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TESTOSTERONE PHENYL PROPIONATE (TPP): BIOLOGICAL TRIALS WITH A NEW ANDROGEN

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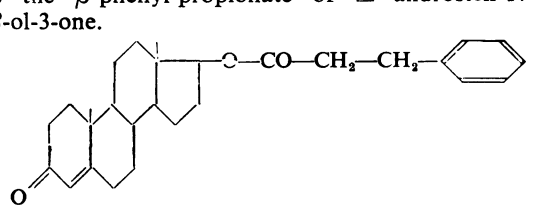
Testosterone propionate (TP) has been for a long time the most effective androgenic substance available. The search for a more potent compound has not, however, been allowed to lapse, as the need for such a therapeutic agent has been apparent for some years; during this time a number of highly active and easily made oestrogens have been discovered, and it has been hoped that parallel advances would widen the field of use of the male sex hormones. So far no synthetic non-steroid substance has been found to have marked androgenic activity. Attempts to increase the potency and duration of action of testosterone compounds have been limited to efforts to delay the absorption by forming crystalline depots or by adsorbing the androgen on aluminium phosphate, but these have not been uniformly successful. It was not until 1951 that a more potent compound, testosterone cyclopentylpropionate (TCP) was found by Ott, Kuizenga, Lyster, and Johnson (1952) and first reported by Sakamoto, Gordan, and Eisenberg (1951) and Lloyd and Fredericks (1951).

At the same time in these laboratories, Dr. C. L. Hewett (1951) produced another compound with a long side chain, testosterone phenyl propionate (TPP), which is easily made, and as far as can be ascertained has not been mentioned previously in the literature. This ester occurs as colourless needles melting at 116.5° C., $[\alpha]_D^{20°} + 88.7°$, and is readily soluble in the usual solvents. Chemically it is the β-phenyl-propionate of Δ^4-androsten-17 β-ol-3-one.



It was decided to investigate the maximal effect and duration of effect of single doses of TPP as compared with TP, and also to find the most suitable medium for injection of TPP and the most effective route of administration. A study of the morphological changes resulting from administration of TPP was also undertaken, with particular attention to any toxic effects from excess dosage.

METHODS

Assay Methods.—Normal immature 40- to 50-g. male albino rats from Wistar stock were used while being fed on Aberdeen Standard diet. The animals were castrated through a scrotal incision, and were injected 14 days after castration. Each animal received a single injection either subcutaneously in the back or intramuscularly in the hind leg. At intervals after the injection, groups of 3 to 6 animals were killed and weighed. The seminal vesicles and prostate were then dissected out, by cutting through the vasa deferentia and the urethra near the base of the bladder; the coagulating glands were then teased from the seminal vesicles, which were cut off as near as possible to the ejaculating ducts, and weighed, while fresh, on a torsion balance to the nearest 0.1 mg. The prostate and urethra were fixed overnight in Bouin's fluid, and on the next day the urethra was removed and the whole prostate dried on blotting-paper and weighed to the nearest 0.1 mg.

In all these experiments 1,012 male rats were used.

The preliminary tests were carried out by the method of Mathieson and Hays (1945), using subcutaneous injections of testosterone propionate (TP) in sesame oil as a standard of known androgenic activity whose effects were compared with those of TPP in sesame oil at two dose levels, 0.2 mg. and 0.8 mg., both in 0.2 ml., the ratio between the two dose levels being 1:4, and the lower dose being near the minimum for obtaining a measurable response. Other vehicles for the two androgens were also compared at these dose levels, by both intramuscular and subcutaneous routes.

In the second series of experiments a higher dose of 2.5 mg. in 0.1 ml. was used throughout, and readings were taken over a longer period to investigate the rate of involution of the glands. Readings were also made at very short intervals after injection, to

examine more fully the rate of growth to the peak. In these experiments TPP was again compared with TP, and it was also desired to compare the effects of different solvents and different routes of injection.

Control groups of castrated animals were untreated or injected intramuscularly with placebo solvents (ethyl oleate, sesame oil, and blank emulsion). These were killed on the 3rd and 10th days after injection.

