CLXXIV. EFFECT OF ANDROSTERONE AND OF MALE HORMONE CONCENTRATES ON THE ACCESSORY REPRODUCTIVE ORGANS OF CASTRATED RATS, MICE AND GUINEA-PIGS.

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I. INTRODUCTION.

THE accessory reproductive glands of castrated rodents have long been recognised as suitable objects for testing the activity of male hormone preparations. A full bibliography will be found in Koch [1932] and the other literature cited below. Moore and Gallagher [1930, 2], enumerating mammalian indicators for male hormone, state that, after the spermatozoon motility test (one not in general use), the restoration of normal prostatic structure is the most sensitive test, the next being cytological restoration of the seminal vesicles. Many tests of this kind have been made, but although histological changes in the glandular epithelium have been brought about by a few days' treatment, yet the stimulation or maintenance of glands of normal adult size in groups of animals castrated for as long as 10 or 14 days has not been quantitatively demonstrated, until recently by Dingemans *et al.* [1935]. These workers give the average seminal vesicle weights in 5 immature castrated rats injected for 24 days with a testis extract as 538 mg., approximately equivalent to the glands in a normal 175 g. rat (Fig. 3) and about two-thirds of the full size. Now that androsterone, the male hormone first extracted from urine, can be obtained artificially in larger quantities, by the method of Ruzicka *et al.* [1934, 2; Ruzicka, 1935], it has been possible to make an extended quantitative investigation of the responses in weight of the prostate and seminal vesicles. For comparative purposes, a number of experiments have also been made with several urine concentrates.

The two types of experiment which have been carried out are: (1) maintenance of normal size glands in rats and mice castrated as adults, and (2) production of growth in the glands of prepubertally castrated rats, mice and guinea-pigs.

An experiment of the first type was reported by Moore and collaborators [1930], who state that they maintained in normal condition the accessory glands of two adult castrated rats for 20 days by injections of crude male hormone begun on the day following castration; unfortunately no figures are given for either the weight of the rats or the weight of the glands. Hansen [1933] also carried out maintenance experiments on rats for 10-day periods but the accessory glands were not weighed, only examined histologically. Martins [1930] carried out similar partial maintenance experiments on mice of about 18 g. weight, using a length-breadth index of seminal vesicle size.

Many experiments of the second type have been reported on rats [Moore and collaborators, 1930; Freud *et al.*, 1930; 1932; Korenchevsky and collaborators, 1932-1935] and others have been carried out on mice [Voss and Loewe, 1931].

The actual increases in prostate and seminal vesicle weights (particularly the latter) caused in these prepubertally castrated rats and mice have in most cases been so small as to be almost negligible (a point emphasised by Voss and Loewe) and consequently the majority of these workers have relied on histological changes as an index of the activity of their extracts. Korenchevsky and his co-workers alone have attempted to base a quantitative method of assay on the increase in weight of the atrophied glands.

Freud and his collaborators have tried to obtain improved results in this type of experiment by combinations of oestrin with male hormone urine concentrates. They have also tested various oestrin derivatives [David *et al.*, 1934]. For assaying purposes these workers rely on the capon comb test, but Freud [1933] notes that in the purification of extracts male hormone activity could be lost as regards rats while being retained as regards capons [*cf.* also Laqueur, de Fremery *et al.*, 1931].

The present experiments were designed not only to test the biological activity of pure androsterone on rodents but also to compare it with the activity of crude urine extracts, since a significant difference between the two might imply the natural occurrence of more than one active substance.

II. MATERIAL.

Note on nomenclature. The term androsterone is applied to the crystalline compound first isolated by Butenandt from urine and prepared artificially from cholesterol by Ruzicka and his co-workers. The term "male hormone" is applied generally to this substance and to concentrates from natural sources without implying that they are necessarily identical. It seems desirable to avoid such terms as "testicular hormone from urinary sources" in the present state of knowledge.

Crystalline androsterone. The method described by Ruzicka *et al.* [1934, 2] for the preparation of androsterone from cholesterol was followed closely except in the preparation of *epicholesterol* and in the isolation of androsterone in the final stage. The reduction of cholestanone was carried out in quantities of 100 g. dissolved in 3 litres of amyl ether. To this were added 100 ml. of concentrated hydrochloric acid and platinum black prepared by Vavon's method [1914] from 35 g. of platinum chloride. Absorption of hydrogen was complete in 4 hours with fresh catalyst. After filtering the hot solution from the catalyst, the *epicholesterol* was allowed to crystallise out and the mother-liquors were used for a second reduction. The addition of fresh catalyst was necessary after four reductions. A yield of 397 g. of recrystallised *epicholesterol*, m.p. 180–183°, was obtained from 786 g. of cholestanone in one series of reductions.

Some attempt was made to modify the conditions of the oxidation of *epicholestanyl* acetate, the most wasteful stage in the preparation. Oxidation at a higher temperature (102–105°) for a shorter time yielded however a ketonic fraction containing much oily material. The isolation of the ketonic fraction was carried out with the help of the new reagent trimethylaminoacetohydrazide chloride, $\text{Cl} \cdot \text{Me}_3\text{N} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NHNH}_2$ [Girard and Sandulesco, 1934]. The neutral fraction of the oxidation product from one batch (80 g.) of *epicholestanyl* acetate, freed from the bulk of unchanged material and methylheptanone, was evaporated to dryness and dissolved in 25 ml. of glacial acetic acid. To this solution was added a solution of 10 g. of Girard's reagent in 25 ml. of acetic acid and the mixture was warmed gently for a short time. The mixture was then poured into water and oily material extracted with ether. The aqueous layer, containing the ketonic fraction as a water-soluble hydrazone, was

then warmed on the water-bath with a slight excess of dilute sulphuric acid; the material which separated was extracted with ether, and the extract evaporated. The crude acetate thus obtained was hydrolysed with alcoholic sodium hydroxide and yielded 1.4 g. of crude ketone, M.P. about 120°, which was recrystallised from ethyl acetate. Crude androsterone (0.4 g., M.P. 168–176°) separated, mixed with flocculent material. Recrystallisation from ethyl acetate, a good, though rather wasteful, solvent for the purpose, yielded pure androsterone, M.P. 184–185°, $[\alpha]_D^{20} + 93.6^\circ$, $[\alpha]_{5461}^{20} + 115.8^\circ$ in absolute alcohol. About 3 g. of androsterone were obtained from 3 kg. of cholesterol by this method. Under good conditions the over-all yield was about 0.2 % of the theoretical.

Fractional crystallisation of the product from the mother-liquors and the flocculent material accompanying the crude androsterone yielded a *ketone*, M.P. 166–167°, from aqueous alcohol, $[\alpha]_D^{20} + 20^\circ$, $[\alpha]_{5461}^{20} + 22^\circ$ in absolute alcohol. It showed no selective absorption in the ultra-violet in 0.1 % solution. This material did not induce measurable comb growth in capons in a total dose of 10 mg. over 5 days. Its composition has not yet been further investigated, but it seems likely that it is a product of incomplete removal of the sterol side-chain, possibly *epiallopregnanolone*.

