 0·010–0·005). Details of the growth of the regenerates of females treated with androgens are given in Table 2.

The ovaries of animals treated with a high dose of NPP showed no atypical

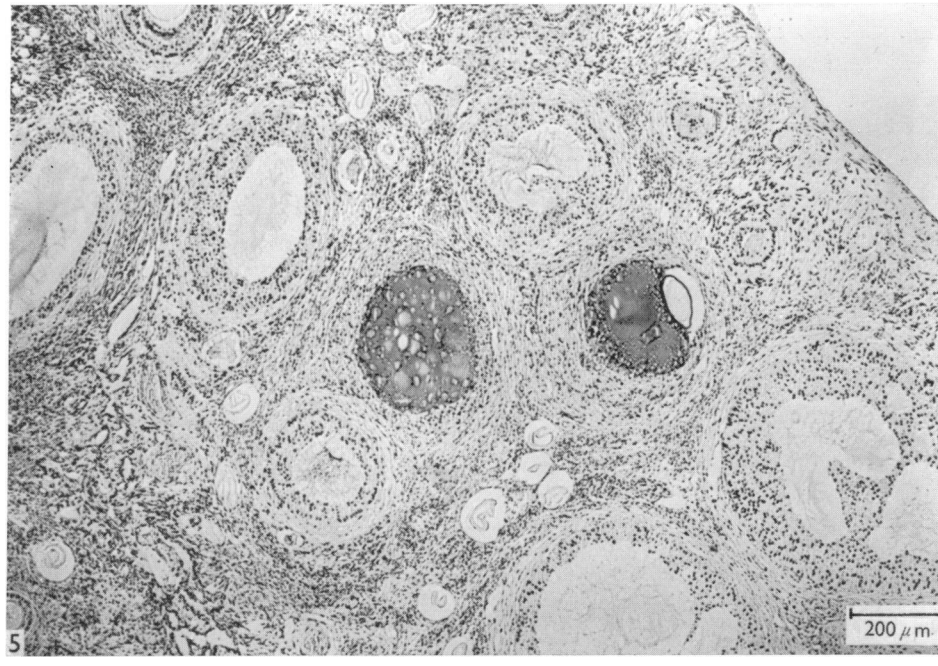


Fig. 5. Appearance of the ovary of a rabbit treated with a high dose of TPP for 112 d. A basophilic vacuolated secretion can be seen in certain follicles. (Toluidine blue.)

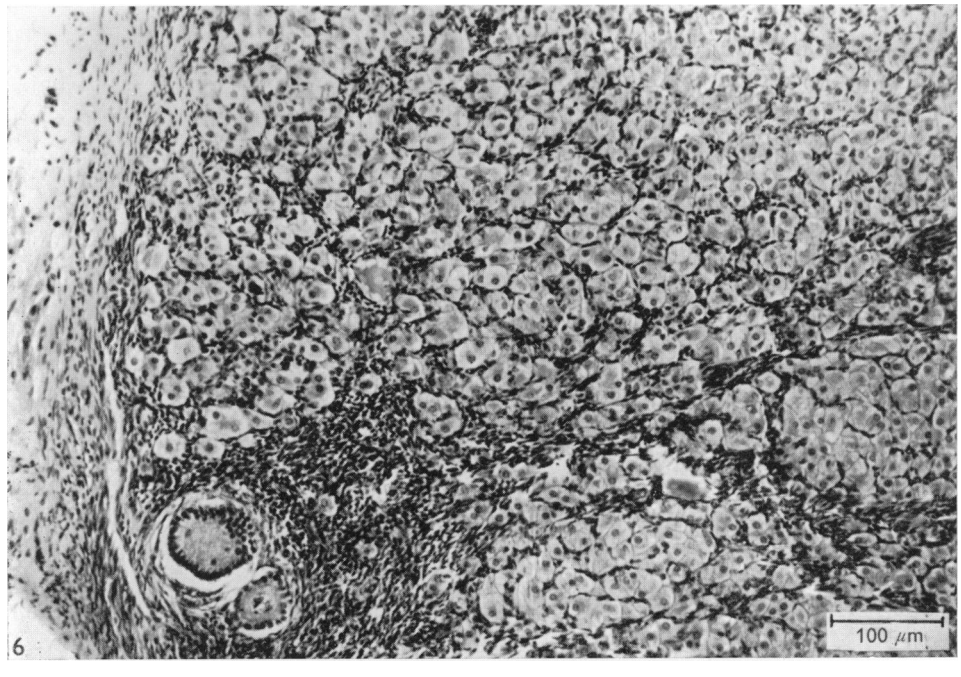


Fig. 6. Secretory cells in the ovary of a rabbit treated with low dose NPP for 49 d (H. and E.).