The preliminary experiment carried out at two dose levels was treated as a 4-point test on the assumption that TPP and TP had qualitatively similar effects. The response at 5 days after injection was arbitrarily selected for the comparison.

It was felt, however, that none of the usual methods of comparison would give a clear expression of relative potencies, since both intensity and duration of activity were involved; it was possible to compare these independently, but preferable to find a single expression of potency. Accordingly, the weight of glands was plotted against time, and the area enclosed by each graph up to the point of 50% involution from peak stimulation, using the control level as ordinate, was used as an expression of the potency. The activity-duration index arrived at is in "milligram-day" units (from the graphical axes). This can readily be expressed as a ratio to the effect of TP in sesame oil, which was taken as unity at each dose level.

The activity-duration index estimated from the graphs in "milligram-day" units is included in the tables, except where no normal growth-time curve could be obtained, either through too little stimulation (doses of 0.2 mg.) or through irregular results (as were obtained through using the supersaturated solution of TPP in propylene glycol). The activity-duration as a ratio to TP in sesame oil as unity (at each dose level) is also shown in the tables.

Histological Examination. — The morphological effect of TPP on the testes in immature rats was studied by comparison of 12 treated animals with 12 untreated controls, the animals being of about 45 g. body weight. The treated animals were injected subcutaneously with 0.8 mg. of TPP in 0.2 ml. of sesame oil, and 3 of each group were killed on the 3rd, 4th, 7th, and 14th days after injection, and the testes completely sectioned and microscopically examined.

Toxicity tests were carried out by gross and microscopic examination of various organs in rabbits of both sexes. Six young rabbits (litter-mates) weighing approximately 1,300 g. were used. Four of these (2 male, 2 female) were injected subcutaneously with 30.5 mg. of TPP in 0.5 ml. sesame oil three times weekly for four weeks, the total dose of 366 mg. per rabbit being equivalent in testosterone content to 300 mg. of testosterone propionate; two rabbits (1 male, 1 female) served as controls. Body weights were recorded once weekly, and on the 30th day all animals were killed for histopathology. Tissues were fixed in Bouin's fluid and paraffin slices about 5 μ in thickness were cut. The haemalum-eosin method was used for general purposes. Bone-marrow was fixed in Zenker-formol solution, smears of bone-marrow being stained by Leishman's stain; for bone-marrow sections haemalum-eosin, haematoxylin-azo eosin, and eosin-methylene blue methods were used, some sections from each animal by each method.

Drugs and Media

Subcutaneously: Preliminary experiments: TP and TPP in sesame oil and in emulsions prepared by the method of Lens, Overbeek, and Polderman (1949) (all 1 mg./ml. and 4 mg./ml.). Higher-dosage experiment: TPP in ethyl oleate, propylene glycol, and aqueous suspension (all 25 mg./ml.). Histological experiment: TPP in sesame oil (4 mg./ml. for rats; 61 mg./ml. for rabbits).

Intramuscularly: Preliminary experiments: TP and TPP emulsions (1 mg./ml. and 4 mg./ml.). TP aqueous suspension (BDH) (5 mg./ml.). Higher-dosage experiment: TP and TPP in sesame oil, ethyl oleate, and emulsion (all 25 mg./ml.). TPP in propylene glycol (25 mg./ml. supersaturated). TP aqueous suspension (BDH) and TPP aqueous suspension (both 25 mg./ml.).