We are indebted to Mr A. L. Bacharach of Glaxo Laboratories for the supply on special terms of the cholesterol used in this preparation.

In addition to the material prepared in the laboratory, about 1.4 g. of androsterone were placed at our disposal by Messrs Ciba of Basle, to whom our very best thanks are due for this most generous assistance. A supply of androsterone from natural sources (50 mg. of crystalline “Proviron”) was very kindly made available by Prof. Schoeller, Schering Kahlbaum A.G., Berlin.

Solutions of androsterone in arachis oil for injection were prepared by mixing at 100–150°. Greenwood *et al.* [1935] have shown that androsterone is stable under these conditions.

Male hormone concentrates.

(a) 2300 capon units of “Proviron” urine concentrate were given by Prof. Schoeller.

(b) 1000 capon units of “Enarmon” urine concentrate were given to us by Dr Itoh, Teikokusha Institute, Kawasaki, whom we also take this opportunity of thanking.

(c) A small supply (240 capon units) of “Hombreol” Organon was purchased, but only two experiments were possible with this material because of its high price.

isoAndrosterone. 3-Hydroxy-17-keto-aetioallocholane, isomeric with androsterone, referred to as *isoandrosterone* for convenience, was prepared from cholestanyl acetate as described by Ruzicka *et al.* [1934, 1], but was isolated with the help of Girard’s reagent.

Oestrin was administered in the form of oestrone (ketohydroxyoestrin) or, in earlier work, oestriol (trihydroxyoestrin) for which we are indebted to the British Drug Houses, Ltd.

Comb growth promoting activity of the materials. As a basis for comparison of the results obtained on rodents, the various preparations were first tested on Brown Leghorn capons belonging to Dr Greenwood’s stock, but kept in London. The assay of pure androsterone differs slightly from that carried out in Edinburgh by Dr Greenwood [Greenwood *et al.*, 1935], probably because of differences in environmental factors, but the assays in London were all carried out under identical conditions, and should thus be comparable with each other, the only essential point. The results of these assays are given in Table I.

Table I. *Assays of male hormone preparations on capon combs.*

Material	Daily dose for 5 days	Av. comb increase <i>L+H</i> mm.	Wt. or volume of capon unit (approx.)	Capon units per mg. or per ml.
Androsterone (Ciba)	0.2 mg.	10.9	100 γ	10 per mg.
Androsterone (present paper)	0.2 mg.	9.3		
<i>iso</i> Androsterone	1.4 mg.	12.0	600 γ	1.6 per mg.
"Enarmon"	0.2 ml.	10.2	0.1 ml.	10 per ml.
"Proviron"	0.1 ml.	9.7	0.05 ml.	20 per ml.
"Hombreol"	0.1 ml.	11.1	0.045 ml.	22 per ml.

Each assay was carried out on a group of 5 capons, and the capon unit is defined as that amount of preparation which, injected daily for 5 days, produces an average increase in $L+H$ of 5 mm. one day after the last injection. For purposes of calculation, it is assumed that the dose/response relation is linear up to a $L+H$ increase of 12 mm., a quite warrantable assumption [Greenwood *et al.*, 1935]. The dosage in each case was an amount expected to give about 10 mm. increase in $L+H$ in the 5 days, *i.e.* a good accurately measurable response.

The weight of the capon unit of crystalline androsterone agrees closely with that obtained by Ruzicka *et al.* [1934, 2], using a slightly different method of assay, involving however a very similar amount of growth. In the case of *iso*androsterone, close agreement is also seen with Ruzicka's assays, in which it was found that the isomeride had about one-seventh the activity of the hormone; the above assays make the ratio 1 : 6. For comparative purposes the activity of crystalline androsterone is taken as 10 capon units per mg. Only two samples of androsterone were assayed in London, but a series of assays carried out by Dr Greenwood failed to show any significant difference in activity between androsterone "Ciba", androsterone prepared in this laboratory and androsterone "Proviron" from urine.

The "Enarmon" preparation, a urine concentrate in oil, was labelled as 10 capon units per ml., no information being available as to the method of testing. The above assay entirely agrees with this definition of its strength. "Enarmon" was also slightly oestrogenic. 1 ml. of "Enarmon" oil produced cornification in an ovariectomised rat, and 0.1 ml. oil injected into each of three ovariectomised mice gave a positive response in two.

The batch of "Proviron" urine concentrate used in this test had been in the laboratory for nearly 2 years and was labelled as 25 capon units per ml. (for method of assay, see Schoeller and Gehrke [1931]). Actually, it assayed at almost exactly 20 units per ml. by our method. The difference, if significant, may be due to difference in assay method or deterioration of the preparation.

The "Hombreol" urine concentrate was labelled as 20 units per ml. (for method of assay, see Freud *et al.* [1932]). Our assay, recorded above, agrees substantially with this description; the difference is doubtfully significant.

The remarkable thing about these assays is the close agreement between the standardisations of the four different preparations carried out in as many different countries. It is doubtful if any of the differences between the observed and labelled activities are significant: there appears to be greater unanimity as to what constitutes a capon unit than might have been expected. In view of these findings it is rather surprising that the androsterone tests carried out in Edinburgh [Greenwood *et al.*, 1935] should have given uniformly lower values, especially since the birds were from the same stock as those used in London.

III. DESCRIPTION OF PROSTATE AND SEMINAL VESICLES; METHODS OF DISSECTION.

(a) *The rat.* The rat prostate has been frequently described; references will be found in Walker [1910]. It is illustrated in Figs. 1 *a* and 1 *b*, which show the terminology adopted in this work. The gland consists essentially of three pairs of lobes:

(1) Cranial lobes or coagulating glands attached to the length of the seminal vesicles. These are termed the anterior lobes by Moore, Price and Gallagher [1930],

Fig. 1. Prostate and seminal vesicles of the rat. *a*, ventral view; *b*, dorsal view. The ventral lobes have been drawn back to expose the rest of the glands.

as were similar lobes in the mouse prostate by Deanesly and Parkes [1933]; but since Walker [1910] uses the term in another sense it seems better to discard it.

(2) Dorso-lateral lobes lying round the base of the seminal vesicles and almost encircling the urethra. Moore, Price and Gallagher [1930] call these the middle lobes, whereas Walker calls them the posterior lobes.

(3) Ventral lobes attached to the caudal end of the prostate only by their ducts. These are the posterior lobes of Moore, Price and Gallagher [1930] and the anterior lobes of Walker [1910].