features. The uterine endometrium was highly vascular, with enlarged, branched and actively secreting glands. Three of the rabbits were pseudopregnant and one had oviducal cysts. One of the adrenals examined had numerous cells with pyknotic nuclei in the zona fasciculata of the cortex but the others were normal in appearance. All the kidneys examined were normal.

Table 2. *Effect of synthetic androgens on regenerative growth in females*

Treatment	Days after excision	*N*	Mean area of regenerate as % of initial deficit	S.E.
High-dose TPP	14	5	39·6	±6·8
	21	6	58·0	±5·1
	28	6	68·2	±7·5
	35	6	77·5	±8·9
	42	6	80·2	±8·9
	49	6	85·5	±7·9
Low-dose TPP	14	4	41·0	±8·4
	21	6	48·5	±4·9
	28	6	65·7	±1·5
	35	6	78·3	±4·8
	42	6	83·3	±5·0
	49	6	85·8	±4·6
High-dose NPP	14	5	35·8	±5·5
	21	6	62·5	±3·0
	28	6	79·5	±5·0
	35	6	89·5	±3·9
	42	6	90·3	±4·6
	49	6	91·8	±5·5
Low-dose NPP	14	4	41·8	±7·8
	21	6	55·8	±7·5
	28	6	72·2	±4·7
	35	6	78·3	±4·4
	42	6	84·8	±3·2
	49	6	87·2	±3·5
Carrier injections	14	3	23·7	±2·3
	21	5	33·1	±4·2
	28	5	34·7	±4·9
	35	5	47·0	±8·1
	42	5	50·9	±8·9
	49	5	62·9	±7·5
Unrelated controls	14	11	21·6	±4·1
	21	11	38·9	±5·0
	28	12	45·3	±5·6
	35	12	57·1	±5·6
	42	12	63·2	±5·5
	49	12	67·2	±4·9

The ovaries of rabbits treated with NPP at a low dose level showed normal follicle maturation but their endocrine-secreting cells were larger and more numerous than those of control ovaries (Fig. 6). Four of the uteri examined were pseudopregnant. All the kidneys and adrenals examined were normal.

(2) *The effect of synthetic androgens on males*

(*a*) *TPP.* The treatment of males with TPP at a low dose level did not affect the growth of the regenerate significantly. By 49 d the mean area of the regenerates of untreated control males was 85·2 ± 2·6 % while that of TPP-treated males was 81·0 ± 5·6 % (see Table 3). Comparison of these two means gave a value of $t = 0{\cdot}764$, D.F. = 22, $P = 0{\cdot}5$–$0{\cdot}4$.

Table 3. *Effect of synthetic androgens on regenerative growth in males*

Treatment	Days after excision	*N*	Mean area of regenerate as % of initial deficit	S.E.
Low-dose TPP	14	6	35·1	±5·7
	21	6	47·5	±8·1
	28	6	54·3	±8·2
	35	6	65·2	±7·3
	42	6	73·1	±7·3
	49	6	81·0	±5·6
Low-dose NPP	14	6	32·1	±4·1
	21	6	48·7	±2·7
	28	6	57·1	±4·8
	35	6	66·4	±5·8
	42	6	69·7	±4·9
	49	6	76·0	±5·2
Untreated controls	14	18	22·3	±3·5
	21	18	49·1	±2·4
	28	18	63·9	±2·8
	35	18	72·8	±2·6
	42	18	77·0	±2·8
	49	18	85·2	±2·6

No histologically detectable abnormalities were found in the organs examined.

(*b*) *NPP.* The treatment of males with NPP at a low dose level resulted in a decrease in the amount of regenerative growth achieved by 49 d, 76·0 ± 5·2 % as compared with 85·2 ± 2·6 % in the untreated males ($t = 1{\cdot}752$, D.F. = 22, $P = 0{\cdot}1$–$0{\cdot}05$). At 35 d the growth of the regenerates of treated and untreated males was not significantly different ($t = 1{\cdot}180$, D.F. = 22, $P = 0{\cdot}3$–$0{\cdot}2$).

