XLVI. COMPARATIVE ACTIVITIES OF COMPOUNDS OF THE ANDROSTERONE-TESTOSTERONE SERIES.

BY RUTH DEANESLY AND
ALAN STERLING PARKES.

From the National Institute for Medical Research, London.

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I. Introduction.

THE first isolation of a crystalline substance with male hormone properties was carried out by Butenandt [1931], who obtained from male urine a small amount of the substance to which he subsequently gave the name androsterone. Later, he was able to prepare larger quantities and to suggest that the substance was a fully hydrogenated *cyclopentenophenanthrene* derivative with a hydroxyl group in position 3 and a keto-group in position 17 [Butenandt, 1932; 1933]. Ruzicka *et al.* [1934] were able to confirm this hypothesis by the chemical preparation of androsterone from *epicholesterol*, thus determining the configurations of carbon atoms 3 and 5.

In the meantime evidence had been accumulating that the male hormone activity of male urine differed quantitatively from that of testicular extracts, notably in its smaller activity per capon unit on the seminal vesicles of castrated rats (see Dingemans *et al.* [1935] for refs.), and it was soon evident that androsterone was not the chief active principle of testicular extracts [David and Freud, 1935; Callow and Deanesly, 1935, 1]. The search for other male hormone substances therefore continued. Butenandt and Dannenbaum [1934] had previously isolated from male urine an unsaturated substance closely related to androsterone and having about one-third of its activity, *transdehydroandrosterone*. Ruzicka was led to believe that the testicular hormone itself, by analogy with progesterone, might be an unsaturated compound related to androsterone [Ruzicka and Wettstein, 1935, 1]; having produced *transdehydroandrosterone* ($\Delta^{5,6}$ -*trans*-3-hydroxy-17-keto-androstene) by degradation of cholesterol, he set about the systematic production of other compounds of the series. While these developments were taking place, David *et al.* [1935] had obtained from testis a crystalline substance having high activity on both capons and rats, and apparently of sterol type, to which they gave the name testosterone. On the basis of his biological tests of the substances prepared by Ruzicka, Tschopp [1935, 1] was able to suggest that the testosterone of Laqueur and his co-workers might be the androstenedione already prepared by Ruzicka, or possibly the corresponding 17-hydroxy-3-keto-compound. David [1935] then showed that oxidation of testosterone yielded the unsaturated diketo-compound and it seemed certain that testosterone must be Δ^4 -17-hydroxy-3-keto-androstene. The next step in this remarkable story was the report by Butenandt and Hanisch [1935, 1, 2] of the artificial production of this compound from dehydroandrosterone and its identification with Laqueur's testosterone. Simultaneously, Wettstein [1935], and soon afterwards Ruzicka and Wettstein [1935, 2], published details of the completion of their projected synthesis of Δ^4 -17-hydroxy-3-keto-androstene.

Allowing for transposition of the hydroxyl and ketone groups at positions 3 and 17, for *cis* and *trans* configurations of the 3-hydroxyl groups, for the diketone and the dihydroxy-compounds, but not for differences in configuration of the 17-hydroxyl, there are twelve possible derivatives in the series, six from androstane and six from androstene. Of these twelve, ten have so far been produced artificially from cholesterol. (See Table I.) The remaining two involve the

Table I.			
Androstane derivatives (saturated)		Androstene derivatives (unsaturated: Δ^4 or $\Delta^{5,6}$)	
	3- <i>cis</i>	3- <i>trans</i>	
3-hydroxy-17-keto-	Androsterone	<i>trans</i> Androsterone	Dehydroandrosterone (not known)
3:17-dihydroxy-	Androstanediol	<i>trans</i> Androstanediol	Androstenediol (not known)
17-hydroxy-3-keto-		3-Keto-androstanol	Testosterone
3:17-diketo-		Androstanedione	Androstenedione

Androstane

technical difficulty of securing a *cis*-3-hydroxyl with a 5 : 6 double linking. Of the ten now available, androsterone, *trans*androsterone, *trans*dehydroandrosterone, testosterone and androstenedione have been referred to above. The others include *cis*androstanediol [Ruzicka, Goldberg and Meyer, 1935, 2; Butenandt and Tscherning, 1935], *trans*androstanediol [Ruzicka, Goldberg and Rosenberg, 1935], androstanedione [Butenandt and Tscherning, 1934, 2; Ruzicka, Goldberg and Meyer, 1935, 1], and *trans*androstenediol [Ruzicka and Wettstein, 1935, 1]. Further, Ruzicka and his co-workers have made various esters of the compounds [see Ruzicka, Wettstein and Kägi [1935] in addition to papers cited above], and also derivatives methylated or ethylated at position 17, including methyltestosterone.