In addition to these lobes there is a pair of small ventral ampullary glands at the point of entry of the vasa deferentia, which for weighing purposes may be included with (2).

The bulk of the rat prostate is made up of the dorso-lateral and ventral lobes, which are much better developed than the corresponding structures in the mouse. Since these react more readily to male hormone than the cranial lobes or the seminal vesicles, the rat is a more satisfactory test animal for small doses than the mouse.

The rat seminal vesicles are shown in Figs. 1 *a* and 1 *b* and require no description here.

The dissection and weighing of the accessory glands were carried out as follows: the entire reproductive tract was dissected free of fat, fixed in Bouin's fluid and then transferred to 70 % alcohol. At this stage the cranial prostate lobes were dissected off the seminal vesicles and the latter freed from the dorso-lateral lobes and removed for separate weighing. Next, the ventral and dorso-lateral lobes were freed from the urethral musculature by dissection from the caudal region and the vasa deferentia were cut off at their points of entry. After removal of the bladder and of any remaining connective tissue, the dissected

prostate consisted almost entirely of glandular substance. In the larger prostates the three pairs of lobes were weighed separately but this was not done for the smaller glands. Dissection was carried out as rigorously as possible, but naturally prostates of 15–40 mg. were more difficult to dissect cleanly and accurately than the larger glands. All glands were thoroughly drained before weighing. No glandular tissue from inside the urethral muscle was included with the prostate, since it was desirable to simplify and standardise the dissection as far as possible.

(b) *The mouse.* The mouse seminal vesicles and prostate have already been described [Deanesly and Parkes, 1933]. The method of dissection used in this work was the same as before; the seminal vesicles, with the cranial (anterior) lobes of the prostate attached to them, were cut off after fixation at the level of the entry of the vasa deferentia and weighed together. The mouse prostate, particularly when atrophied, is unsuitable for clean dissection and weighing.

(c) *The guinea-pig.* The guinea-pig prostate has been described by Walker [1910] and Engle [1926]. Although consisting of the same three pairs of lobes it is less compact and well-developed than that of the rat and accordingly less suitable for quantitative work with male hormone, since its sensitivity is much the same as that of the rat.

The seminal vesicles of the guinea-pig are elongated tubular structures which regress after castration more slowly than those of the rat or mouse. They were dissected out from the prostate after fixation and weighed separately.

IV. GROWTH OF THE PROSTATE AND SEMINAL VESICLES OF THE RAT.

The growth and post-castration atrophy of the accessory reproductive glands of the normal mouse have already been dealt with [Deanesly and Parkes, 1933], and the growth of the seminal vesicles of the guinea-pig will be described elsewhere [Deanesly and Rowlands]. As a control on the experiments in Sections V and VI however it was necessary to make an extensive study of the growth of both the prostate and seminal vesicles of the rat. All animals used were albino and belonged to the Institute's stocks.

Growth of the prostate and seminal vesicles. The weights of the prostate and seminal vesicles were obtained in 79 normal rats, ranging in body weight from 42 g. to 250 g. The gland weight, plotted against body weight, is shown in Figs. 2 and 3, and the curves drawn through the same points are shown in Figs. 4 and 5 as a standard against which the experimental results described in Section V can be compared. In order to facilitate comparison, the growth curve for the seminal vesicles has been superimposed as an interrupted line on the prostate diagram and *vice versa*.

Although the figures show considerable variations in gland size in relation to body weight, especially in the smaller rats, the main trend of growth is quite clear. In both prostate and seminal vesicles little appreciable enlargement occurs before the body weight is 100 g. In this slight early growth the seminal vesicles, which start very much below the prostate, lag behind still farther, so that at 90 g. body weight the prostate averages about 80 mg. while the seminal vesicles are only about 20 mg. Above 100 g. body weight both the glands enlarge very rapidly and tend to approximate in size. At 150 g. body weight the prostate is rather above and the seminal vesicles rather below 300 mg. At 180 g. body weight both are about the same at 500 mg., while soon after 200 g. body weight the glands attain what appears to be their full weight of 700–800 mg. each. Above a body weight of 250 or 275 g. the prostate and seminal vesicles of the

rat do not seem to show any regular increase in size although both may occasionally reach a weight of 1 g. in large rats. In some large animals however, presumably old ones, both seminal vesicles and prostate have obviously regressed. The initial lag in the growth of the seminal vesicles suggests that they have a threshold requirement different in kind or degree from that of the prostate.

Fig. 2.

Fig. 3.

Fig. 2. — Growth of prostate in the normal rat; ---- growth of seminal vesicles for comparison.

Fig. 3. — Growth of seminal vesicles in the normal rat; ---- growth of prostate for comparison.

V. MAINTENANCE OF THE PROSTATE AND SEMINAL VESICLES OF THE RAT AFTER POSTPUBERTAL CASTRATION.

Previous workers have shown that in the adult rat, as in the mouse, considerable atrophy of the accessory glands takes place in the first fortnight after castration. Certain "maintenance" experiments have been carried out in which attempts have been made to replace testicular activity in sexually mature rats

by injections begun immediately after castration. Since some body growth takes place in the 14 days' course of the experiments, complete maintenance of the accessory glands in their normal relation to body size implies not merely maintaining the size of the glands at the time of castration but actually inducing normal growth.

Fig. 4.

Fig. 5.

Fig. 4. Maintenance of prostate in postpubertally castrated rats by injection of androsterone.

Fig. 5. Maintenance of seminal vesicles in postpubertally castrated rats by injection of androsterone.

Three groups of five rats each of 130–155 g. body weight were castrated and injected with androsterone in arachis oil for 14 consecutive days and then killed 24 hours after the last injection. Rats of the first group were given 0.5 mg. daily, those of the second 1 mg. daily and those of the third 2 mg. daily. The weights of the prostate and seminal vesicles at autopsy are shown in Figs. 4 and 5 and Table II in relation to the normal growth curve and to their weights in uninjected normal and castrated groups.

It will be seen that:

(a) The prostates of all the rats receiving 1 or 2 mg. daily and of 4 of those receiving 0.5 mg. are substantially normal in size. Considered as groups, those receiving 2 mg. are rather above normal size, those receiving 1 mg. (slightly

smaller animals) are almost normal, whilst those receiving 0.5 mg. are below normal (Table II).

Table II. *Growth and maintenance of rat accessory glands after postpubertal castration.*

No. of rats	Condition	Daily injection of androsterone mg.	Av. body wt. at castration g.	Av. body wt. at autopsy g.	Av. prostate wt. mg.	Av. seminal vesicles wt. mg.
5	Normal	—	—	139	283	230
5	Normal	—	—	162	511	474
5	Normal	—	—	198	624	650
5	Castrated	3	199	212	867	665
1	Castrated	—	220	205	128	168
5	Castrated	2	138	167	577	431
5	Castrated	1	138	155	429	204
5	Castrated	0.5	141	157	291	122
5	Castrated	—	138	165	55	51

(b) The seminal vesicles have been less well maintained. All rats receiving 2 mg., 3 of those receiving 1 mg., and 2 of those receiving 0.5 mg. have seminal vesicles approximately normal in size, but the average seminal vesicle weight in all groups is below that of the controls, although the difference is probably not significant for the group receiving 2 mg. daily.