The kidneys of all NPP treated males were normal in appearance. Small necrotic areas were found in the liver of one of the animals, though the livers of the rest were undamaged. In two animals the testes contained few mature spermatozoa; the testes of the other rabbits were normal.

(3) *The effect of the carrier on females*

Although animals receiving regular injections of the steroid carrier (sesame oil plus 0·5 % phenol) had a lower regeneration rate initially than the control females, the difference in the mean areas of the regenerates of the two groups at 49 d was not significant. The mean area of the carrier-treated regenerates was 62·9 ± 7·5 % at 49 d, and that of the regenerates of untreated control females was 67·2 ± 4·9 % ($t = 0{\cdot}464$, D.F. = 15, $P = 0{\cdot}7$–$0{\cdot}6$).

The histological appearance of the liver, kidney, uterus, ovary and adrenal was normal.

The effect of synthetic androgens on the regenerative rate is summarized graphically in Figs. 7 and 8.

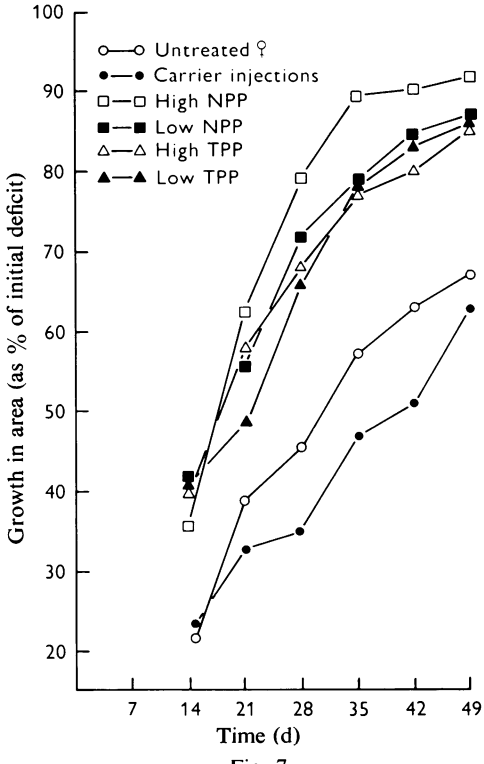


Fig. 7

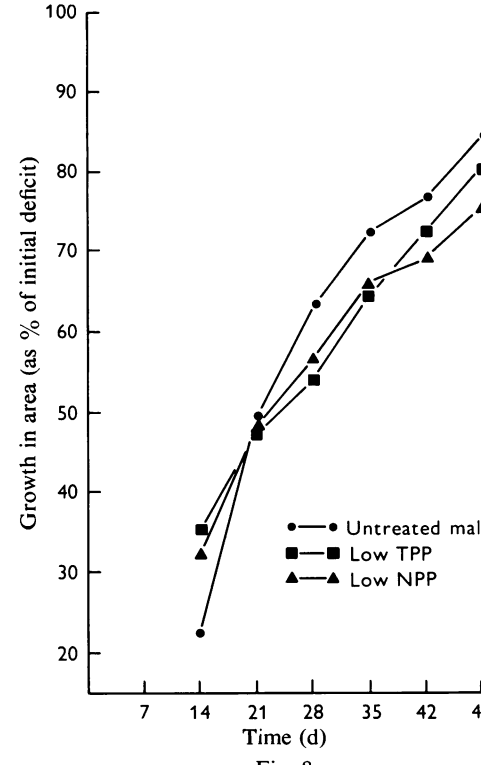


Fig. 8

Fig. 7. Graph showing the effect of synthetic androgens on the regenerative rate in females.
Fig. 8. Graph showing the effect of synthetic androgens on the regenerative rate in males.

C. *Orchidectomy*

(1) *The effect of orchidectomy immediately before excision of ear tissue*

The mean size of the regenerate produced by 49 d in the experimentally castrated animals was not significantly less than that of the control males (t = 0·099, D.F. = 22, P > 0·45). The amount of growth achieved is recorded in Table 4. There was a higher replacement rate initially in the experimental group, resulting in more growth than in the controls by 14 days (Fig. 9). The growth rate of the experimental group then decreased and for the last 21 d of the regenerative period studied, that is from 28 to 49 d, the growth rate of the tissue in the experimental group was less than that in the control males.