TABLE I

PRELIMINARY EXPERIMENT

Mean responses ± S.E. (3–6 rats) of seminal vesicles and prostates of castrated rats to doses of 0·2 mg. and 0·8 mg. of TP and TPP given subcutaneously in 0·2 ml. sesame oil

Days after Injection	Testosterone Propionate				Testosterone Phenyl Propionate			
	0·2 mg.		0·8 mg.		0·2 mg.		0·8 mg.	
	Sem. Ves. (mg.)	Prostates (mg.)	Sem. Ves. (mg.)	Prostates (mg.)	Sem. Ves. (mg.)	Prostates (mg.)	Sem. Ves. (mg.)	Prostates (mg.)
3	17·0±2·8	31·6±3·1	22·0±3·7	42·0±6·6	17·3±2·5	30·0±6·1	24·4±4·1	49·8±4·2
4	19·6±1·8	32·3±3·7	*31·5±3·8	47·0±6·1	25·5±3·9	37·5±4·0	34·8±9·6	51·5±14·0
5	18·8±5·8	37·0±5·8	31·1±4·5	49·7±11·9	26·7±4·2	43·7±7·9	45·6±10·0	73·3±11·1
7	—	—	26·0±14·2	*55·1±14·3	—	—	*60·5±12·4	*118·8±14·4
14	—	—	15·5±1·3	33·0±10·7	—	—	49·0±14·7	90·8±9·5
21	—	—	13·0±4·8	24·5±8·0	—	—	25·8±4·4	48·0±16·0
28	—	—	13·0±1·0	17·5±0	—	—	24·3±3·2	27·7±4·3
Activity-duration Index	—	—	250	575	—	—	700	1,400
Activity-duration Ratio	—	—	1·0 (Standard)	1·0 (Standard)	—	—	2·8	2·4

* Peaks.

RESULTS

Comparative Tests.—The most important of the results from the preliminary experiments on rat seminal vesicle and prostate growth are given in Table I, from which Figs. 1 and 2 have been constructed.

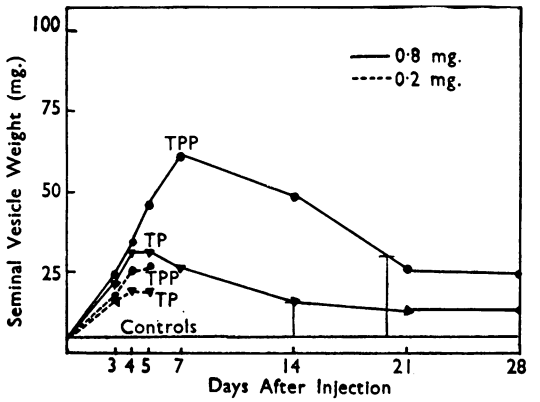


FIG. 1.—Effect on seminal vesicle weight of doses of 0.2 mg. and 0.8 mg. of TP and TPP in sesame oil injected subcutaneously in castrated rats. Vertical lines are drawn through the points of 50% involution from the peak readings to give the areas taken as the activity-duration indices.

It was found, by applying 4-point procedure to the readings at 5 days for TP and TPP given to rats subcutaneously in sesame oil in doses of 0.2 mg. and 0.8 mg., that TPP was about 2.7 times as potent an androgen as TP, the result being the same for both seminal vesicles and prostates. On the other hand, when the activity-duration index was adopted for this experiment the potency of TPP was found to be about 2.8 (seminal vesicles) and 2.4 (prostates) times that of TP. None of the emulsions or suspensions tried at these dose levels was as potent as TP in sesame oil.

From the results of the second series of experiments the activity-duration indices and ratios have been calculated (Table II), and Figs. 3 and 4 have been constructed.

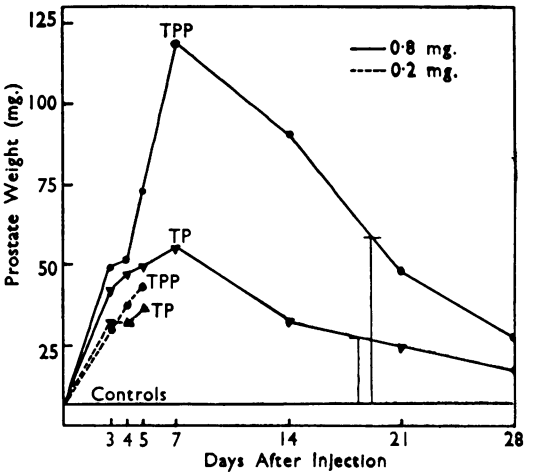


FIG. 2.—Effect on prostate weight of doses of 0.2 mg. and 0.8 mg. of TP and TPP in sesame oil injected subcutaneously in castrated rats. Vertical lines are drawn through the points of 50% involution from the peak readings, as in Fig. 1.