It is apparent that while 1 mg. of androsterone daily will maintain the prostate in rats of this size after removal of the testes, about twice as much is required to maintain the seminal vesicles. This obvious difference in threshold requirement (noted in earlier qualitative experiments by Moore and Gallagher, [1930, 2; Hansen, 1933]) can be associated with the difference in size in the early stages of growth.

Hansen [1933] estimates the maintenance requirements of seminal vesicles of adult rats at six or seven of Gallagher and Koch's capon units of testicular extract [Koch, 1932], whereas two such capon units are ample for the prostate.

In order to ascertain whether the larger glands of larger rats required a correspondingly increased amount of hormone for maintenance, a group of 5 rats 185–230 g. in weight were castrated and given 3 mg. daily for 14 days and then killed. The seminal vesicles averaged 665 mg., and, allowing for normal variation, were apparently fully maintained (Table II). The prostates averaged 867 mg. and were, if anything, exceptionally large. Only one uninjected castrated control was used for this group, since the previous experiments had shown that the glands of rats receiving injections should be compared with normal ones rather than with those of castrates.

VI. STIMULATION OF THE PROSTATE AND SEMINAL VESICLES OF THE RAT AFTER PREPUBERTAL CASTRATION.

(a) *Body weight at castration; size of prostate and seminal vesicles.* In the experiments recorded below an attempt was made to stimulate rudimentary accessory glands with male hormone preparations. Rats were castrated at 35–70 g. weight, the majority at 40–50 g., before prostate or seminal vesicles had grown appreciably. In ten normal rats, 51–67 g. in weight, the average prostate size was 34 mg. and that of the seminal vesicles 4 mg. A control series of 16 uninjected rats, castrated at 35–70 g. and killed 16–68 days afterwards (with the same body weight range as the experimental animals), had prostates averaging

16 mg. (9–25 mg.) and seminal vesicles of 4 mg., the latter unaffected in weight by castration. These rats obviously provide a very low starting point for growth experiments. The figures given by Freud [1933] and David *et al.* [1934] for rats of this size and smaller are of the same order, but are rather lower for the prostate and higher for the seminal vesicles. Apparently in their method the whole prostate is not weighed together; the seminal vesicles are weighed (fresh) with a connecting bridge of prostatic tissue, and of the rest of the prostate only the weight of the “anterior” lobes is given. The peri-urethral tissue is weighed separately. Exact comparisons with the present experiments cannot therefore be made. To the differences in presenting the data must be added the fact that the Amsterdam workers in earlier experiments inject the young rats only 3 days after castration. More recently, Dingemanse *et al.* [1935] give the average weight of the prostate and seminal vesicles in 5 prepubertally castrated rats as 8 mg. and 6 mg. respectively.

The figures (50–70 mg.) given by Korenchevsky *et al.* [1932, 1934, 1, 2], although relating to Bouin-fixed prostate and seminal vesicles from prepubertally castrated rats, are so much higher than our own that they imply a difference of technique.

(b) *Method of testing.* Rats were injected, generally in groups of 5, beginning 20–90 days after castration. No attempt was made to standardise either the time after castration or the body weight of the animals at the time of injection, since the series of control rats showed that these factors did not influence appreciably the size of the rudiment. Most of the rats were injected at 100–220 g. weight [*cf.* Dingemanse *et al.*, 1935]. The dose is generally expressed as the total dose per rat.

Different preparations and amounts of male hormone were injected into groups of 5 prepubertally castrated rats, for a period of 10 days unless otherwise stated, after which the animals were killed and the growth and anatomical changes in the accessory glands were noted.

(c) *Administration of androsterone.* Ten groups of rats were given androsterone alone; the details are set out in Table III.

Table III. *Effect of androsterone on the immature castrated rat.*

No. of exp.	Days injected	Total dose per rat mg.	Medium and amount daily (ml.)	Average weight of	
				Prostate mg.	Seminal vesicles mg.
34	5	3.5	2.5 10% alcohol	37	9
33	10	3.5	1.0 „	67	11
44	10	4.0	2.0 „	57	8
42	10	20.0	0.1 70% „	170	56
38	10	3.5	0.1 arachis oil	44	9
56	10	6.0	0.1 „	64	14
45	10	10.0	0.1 „	112	32
57	10	15.0	0.1 „	129	17
46	10	20.0	0.2 „	129	26
63	28	28.0	0.1 „	394	46

The average weights of prostate and seminal vesicles in control rats are 16 and 4 mg. respectively. There are 5 rats in each experiment except No. 56 where there were 6.

It is immediately obvious that androsterone does not produce such striking growth in the accessory glands of the castrated rat as can be produced by oestrin in the uterus of the ovariectomised female. In the case of the seminal vesicles

particularly, the growth is trifling compared with that of the normal gland. The following observations may be made with reference to these experiments.

(i) From experiments 33 and 34, it is clear that a small dose is more effective when spread over 10 days than over 5 days. From experiments 46 and 63, it also seems that the efficiency of large doses on the prostate can be greatly increased by extending the time over which administration is spread.

(ii) A comparison of the five groups receiving graduated amounts of androsterone in arachis oil over 10 days (Exps. 38, 56, 45, 57, 46, Fig. 6) suggests

Fig. 6. Growth of prostate in prepubertally castrated rats by injection of male hormone.

that the maximum growth in that period will produce a prostate of only about 130 mg. Certainly, very little is gained by increasing the total dose from 10 mg. to 20 mg. It is clear however that a prostate can actually grow to a greater weight than 130 mg. in 10 days. Thus in experiments with immature 40–50 g. normal rats, prostates averaging nearly 200 mg. were produced as the result of testicular stimulation for 10 days with prolan injections. In other experiments with androsterone (*e.g.* Exp. 42), prostates of more than 130 mg. were produced. The response of the seminal vesicles was poor in all the five groups referred to above, a total dose of 10 mg. androsterone actually giving the greatest effect.