No histological abnormalities were found in the liver or kidney. The zona reticularis of the adrenal cortices of five of the group contained enlarged cells which had prominent nucleoli and appeared to be distended with secretory products (Fig. 10).

(2) *The effect of orchidectomy followed by delayed excision of ear tissue*

Excision of ear tissue was delayed for 14, 21 and 28 d respectively in three groups of castrated males. In those in which excision was delayed for 14 d there was an extremely rapid initial growth of new tissue so that at 14 d after excision the area of the regenerates from the experimental ears (52·6 ± 9·8 %) differed considerably from that of the control males (22·3 ± 3·5 %). This difference is significant (t = 3·228, D.F. = 19, P = 0·01–0·001). The growth rate then fell gradually and by the end of the regenerative period the areas of both experimental (87·6 ± 0·4 %) and control (85·2 ± 2·6 %) regenerates were not significantly different (t = 0·380, D.F. = 19, P = 0·8–0·7).

Table 4. *Effect of orchidectomy on regenerative growth*

Treatment	Days after excision	*N*	Mean area of regenerate as % of initial deficit	S.E.
Immediate excision	14	4	35·5	± 5·0
	21	6	54·1	± 6·5
	28	6	76·5	± 7·9
	35	6	81·9	± 6·2
	42	6	81·9	± 4·4
	49	6	84·7	± 4·7
Delayed excision				
(*a*) 14-d delay	14	3	52·6	± 9·8
	21	3	59·5	± 11·3
	28	3	69·1	± 13·0
	35	3	71·6	± 12·0
	42	3	75·7	± 8·3
	49	3	87·6	± 0·4
(*b*) 21-d delay	14	3	43·7	± 1·9
	21	3	66·7	± 2·6
	28	3	68·9	± 2·8
	35	3	73·5	± 4·1
	42	3	77·5	± 3·7
	49	3	78·0	± 3·9
(*c*) 28-d delay	14	6	34·8	± 8·7
	21	6	46·7	± 10·2
	28	5	53·2	± 6·0
	35	6	63·1	± 7·3
	42	6	65·1	± 7·6
	49	5	69·5	± 7·4

In those animals with a 21-d and a 28-d delay in excision after castration the amount of growth achieved by 14 d was less than that in the 14-d delay group, but greater than that in the control males. The mean areas of the regenerates of both 21-d and 28-d delay groups were less than that of the control males at 49 d (t = 1·087, D.F. = 19, P = 0·3–0·2 for the 21-d delay group and t = 2·528, D.F. = 21, P = 0·02–0·01 for the 28-d delay group), but only in the latter group was the difference significant.

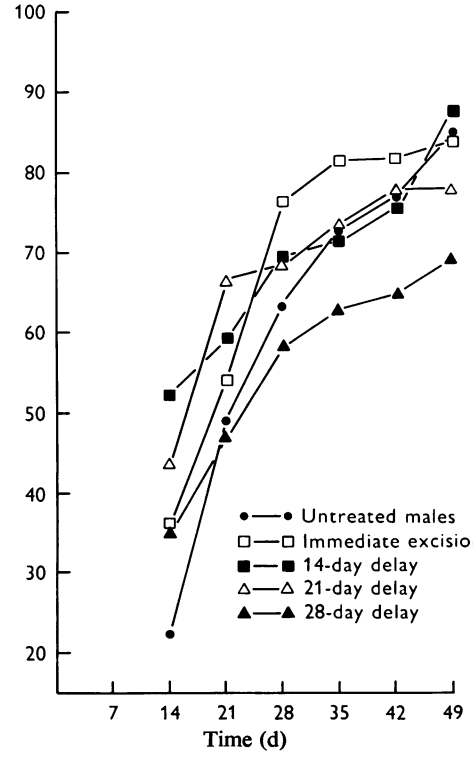


Fig. 9. Graph showing the effect of orchidectomy on regenerative growth after immediate and delayed excision.