The mean control values for seminal vesicles varied from 3.7 mg. to 5.7 mg. and for prostates from 5.3 mg. to 10.8 mg., none of the groups injected with placebo solvents showing any significantly different results from the untreated group.

The figures obtained from the activity-duration indices showed that in sesame oil TPP was about 3.2 (seminal vesicles) and 4.8 (prostates) times as potent as TP, and in ethyl oleate TPP was about 4.2 (seminal vesicles) and 4.4 (prostates) times as potent as TP, when given to rats intramuscularly at a dose of 2.5 mg./0.1 ml.

Ethyl oleate was about 1.6 (seminal vesicles) and 1.6 (prostates) times as effective as sesame oil as a solvent for TPP when used intramuscularly and about 2.3 (seminal vesicles) and 2.1 (prostates)

TABLE II

HIGHER-DOSAGE EXPERIMENT

Activity-duration indices and ratios based on response/time graphs from mean responses (5 rats) of seminal vesicles and prostates of castrated rats to a dose of 2·5 mg. of TP and TPP in 0·1 ml. Groups killed at 8 hrs. and 1, 3, 5, 7, 9, 11, 14, 21, 28, 35, and 42 days after injection

Androgen	Medium	Route of Injection	Activity-duration Index		Activity-duration Ratio	
			Seminal Vesicles	Prostate	Seminal Vesicles	Prostate
TP	Sesame oil	Intramuscular	500	425	1·0 (Standard)	1·0 (Standard)
TPP	,, ,,	,,	1,575	2,025	3·2	4·8
TP	Ethyl oleate	,,	625	725	1·25	1·7
TPP	,, ,,	,,	2,600	3,175	5·2	7·5
TPP	,, ,,	Subcutaneous	3,950	4,225	7·9	9·9
TP	Aqueous emulsion	Intramuscular	500	400	1·0	0·9
TPP	,, ,,	,,	750	675	1·5	1·6
TP	Aqueous suspension	,,	450	475	0·9	1·1
TP	,, ,,	Subcutaneous	850	725	1·7	1·7
TPP	,, ,,	Intramuscular	575	500	1·2	1·2
TPP	,, ,,	Subcutaneous	1,700	1,475	3·4	3·5