(iii) It is possible that the relative ineffectiveness of the large doses may have been due, at least in part, to inefficient absorption of the material from oil. The old observation that oil is a bad medium for subcutaneous injection of rodents is amply confirmed by these experiments. Thus 3.5 mg. given as a colloidal solution in aqueous alcohol caused apparently twice as much growth in the prostate as the same amount of androsterone given in 0.1 ml. of oil daily. Similarly a total dose of 20 mg. in 70 % alcohol produced a much better effect than a total of 20 mg. in 0.2 ml. of oil daily. The increased growth of the seminal vesicles was particularly noticeable in Exp. 42. Both prostate and seminal vesicles in this group of rats show a greater approach to their maximum 10-day growth. It is difficult to ascribe these differences to any factor other than better absorption from the aqueous-alcoholic solutions. Unfortunately, only very dilute colloidal

solutions in 10 % alcohol can be made, and the stronger alcoholic solutions caused bad local reactions. It should be noted that even where intramuscular injection of the oil is possible, as in the capon, aqueous media appear to be more efficient.

(iv) Exp. 63, in which the rats were injected with 1 mg. androsterone daily for 28 consecutive days, gave a very interesting result. The prostate size was nearly 400 mg., more than three times as large as that produced by a daily dose of 1.5 mg. for 10 days, but the seminal vesicles, as in all the experiments recorded in Table III, were small, weighing 46 mg., and consequently the relationship between the size of the two glands was quite abnormal for that stage of prostate development [*cf.* Korenchevsky, 1935]. In a normal rat with a 400 mg. prostate the seminal vesicles would be very similar to it in size.

Fig. 7. Growth of seminal vesicles in prepubertally castrated rats by injection of male hormone.

This suggests that although androsterone in sufficient doses will maintain the seminal vesicles in a normal condition after castration and will also grow them from a rudimentary size up to a stage containing secretion, yet it is not as active on these glands as male hormone preparations from testis [Dingemanse *et al.*, 1935] or urine (Table IV). It is noteworthy however that Moore, Hughes and Gallagher [1930], who injected immature castrated rats for as much as 20 or 40 days with testis extract, never produced seminal vesicles of anything approaching normal adult size. This of course may have been due to absorption difficulties.

(d) *Administration of male hormone urine concentrates.* Five experiments were carried out, one each with "Hombreol" and "Proviron", and three with "Enarmon". The details are given in Table IV and Figs. 6 and 7. The results relating to both prostate and seminal vesicles are quite clear. Assuming 10 capon units per mg. of androsterone, Fig. 6 shows that a capon unit of either "Proviron" or "Hombreol" has about the same effect on the prostate as a capon unit of crystalline androsterone. The same conclusion may be drawn from Fig. 7 for the seminal vesicles. There is thus presumptive evidence that the only active principle of the two concentrates is androsterone. The activity of "Enarmon"

Table IV. *Effect of male hormone oil concentrates on the immature castrated rat.*

No. of exp.	No. of rats	Male hormone	Capon units per ml.	Total dose per rat in c.u.	Days injected	Average weight	
						Prostate mg.	Seminal vesicles mg.
52	5	"Proviron" (1933)	20	20	10	28	3
51	5	"Hombreol"	22	40	9	62	10
37	3	"Enarmon"	10	20	10	78	27
47	5	"Enarmon"	10	50	10	125	68
55	4	"Enarmon"	10	70	10	162	67

per capon unit however is more than twice as great as that of crystalline androsterone, "Hombreol" or "Proviron". The effect on the size of the seminal vesicles is more than 4 times as great. This clearly indicates that the comb growth promoting principle in "Enarmon" is not ordinary androsterone, or else that one or more supplementary substances with little or no effect on comb growth, but active on the accessory glands of rats, are present in addition to androsterone. In view of the work of Freud [1933] and David *et al.* [1934] confirmed by Korenchevsky and Dennison [1934, 1, 2], an obvious suggestion seemed to be that it was the small amount of oestrogenic substance present in "Enarmon" which was causing the difference in response. On the other hand, the fact that the prostate, which is much less affected by oestrone over short periods than the seminal vesicles, also showed the increased response, made this doubtful. The possibility was investigated by experiments on the effects of oestrone alone and of androsterone *plus* oestrone.

(e) *Administration of oestrone alone.* Two experiments were carried out with oestrone. The first group of rats was given 0.1 mg. oestrone each over 10 days, and the second 1.0 mg. The results in the two groups are identical and may be considered as representing the maximum development of rudimentary male accessory glands on oestrone alone in 10 days. The prostates showed some enlargement, possibly up to 50 %, while the seminal vesicles showed an increase to about 3 times the control weight (Table V). Allowing for the differences in the

Table V. *Effect of oestrone alone and of androsterone plus oestrone on groups of immature castrated rats.*

No. of exp.	Total dose per rat		Medium and amount daily (ml.)	Average weight	
	Oestrone mg.	Androsterone mg.		Prostate mg.	Seminal vesicles mg.
40	0.1	—	0.1 olive oil	26	16
60	1.0	—	0.1 arachis oil	24	18
53	0.1	5	0.1 "	42	15
61	0.025	10	0.1 "	87	23
62	1.0	10	0.1 "	90	32
41	0.1	20	0.1 arachis oil + 0.1 70 % alcohol	175	73

5 rats in each experiment.

control weights and the duration of injection, these results are entirely similar to those obtained by David *et al.* [1934] and Korenchevsky and Dennison [1934, 1] except that the former obtained a rather greater effect on the prostate.

(f) *Administration of androsterone plus oestrone.* Four experiments were carried out on the simultaneous administration of the two hormones. Amounts

Fig. 8. Relation between growth of prostate and log dose.

Fig. 9. Relation between growth of seminal vesicles and log dose.

of oestrone varying from 25 γ (250 international units) to 1 mg. were given over 10 days; the amount of androsterone varied from 5 mg. to 20 mg. From the results in Table V it will be seen that in 3 of the 4 experiments no increase as compared with the androsterone series in Table III was evident in either seminal vesicles or prostate. In Exp. 41 the maximum dose of androsterone, *plus* 100 γ oestrone, produced a prostate almost identical in size with that produced by the same dose of androsterone alone (Table III). The combined dose produced seminal vesicles slightly larger than the same dose of androsterone alone, but even so, 200 capon units of androsterone *plus* oestrone produced seminal vesicles hardly larger than those resulting from 50 capon units of "Enarmon". These results show that, under the conditions of the experiments, there is no synergistic action between androsterone and oestrone. At most there is a summation of the two different responses, the size increase of the seminal vesicles with oestrone being more marked than that of the prostate. These experiments lend no support to the idea that the extraordinary efficiency of "Enarmon" per capon unit can be due to its slight oestrin content. In Figs. 8 and 9 the responses of the prostate and seminal vesicles, respectively, have been plotted against the logarithm of the dose in terms of capon units, on the supposition that the logarithmic relation might be followed. It is actually found that in the case of the prostate (Fig. 8) the points fall roughly on two lines. There is no clear distinction between the points corresponding to androsterone, whether in oil or alcohol, androsterone *plus* oestrone, "Hombreol" or "Proviron". The results with "Enarmon" however fall quite outside the range of the others on a separate line corresponding to an activity per capon unit about three times that of the other substances. In the case of the seminal vesicles (Fig. 9) the inherent variability of the test object causes difficulty, but it is possible to suggest a line passing through the "Enarmon" points corresponding to about four times the activity per capon unit of the other materials.