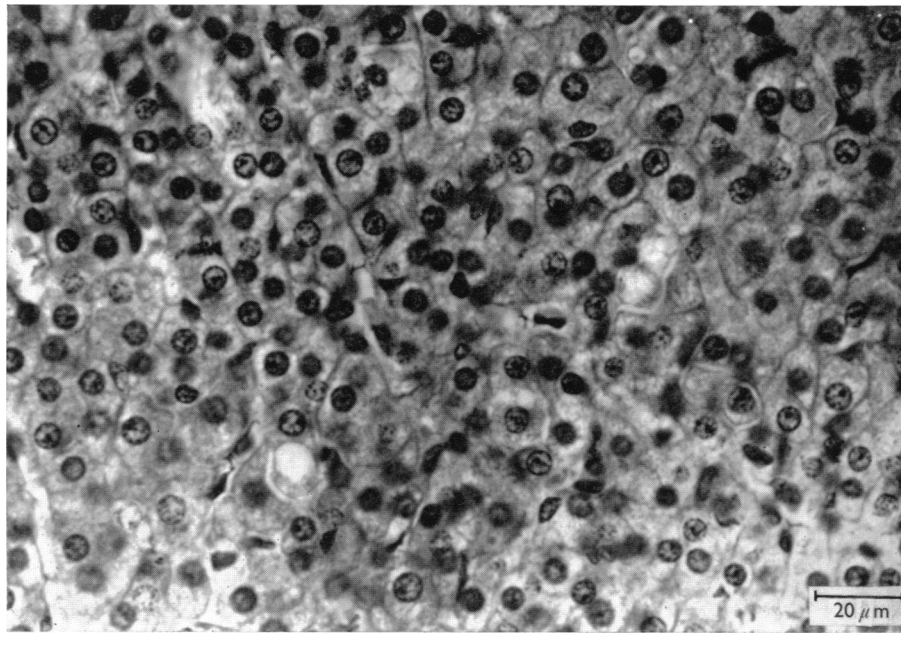


Fig. 10. Cells of the zona reticularis of the adrenal cortex of an orchidectomized male. (H. and E.)

Two-sided tests were applied to the differences in means recorded in this experiment.

D. *Correlation of regenerative growth with 17-oxosteroid excretion*

There is a positive correlation between 17-oxosteroid excretion, expressed as $\log_{10}$ of the 24-h production in μg, and the area of the regenerate at 49 d ($r = 0{\cdot}811$, $N = 13$, D.F. $= 11$, $P < 0{\cdot}001$). Fourteen rabbits were used in the experiment but one lost weight rapidly during the first two postoperative weeks and was therefore omitted from the calculations, though the results from it are shown on the graph (see Fig. 11).

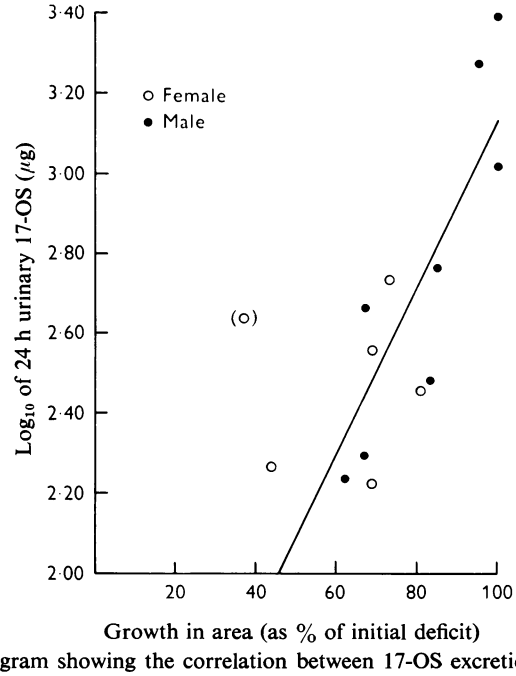


Fig. 11. Scatter diagram showing the correlation between 17-OS excretion and the extent of growth at 49 d. The result omitted from the calculation of the correlation coefficients is shown in brackets.