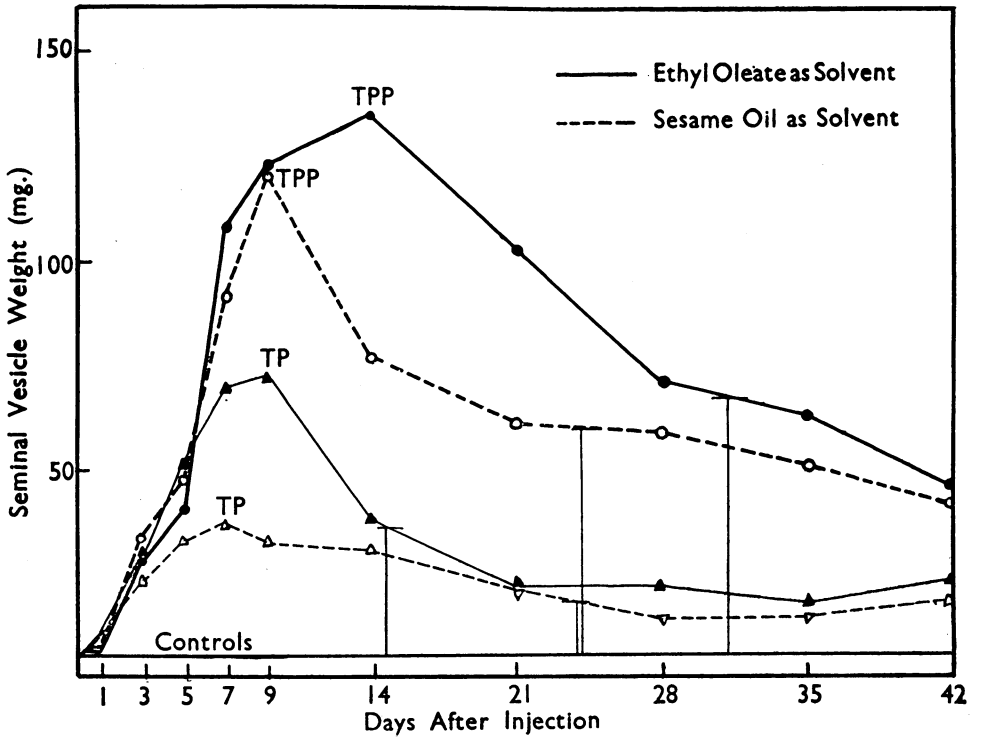


FIG. 3.—Effect on seminal vesicle weight of doses of 2.5 mg. of TP and TPP in sesame oil and ethyl oleate injected intramuscularly in castrated rats. Vertical lines as in Fig. 1.

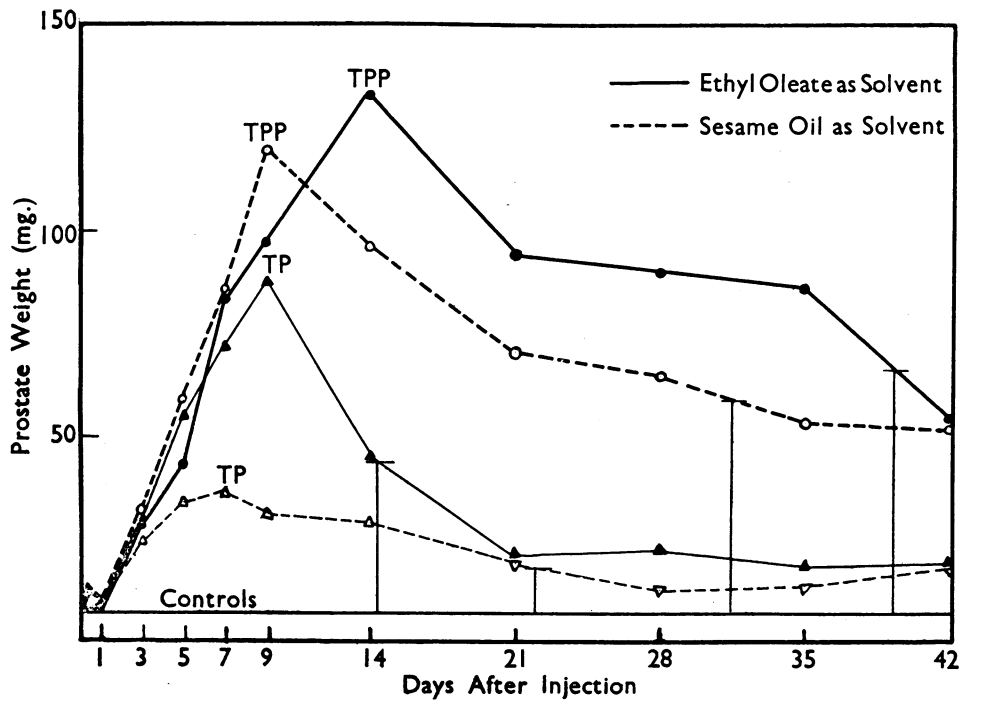


FIG. 4.—Effect on prostate weight of doses of 2.5 mg. of TP and TPP in sesame oil and ethyl oleate injected intramuscularly in castrated rats. Vertical lines as in Fig. 1.