(g) *Administration of isoandrosterone.* Five immature castrated rats each received a total dose of 24.5 mg. of *iso*androsterone given in 0.2 ml. arachis oil daily for 10 days. This was equivalent in capon units to 4.0 mg. of androsterone, and although the reaction to the *iso*-hormone was slightly less than to 3.5 mg. of androsterone in oil (prostates 37 mg., seminal vesicles 6 mg.), it is doubtful if it was significantly different, especially in view of the larger volume of oil used for the *iso*-hormone. It is hoped to make further experiments on this substance.

VII. EXPERIMENTS ON MICE.

(a) *Stimulation of growth in rudimentary or vestigial seminal vesicles.* Attempts have been made to grow the rudimentary (2 mg.) seminal vesicles in mice, some time after prepubertal castration. Crude testis and urine extracts, administered in various forms, have been employed, the mice being usually injected for 10 days before killing. Many of the extracts either were not absorbed or had a deleterious effect on the animals, and insufficient material has been available up to date in a suitable form for the preparation of a dose-response curve on mice. Although it is easy to produce cytological changes in mouse seminal vesicles, such as have been described by Voss and Loewe [1931], the organs have in most experiments shown very small weight increases (up to 8–10 mg.) after 10 days' injection. Such a period is long enough under optimum conditions of dosage to produce seminal vesicles up to 140 mg. Ten days' stimulation of the testes of 10 normal immature 10–15 g. mice by prolan produced seminal vesicles having an average weight of 101 mg. The largest seminal vesicles so far developed in an immature castrated mouse weighed 46 mg.; this animal had

received about 100 capon units of a urine concentrate in 10 days. A crude testicular extract produced growth of seminal vesicles to a weight of 35 mg. in another mouse, which was given 6 injections on alternate days and then killed 10 days after the last injection. A group of five immature castrated mice each received a total of 3.5 mg. androsterone in dilute alcohol during 10 days; the seminal vesicles were very small, averaging just over 5 mg., but the epithelium was glandular in comparison with that of a castrated control.

Experiments with crude extracts were also carried out on mice castrated post-pubertally at various body weights; in these the seminal vesicles had atrophied completely and weighed 5–20 mg. Such seminal vesicles naturally reached a larger size than those of the immature castrates, but the extent of their reaction was more difficult to gauge owing to the greater variation of the vestigial gland. In these tests seminal vesicles of 30–40 mg. were not uncommonly produced, but the largest ones obtained were only 58 mg. after 10 days' injection with testis extract, and 50 mg. after a total of 16 capon units of "Enarmon", compared with 20 mg. controls. On the whole the larger initial size of the atrophied adult, as against the rudimentary, seminal vesicles, probably does not counterbalance the disadvantage of their greater variability, although the weighing of the seminal vesicles after small doses of male hormone can be more readily carried out.

(b) *Maintenance of seminal vesicles in adults.* Several attempts were made to maintain the size of the seminal vesicles for 10 or 14 days in castrated adult mice by means of injections of male hormone and of oestrin begun on the day of castration.

It was assumed from earlier work [Deanesly and Parkes, 1933] that there would be less variation in initial seminal vesicle weight if large mice with a body weight of 24–30 g. were selected for these experiments; they therefore differ from the maintenance experiments on rats (Section V), since they are concerned not with the period of rapid growth, but with the later phase of seminal vesicle enlargement in which the rate of increase is slackening. Unfortunately it is found that in such mice, 10 days after castration, the range of variation in seminal vesicle weight is so great that even in groups of 20, one or two animals in which the seminal vesicles have remained large will materially affect the group average, and in groups of 10 or less only a large difference in seminal vesicle weights from the controls can be considered significant. The results of these experiments, which can only be regarded as preliminary, are set out in Table VI; the controls include those mentioned in an earlier paper

Table VI. *Maintenance of mouse seminal vesicles after postpubertal castration.*

Average normal seminal vesicle weight 246 mg. at an average body weight of 27 g.

No. of exp.	Average body weight at castration g.	Days after castration	No. of mice	Daily injection	Average seminal vesicle weight mg.
Controls	27.5	10	39	None	100
Exp. 20	27.5	10	9	10 γ oestriol in aqueous alcohol	72
Exp. 20	27.5	10	10	1 γ oestriol in aqueous alcohol	58
Exp. 13	27.0	10	18	1 capon unit ("Proviron") in oil emulsion	114
Exp. 12	25.5	10	20	2 capon units ("Proviron") in oil emulsion	115
Exp. 14	25.5	10	20	5 capon units ("Proviron") in 0.4 ml. oil	124
Controls	25.5	14	25	None	64
Exp. 50	26.0	14	10	1 mg. androsterone in 0.1 ml. arachis oil	117
Exp. 58	30.0	14	3	2 mg. androsterone in 0.2 ml. arachis oil	157

[Deanesly and Parkes, 1933] with some additional mice. In all the experimental groups, except the 3 mice in Exp. 58, the average seminal vesicle weight is nearer to that of the corresponding castrated controls than to that of normal mice of the same body weight. Exp. 20, in which the seminal vesicle weights are below those of the controls, should probably be regarded as negative, since it is unlikely, though not impossible, that injections of oestriol would accelerate the shrinkage of the glands which at this stage is brought about mainly by the loss of secretion [*cf.* Moore and Price, 1932]. Exps. 12-14, 50 and 58, though far from conclusive, seem to show partial maintenance of the glands, and this is corroborated for the last two experiments by the histological appearance of the cranial (anterior) prostate attached to the seminal vesicles. It should certainly be possible to maintain the size of the mouse seminal vesicles after castration by injections of male hormone; the relatively large dose which seems to be needed is probably due to the conditions of absorption from oily solutions which are more unfavourable in the mouse than in the rat.