DISCUSSION

If androgen level does play some part in controlling the rate of regeneration the administration of androgens to females should increase the rate at which they replace lost tissue. The first method of administration was by pellet implantation. The results following the implantation of pellets of fused testosterone into one ear, while leaving the other ear untreated, indicate that the effects are systemic and not local since both ears showed an accelerated rate of growth. The increased rate is caused by the steroid and not simply by the presence of a foreign body (the pellet) close to the regenerate since implantation of dummy pellets does not result in an increased growth rate. Administration of synthetic androgens, namely testosterone phenyl propionate and nandrolone phenyl propionate, also increased the regeneration rate in females, while administration of the carrier did not. Nandrolone phenyl propionate is more anabolic and less androgenic than testosterone phenyl propionate,

and the results reported here show that the former is significantly more effective than the latter in promoting regeneration in females. Further supporting evidence is provided by the significant positive correlation found between the urinary excretion of 17-oxosteroids and the amount of regeneration by 49 d in different animals. The 17-oxosteroids are the waste metabolic by-products of gonadal androgens and adrenal corticosteroids, some of which are also anabolic androgens. The daily urinary excretion of 17-oxosteroids is generally greater in males than in females though the two groups overlap. This is of interest since there is also a similar overlap of the distribution curves of the rate of regeneration in males and females, although there is a highly significant difference between their mean rates (Joseph & Dyson, 1966*b*).

Attempts were also made to increase and decrease the androgen level in males. Administration of both TPP and NPP by injection in males did not significantly affect the rate of regeneration. Either there is an 'optimum' level of androgens for regeneration or their injection results in a reduction in the production of androgens by the animals. Removal of the testes may be expected to result in the reduction of androgen production though the adrenals also produce androgens and may possibly increase their production if the testes are removed. It was decided to limit our experiments to castration because of the trauma involved in removing the adrenals, and because of the additional complications of withdrawing from the animals the other important hormones produced by these organs. The experiments involving orchidectomy were of two types, those in which the testes were removed and the ear tissue was excised immediately afterwards, and those in which the removal of ear tissue was delayed for 14, 21 or 28 d. The results obtained are of considerable interest. There was an initial acceleration of tissue replacement in all groups but particularly in those animals with a 14-d delay between orchidectomy and ear tissue excision. This acceleration was followed by a reduction in growth rate so that, in spite of the initial increase, the amount of growth by 49 d was either similar to that of the control males (in the immediate excision and 14-d delay groups) or was significantly and progressively less than that of the controls (in the 21- and 28-d delay groups). The situation is thus more complex than can be explained by simple withdrawal of a source of anabolic androgens. The initial acceleration of repair found in all groups of orchidectomized males suggests that some anabolic effect is exerted by removal of the testes. It may be that the trauma of the operation causes the release of a distantly acting substance which either directly stimulates growth or destroys a growth inhibitor at the site of regeneration, the ear blastema. A further possibility is that removal of one source of anabolic androgen, the testes, stimulates by a negative feedback mechanism the production of such agents elsewhere, in the adrenal cortex for example. One or both of these processes appear to reach a maximum at about 14 days and thereafter decrease, since animals from which ear tissue was excised earlier or later than 14 d after castration showed less acceleration than did animals with a 14-d delay. If the rates of growth following the initial decrease are compared it is found that the highest rate is in the control group of normal males, and that there is a progressive reduction in growth rate in the castrated animals, with the 21- and 28-d delay groups showing a much lower replacement rate than the immediate excision and 14-d delay groups. This suggests

that there is a gradual falling off in the effectiveness of the anabolic agents produced in response to the trauma of castration and that by 70 d after castration their effect is less than that of the total anabolic androgens of the normal male.

SUMMARY

A series of experiments was performed to test the hypothesis that the higher rate of tissue regeneration recorded in males than in females was associated with the higher androgen level of the former. The replacement site chosen was the rabbit's ear. 1 cm^2 of tissue was excised through the whole thickness of the ear under Nembutal anaesthesia and the rate of replacement, in terms of increase in surface area of the regenerate, was recorded. The conclusions drawn from the experiments are listed below:

(1) The treatment of females with testosterone stimulates their regenerative growth.

(2) The synthetic steroids testosterone phenyl propionate and nandrolone phenyl propionate, both closely allied to the naturally occurring androgen, testosterone, also stimulate regenerative growth in females.

(3) The effect of the androgen is systemic rather than local.

(4) Orchidectomy is followed by a decreased rate of regeneration provided that a time lapse of sufficient length is allowed between orchidectomy and excision of ear tissue.

(5) There is a positive correlation between the regeneration rates of both males and females and their 17-oxosteroid excretion.

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