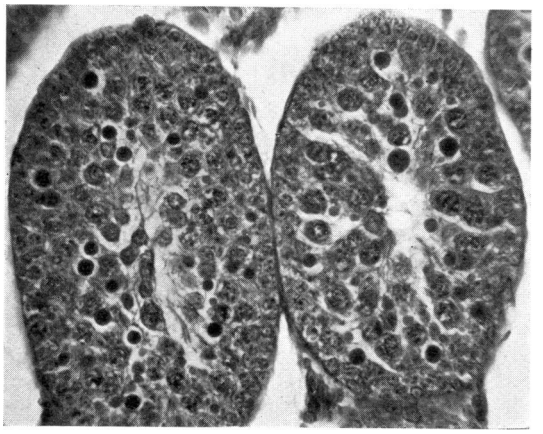
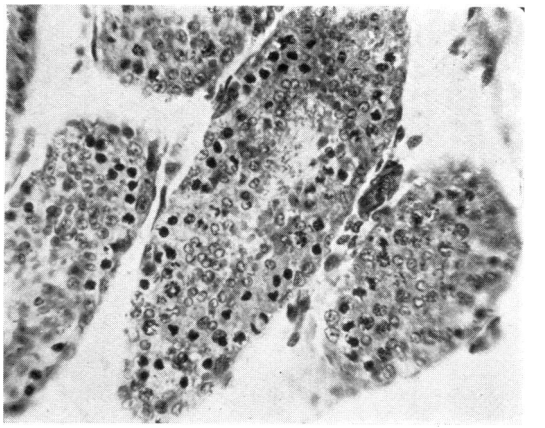


FIG. 5.



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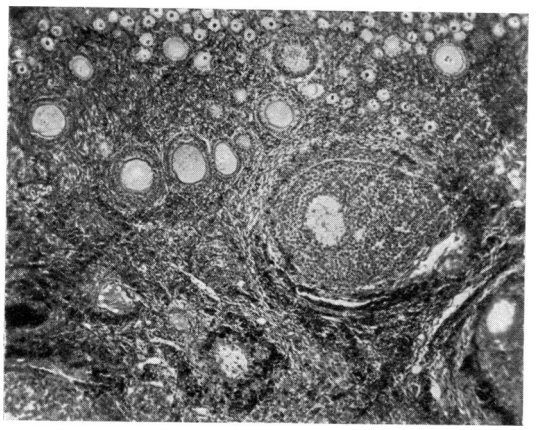

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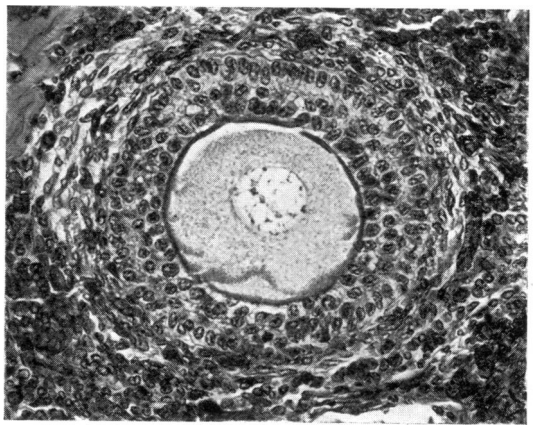

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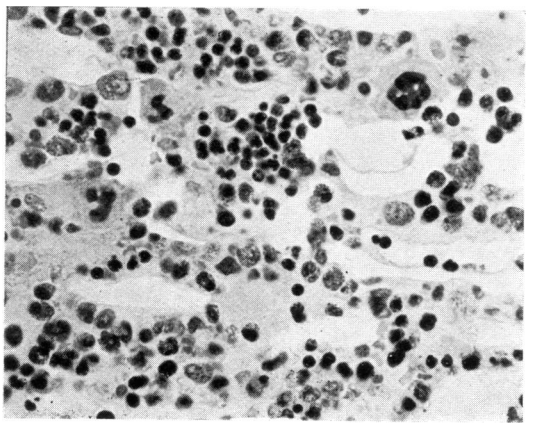

FIG. 9.

FIG. 5.—The seminiferous tubules of prepuberally castrated rat injected with 0.8 mg. testosterone phenyl propionate (× 275).

FIG. 6.—The seminiferous tubules of rabbit injected with 366 mg. testosterone phenyl propionate (× 220).

FIG. 7.—The ovary of rabbit injected with 366 mg. testosterone phenyl propionate (× 60).