VIII. EXPERIMENTS ON GUINEA-PIGS.

Two groups of prepubertally castrated guinea-pigs were injected with androsterone alone and in conjunction with oestrone and were killed 24 hours after the last injection; the results are summarised in Table VII. The accessory

Table VII. *Average weights of prostate and seminal vesicles in control and injected guinea-pigs.*

No. of animals	Body weight at castration g.	Body weight at death g.	Condition at autopsy	Average prostate weight mg.	Average seminal vesicles weight mg.
4	—	200	Normal immature	57	42
3	200	250-450	21-89 days after castration, not injected	76	53
3	193	473	88 days after castration: had 15 daily injections of 1 mg. androsterone in arachis oil	186	121
3	197	442	88-89 days after castration: had 15 daily injections of 1 mg. androsterone + 10 γ oestrone in arachis oil	180	120

glands of guinea-pigs, in our experience, are slower to shrink in weight after castration than those of the rat or mouse. After prepubertal castration there seems to be little or no shrinkage of the comparatively undeveloped glands. The high average weight for the prostate in the control castrated animals in Table VII is due to one animal in which it was exceptionally large. On such small numbers the figures for the average weights can only be regarded as approximate, but the results are very consistent. In the two experimental groups the average weights of prostate and seminal vesicles were almost exactly the same; the addition of oestrone had not reinforced the androsterone, and the experiment confirmed the conclusion reached as the result of experiments with rats (Section VI *f*). A total dose of 15 mg. androsterone caused an increase in the seminal vesicles from 40-50 mg. to 120 mg., which suggests that they are more responsive to this substance than are the rat seminal vesicles: the effect however may be due less to greater sensitivity than to the larger size of the rudiment. The prostate had also tripled in size in the experimental guinea-pigs; its average weight, 183 mg., may be compared with the average weight of the prostate, 129 mg., in rats receiving

the same doses of androsterone over a slightly shorter period (Tables III and V). Allowing for the difference in size of the rudiment, the actual gain in weight in the two species is very similar.

IX. HISTOLOGICAL OBSERVATIONS.

(a) *Rats and mice.* The prostate and seminal vesicles of one or more animals from most of the experimental groups were examined microscopically, all material having been fixed in Bouin's fluid as a routine. Sections were cut at 7μ and 10μ and stained in haematoxylin and eosin. Since the histological changes in the rudimentary accessory glands of rats and mice giving a positive response to male hormone have been described in detail in a number of papers by earlier workers already cited, no account of these will be given here. The present observations were intended to be mainly quantitative and histological examination was used as a means of distinguishing any abnormal growth stage that might be produced experimentally, *e.g.* by oestrin injections [David *et al.*, 1934]. The glands from injected rats were therefore compared with normal seminal vesicles and prostates of similar weight, as well as with those of the appropriate castrate controls. Owing to the variations both in the normal growth rate of the accessory organs and in the structure of different parts of the glands, a histological basis for standardising extracts seems undesirable if not impossible. Hansen [1933] has drawn attention to some of the difficulties involved.

Since the rat prostate lobes differ histologically [Walker, 1910], complete serial sections were cut through the glands of a young animal (one in five being mounted). These showed that, at the early spermatogenetic stage examined, three types of glandular tissue could be distinguished in the cranial, dorso-lateral and ventral lobes respectively. Moore, Price and Gallagher [1930] describe the last two as histologically similar but actually, though both are prostatic in type, they can be readily distinguished in section, since there is less fibrous tissue in the ventral lobes and the alveoli are much larger. For routine examination transverse sections were cut through a ventral prostate lobe, the cranial lobe being similarly examined in some rats and in the adult experimental mice.

The general conclusions drawn from the histological observations may be summarised as follows:

(1) In the rat maintenance experiments (Section V) all prostate sections examined appeared to be histologically normal; they showed none of the castration changes described by Moore, Price and Gallagher [1930]. No attempt was made to section the seminal vesicles in these experiments, since the weights seemed to be an adequate guide to their condition.

(2) The developing prostates of the prepubertally castrated rats injected with androsterone were not obviously different in any way from normal glands of the same size. In other words, androsterone, *isoandrosterone* and the urine concentrates tested will all cause normal development to a greater or lesser extent in a gland ordinarily controlled by the secretion of the gonad. When oestrin is injected in conjunction with the male hormone, the greater activity of the latter on the prostate tends to mask any oestrin effect. Further, if oestrin is injected alone for 10 days the reaction of the rat prostate is very slight [David *et al.*, 1934] and not easily distinguishable in section.

The largest seminal vesicles grown with androsterone in prepubertally castrated rats weighed only 82 mg.; they appeared to be histologically normal for their size and contained secretion. The largest glands produced by the same dose of androsterone (20 mg.) *plus* 100 γ of oestrone weighed 94 mg. and were slightly better developed, but similarly normal. The only essential effect of the

oestrone seems to be the acceleration of the earliest stages of seminal vesicle growth by the development of fibrous tissue [David *et al.*, 1934; Korenchevsky *et al.*, 1934, 1, 2]. This is well shown in the rats receiving oestrone alone, where the seminal vesicle epithelium has not reacted. No fresh observations worth recording were made on the seminal vesicles of immature castrated mice receiving male hormone injections. The cranial (anterior), prostate lobes of the mice injected with 1 or 2 mg. of androsterone daily in the maintenance experiments show some alveolar shrinkage as compared with the prostates of normal mice, but the epithelium is comparatively normal and is still secreting.

The adrenals were examined from 7 injected mice and 5 controls from these experiments (Nos. 50 and 58, Table VI) in order to see whether male hormone had inhibited the changes towards a female type of cortex normally following castration [Howard-Miller 1927; Deanesly, 1928]. The zona reticularis, which tends to atrophy in the 21-day male mouse and can hardly be distinguished in the adrenal of the adult, reappears after castration and forms a dark-staining band round the medulla, consisting of small cells free from fatty inclusions. No distinction could be made however between the experimental and control groups. It is probable that the adrenal is not affected directly by the testicular hormone, but only indirectly through the pituitary, and the present injections of androsterone were inadequate to influence the latter gland.

(b) *Guinea-pigs*. The seminal vesicles and prostate were examined histologically in one animal from each of the groups listed in Table VII. In the immature 200 g. guinea-pig the seminal vesicles and prostate, though small, are already actively secreting. In the corresponding castrated animal, which has the largest accessory glands in the group, the glandular epithelium appears definitely inert, but its regression is much less conspicuous 89 days after castration than in a similar mouse or rat. The prostatic alveoli are as large as in the corresponding normal animal, but the epithelium is disorganised. In guinea-pigs from both experimental groups, the seminal vesicles and prostate, including the coagulating glands, showed well-marked growth and secretion, and there were no obvious differences between the two. The histological changes were of the usual type, including distention of the prostatic alveoli and of the seminal vesicles, and need not be described in detail.

X. DISCUSSION.

The most striking observation in the course of this work is that, although maintenance of the accessory glands of postpubertally castrated rats was achieved, the highest doses of androsterone administered for 10 days failed to produce much growth of the accessory organs of prepubertally castrated rats. In particular, androsterone seems least effective on the seminal vesicles. Although the growth of these glands normally starts later than that of the prostate, yet in the experimental animals receiving androsterone, the seminal vesicles tended to remain disproportionately small. Moreover, when, as in the 28-day experiment, there was considerable growth of the prostate, that of the seminal vesicles lagged far behind. Even when allowance is made for inefficiency in the form of administration and unsatisfactory absorption, there would be some difficulty in assuming that sufficient androsterone is normally secreted by the testis to account for its male endocrine activity.

A clue to the existence of other active substances is provided by comparison of a concentrate from natural sources with androsterone on the basis of two different responses, namely, growth of the comb in the capon and of accessory organs in the rat. It is then found that one concentrate from urine, "Enarmon",

when administered to rats in the same dosage as androsterone, on the basis of capon units, is several times as active as the latter, the contrast being particularly marked in the effects on the seminal vesicles (Figs. 8 and 9). The discrepancy is analogous, in fact, to that which has been observed in the last four years in the comparison of male hormone concentrates from testis and from urine. In 1931, Laqueur, de Fremery *et al.* discussed fluctuations in the ratio rat unit/bird unit in preparations from testis, and, in a later paper Laqueur, Dingemanse *et al.* [1931] speak of a "seminal vesicle hormone". They followed this work by putting forward the view that testicular extracts were superior to urine extracts on mammals [Freud and Laqueur, 1934; Freud, 1934; Dingemanse *et al.*, 1935]¹. The assumption that testicular extracts contain an active substance chemically different from that in urine extracts is confirmed by the work of Gallagher and Koch [1934, 1, 2; *cf.* also Matsuzaki, 1933, 1934; Ogata and Hirano, 1933].

There is some evidence however that crude urine concentrates may also contain a substance active on accessory glands which is lost on further purification. Our own results have shown a significant difference between "Enarmon" on the one hand and "Provion" and "Hombreol" on the other. Korenchevsky also seems to have detected significant differences between urine concentrates of the same capon titre [Korenchevsky *et al.*, 1933, 1, 2; 1934, 2], though he emphasises rather the superiority of his own method of assay as a measure of male hormone activity than the implication that the materials differ in any essential way. Freud [1933] refers to "the loss of potency with respect to seminal vesicles without corresponding loss of potency in capons" when male hormone extracts are purified. This observation actually led the Amsterdam school to the abandonment, for a time, of the "seminal vesicle hormone" hypothesis, and to its replacement by the hypothesis that synergism of oestrin and male hormone stimulated complete seminal vesicle growth.

Undoubtedly, not only is oestrogenic material produced by the male organism, but oestrin itself has a definite stimulating effect on the fibromuscular tissue of the seminal vesicles [Freud, 1933]. The experiments recorded here however convince us that administration of a mixture of androsterone and oestrone does not reproduce the results of administration of a crude urine concentrate of slight oestrogenic potency. This point may be made clear by reference to Figs. 8 and 9, from which it is seen that with one exception the combination of oestrone with androsterone has little or no extra effect on the accessory organs as compared with androsterone alone. The exceptional case is that of the highest dose of androsterone *plus* oestrone, which caused slightly greater growth of the seminal vesicles (Tables III and V), but this is little more than that provoked by one-fourth the number of capon units administered in the form of "Enarmon". The reaction of the prostate to androsterone over 10 days was not increased by oestrone, and the greater activity of "Enarmon" was still obvious.

One is left therefore with the conclusion that crude urine extracts, and presumably testicular extracts also, contain some other androkinetic substance, either (a) supplementary to, or synergistic with, androsterone or (b) acting independently of androsterone, possessing a greater ratio of accessory organ activity to comb growth activity than androsterone and being the chief or only male hormone produced by the testis. The comparative success of the mainten-

¹ Note added to proof. In a further short note, seen after completion of this paper, David and Freud (*Acta Brev. Neerl.* 1935, 5, 31) compare the activities of androsterone, "Hombreol" and testis extract on the growth of the accessory glands of immature castrated rats. Administered in equal capon unit dosage, the first two are of equal potency whereas testis extract is much more active, particularly on the seminal vesicles.

SYN

ance experiments and the nature of the histological findings suggest strongly that androsterone is quantitatively rather than qualitatively deficient and dispose of the necessity for a supplementary or synergistic substance. The existence in the body of a more active form of male hormone present to some extent in urine, and *a fortiori* in testis, is much more likely.

An analogy may be drawn between the male and female hormones in this connection. In each case the characteristic hormonal activity can be exercised by more than one compound. Oestrone and oestriol, both capable of producing the same effects, have a different ratio of activity on different test-objects, *e.g.* cornification of the vagina of the immature rat and of the ovariectomised adult. Oestradiol (so-called "dihydro-oestrin") appears to be the most active oestrogenic compound, and it is likely that it is the primary form of the oestrogenic hormone in the body, since it can be extracted from ovaries [Doisy *et al.*, 1935]. The artificially prepared dihydroxy-derivative of androsterone possesses a similarly enhanced activity in capons [Ruzicka *et al.*, 1935] but there is as yet no evidence of its natural occurrence or of its relative activities in mammals and birds. The analogies between the two hormones suggest strongly the possibility that there remains to be isolated from natural sources some derivative of androsterone which possesses not only a higher degree of activity but also a different ratio of activity on accessory organs in the mammal and on the comb of birds.

SUMMARY.

1. Androsterone, *iso*androsterone, and various urine concentrates have been tested on the accessory reproductive glands of castrated rats, mice and guinea-pigs.

2. Androsterone was injected into groups of adult rats for 14 days, beginning on the day of castration; 1 mg. daily was enough to maintain growth and normal structure in the prostate of 140–160 g. rats but more than 2 mg. daily were required for maintenance of the seminal vesicles. Larger rats, castrated and injected daily with 3 mg. androsterone, had prostates above the normal size and normal seminal vesicles.

In similar experiments with mice 1 and 2 mg. daily of androsterone failed to maintain completely the seminal vesicles or the cranial lobes of the prostate.

3. Androsterone, in both oily and alcoholic solution, was injected into groups of immature castrated rats for 10-day periods in an attempt to grow the rudimentary accessory glands; on a total dose of 20 mg. of androsterone the largest prostate produced in these experiments was 212 mg. or about a quarter of the full size, and the largest seminal vesicles were 82 mg., about one-tenth of the full size. In most of the androsterone experiments however, particularly with oily solutions, the seminal vesicles of the rats gave a very poor reaction as compared with the prostate. This was particularly obvious in a 28-day experiment; on 1 mg. daily the prostate reached a maximum of 433 mg. whilst the largest seminal vesicles were only 63 mg.

4. A comparison of different urine concentrates on the basis of the number of capon units contained in each showed that, whereas in two of them the effect per capon unit on the accessory glands of rats was much the same as that produced per capon unit (100γ) of crystalline androsterone, in the third urine preparation the activity on rats was more than three to four times as great.

5. It was shown that the activity of the most effective urine concentrate could not be reproduced by combined administration of oestrone and androsterone, which gave results little, if any, better than androsterone alone.

6. Experiments were also carried out with castrated mice and guinea-pigs.

7. It is concluded that certain urine concentrates may contain a male hormone other than androsterone, with a much greater activity per capon unit on the accessory glands of mammals.

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