FIG. 8.—High-power view (× 275) of the section shown in Fig. 7.

FIG. 9.—Red bone-marrow of rabbit injected with 366 mg. testosterone phenyl propionate (× 440).

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times as effective as the aqueous suspension as a vehicle for TPP when used subcutaneously in rats at a dose of 2.5 mg./0.1 ml.

The subcutaneous route was about 1.5 (seminal vesicles) and 1.3 (prostates) times as effective as the intramuscular route for TPP in ethyl oleate and about 2.8 (seminal vesicles) and 2.9 (prostates) times as effective as the intramuscular route for TPP in aqueous suspension in rats at a dose of 2.5 mg./0.1 ml.

Combining the most advantageous of the above results it was found that TPP in ethyl oleate given subcutaneously was about 7.9 (seminal vesicles) and 9.9 (prostates) times as potent as TP in sesame oil given intramuscularly to rats at a dose of 2.5 mg./0.1 ml.

Morphology of Testes.—It has been shown that androgens administered to immature and adult rats in low dosage may stimulate spermatogenesis, but that in larger doses they may cause atrophy of the testes and depression of spermatogenesis. TPP lowered testis weight and impaired spermatogenesis in a single dose of 0.8 mg.; this dose was found to affect meiosis, causing pyknotic degeneration of the nuclei of the primary spermatocytes, so that no later stages of spermatogenesis were observed (Fig. 5). The somatic, spermatogonial, Sertoli's, and Leydig cells were not affected. The peak of this activity was observed on the 7th day after injection, but by the 14th day definite recovery was noticed, suggesting that the depressant effect on spermatogenesis in the rat was only temporary.

Toxicity.—In rabbits treated with relatively large doses of TPP no pathological changes were observed on gross examination, except the reduction in size of the testes and the usual enlargement of seminal vesicles and prostate. The average total increase in body weight was 202 g. in rabbits treated with TPP and 228 g. in untreated control animals.

Microscopic examination of the various organs revealed significant changes only in the gonads and bone-marrow, these being observed in all rabbits treated with TPP.

A depressing effect on spermatogenesis was seen in the rabbit similar to that already reported in the rat with lower dosage, except that in the rabbit the pyknotic degeneration affected the nuclei of the secondary rather than the primary spermatocytes (Fig. 6); here again no further stages of spermatogenesis were seen, but in the lumina of the tubules there was some unidentified debris.

It is perhaps of interest that the ovarian response to excess TPP, in the two rabbits examined, was characterized by numbers of primary and growing follicles with the ova in premature stages of meiotic prophase (Figs. 7 and 8).

Investigation of the red bone-marrow of all the treated rabbits revealed no changes other than a stimulation of myeloid tissue with proliferation of eosinophil myelocytes, probably both the true and pseudo-eosinophil myelocytes (Fig. 9). There were no significant changes either in the splenic pulp or its cells, nor in nodules of lymphoid tissue, lymph nodes, or thymus.

There were no detectable pathological changes in other tissues examined (myocardium, lung, intestine, pancreas, liver, kidney) nor in other endocrine organs (pituitary, thyroid, parathyroid, islets of Langerhans, adrenal). Only in one of the female rabbits examined was there found a mild parenchymatous degeneration (cloudy swelling) of renal and hepatic epithelium; this isolated finding may or may not be significant.

In rats injected with the emulsions there was a certain amount of local necrosis at the site of injection.

Discussion

It has been found that the complex androgens testosterone cyclopentylpropionate (TCP) and testosterone phenyl propionate (TPP) show greater potency and duration of effect than any testosterone compounds previously described. Up to the present, TCP and TPP have not been compared on rats, but a recent report from Overbeek (1952) shows that they have about the same potency in inducing capon comb-growth. It is clear from the present results that TPP has a higher potency and more prolonged action than testosterone propionate under the same conditions of administration.

An attempt at a 4-point test comparing TPP and TP gave a very similar result to that obtained from the activity-duration index adopted for this experiment.

It may be seen from the graphs that the weight of the target organs in these experiments increased at an almost identical rate for the first few days no matter what androgen, vehicle, or route was employed, and that the treatments which induced the greatest increase in weight were in general those which had the greatest duration of action.

The comparative failure of the forms designed for slow absorption—the aqueous suspensions and emulsions—must be offset against a number of favourable reports upon similar preparations; it

may be that such forms fail unless a sufficiently large dose is given to deposit a layer of crystals deep enough to impede absorption (as occurs with subcutaneous implants); below this dose level the aqueous medium will naturally not hinder absorption. On the other hand, the low absorption rate of the hormone from an oil solution will probably be effective no matter how small the dose. It is noticeable from these results that the emulsions and suspensions compare particularly badly with the oil solutions when the dose is very low.

The effect of variations in the volume of injected material upon the target organs has not yet been tested, but it seems probable that the concentration of the dose will prove to be an important factor, especially with the crystal-depot type of preparation.

The advantage of ethyl oleate as a solvent was notable. Although its low viscosity makes it easy to inject, it appears not to result in rapid absorption; on the contrary, at the dosage of these experiments it would seem that the hormone is more slowly absorbed from this solvent than from any other medium tried.

Russell (1952) observed that ethyl oleate in rabbits caused local swelling, inflammation, and haemorrhage at the site of injection. In rats no significant local changes were observed by gross examination after subcutaneous or intramuscular injection of this solvent.

It is stated (Gaddum, 1952) that absorption from subcutaneous tissues is slower than from intramuscular ones, and the results clearly confirmed this, since both duration of effect and peak development were greater when the subcutaneous route was employed.

The involution of the target glands was interesting; as might be expected, the most stimulated organs tended to decrease more rapidly than less stimulated ones, as far as one can tell from the evidence of successive readings. More important, perhaps, is the observation that the seminal vesicles or prostates did not return to control size within six weeks of injection of a single moderate dose, the larger organs still being as much as 900% of the control weight.

The interference with spermatogenesis, which was clearly shown, is an indication of the efficacy of TPP as an inhibitor of anterior pituitary activity (Bottomley and Folley, 1938), since in rats this observation was made after a single dose of only 0.8 mg. This inhibition does not long outlast the administration of the hormone, however, and normal spermatogenesis was occurring again 14 days after the injection. The possible stimulating or maintaining effect of very small doses of TPP on the testis was not examined.

The effect of TPP on the ovary, although observed on only two rabbits, may prove to be of some importance in view of the part supposed to be played by ovarian testosterone in follicle ripening (Gaarenstroom and de Jongh, 1946), and has some parallel in the observation of Greep and Jones (1950) on rats.

Summary

1. 1,012 prepuberally castrated rats were used to test the potency of a new ester of testosterone, testosterone phenyl propionate (TPP), by comparison of its effects with those produced by testosterone propionate (TP) under similar conditions.

2. Preliminary tests on its potency, as judged by the stimulation of seminal vesicles and prostates at intervals after subcutaneous injection of small doses (0.2 and 0.8 mg.) in sesame oil, showed that TPP was about 2.7 times as potent an androgen as TP.

3. At a higher dose level of 2.5 mg. the potency of TPP as judged by a combined activity-duration index was at least four times that of TP when injected intramuscularly in ethyl oleate.

4. Ethyl oleate was found to be the most satisfactory vehicle for TPP, giving it greater and more prolonged effect than any other preparation tried; TPP was about one-and-a-half times as active in ethyl oleate as it was in sesame oil.

5. The subcutaneous route was found to give greater and more prolonged effect in rats than the intramuscular wherever these were compared; TPP in ethyl oleate was about one-and-a-half times as potent subcutaneously as it was intramuscularly.

6. TPP was found to be relatively free from toxic effects in rats and rabbits, but it will temporarily depress spermatogenesis even in moderate dosage.

7. Testosterone phenyl propionate appears to be a safe and potent long-acting androgen. It was especially effective in rats when injected subcutaneously in ethyl oleate.

We wish to thank Miss Margaret Harvie for her valuable assistance with the statistical work.

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