The biological properties of androsterone were briefly examined by Butenandt and his co-workers after their initial isolation of the substance (see also Tscherning [1933]), and have since been investigated in detail by Ruzicka, Goldberg *et al.* [1934], Butenandt and Tscherning [1934, 1], Korenchevsky [1935], David and Freud [1935], Callow and Deanesly [1935, 1], Callow and Parkes [1935], Greenwood *et al.* [1935], Tschopp [1935, 2] and Korenchevsky and Dennison [1935, 1]. Androstanediol has been investigated by Tscherning [1934], and by Butenandt and Tscherning [1935], Ruzicka, Goldberg and Meyer [1935, 2], David and Freud [1935], Callow and Deanesly [1935, 2] and Korenchevsky and Dennison [1935, 2].

The biological activities of other compounds of the series, including testosterone, have been examined by Ruzicka and his co-workers in the papers referred to above, by Tschopp [1936] in great detail and to a less extent by Butenandt, in the papers referred to above, and by Parkes [1935]. Reference to their findings is made in the appropriate place below.

The work described below was undertaken as a study of the influence on biological activity of slight changes in molecular structure, for which the

androsterone-testosterone series of male hormone compounds offers scope unrivalled in endocrinology. At the same time it was hoped to find out to what extent compounds known at present, especially testosterone, exhibit the biological properties attributable to the testicular hormone or hormones.

II. *Material and technique.*

The comparative biological activities of the following compounds have been tested:

Androsterone
Androstanediol
*trans*Dehydroandrosterone
*trans*Androstenediol
Testosterone
Androstenedione
Methylandrostanediol
Methyl-*trans*androstenediol
Methyl-3-keto-androstanol
Methyltestosterone
Testosterone benzoate

These compounds were all prepared by Prof. L. Ruzicka and Messrs Ciba.

All the substances were dissolved in arachis oil for injection. Their solubility varied greatly. Androsterone and *trans*dehydroandrosterone are readily soluble at 10 mg./ml.; heating is required to make the solution, but the material does not separate at room temperature. Androstanediol, *trans*androstenediol and methyl-*trans*androstenediol do not stay in solution at room temperature even at 2.5 mg./ml., and heating immediately before injection is necessary if concentrations of more than about 1.5 mg./ml. are being used. Methylandrostanediol is much more soluble. The other compounds remain in solution at the highest concentrations we have made, 5 mg./ml.

Capon tests. The work on the capon comb was carried out as described by Callow and Parkes [1935] and the results were obtained by reference to the standard curve given by Greenwood *et al.* [1935]. Each series of tests was carried out on groups of capons of the same hatch and caponised together. In each experiment a control group received a total dose of 1 mg. androsterone per bird and the activity of the simultaneously tested substance was calculated in comparison with the result on the control group. A total of 1 mg. androsterone gives an average increase in comb size (L+H) of between 6 and 10 mm., depending on the sensitivity of the birds. The doses of the other compounds were adjusted to give a response of similar magnitude before a final comparison was made. In this way the effect of variation in response and of any difference in the slope of the dose/response curves for the various compounds (such as is found in rats) was minimised.

Rat tests. The rats were injected subcutaneously. We have evidence that the volume of oil injected influences the response appreciably and, with one or two exceptions, the volume of oil solution injected daily was standardised at 0.2 ml. The technique of assay on rats was exactly as described by Callow and Deanesly [1935, 1], *i.e.* immature males were castrated at about 40–50 g. body weight and used not less than a month later, the total dose of hormone being given over 10 days. The organs were weighed from 70% alcohol after fixation in Bouin's fluid—a technique employed by us for many years (see *e.g.* Brambell and Parkes [1929]). There is no doubt that the use of rats immediately after castration, before glandular atrophy has set in, greatly increases the apparent response to

treatment, and this fact may explain some of the discrepancies in the results of various workers. Neither the weight of rat nor the time (above 1 month) after castration at injection was strictly standardised, but we have failed to obtain any evidence that within reasonable limits these factors have any regular influence on the result. Groups of 5 rats were used for each test. Many of the tests were duplicated after an interval, giving a total of 10 rats on each dose.

III. Activity on the capon comb.

Table II gives the results obtained with the capon comb test. In addition to preliminary trials, two tests were made with each compound, except *trans*-androsterone (for which the single result is copied from Callow and Deanesly [1935, 1]) and *trans*androstenediol, on which a third test was made. Apart from

Table II. Activity on the capon comb.

Compound	Amount (γ) required to $\equiv$ 100 γ androsterone		International unit as tested on capons (approx.) γ	International units per mg. (approx.)
	1st test	2nd test		
A. Androsterone	100	100	100	10
B. <i>trans</i> Androsterone	700	—	700	1.5
C. Androstanediol	33	30	33	30
D. Methylandrostanediol	27	22	25	40
E. Methyl-3-keto-androstanol	24	23	24	40
F. <i>trans</i> Dehydroandrosterone	310	295	300	3
G. Androstenedione	110	97	100	10
H. <i>trans</i> Androstenediol	175	250, 280	235	4
J. Methyl <i>trans</i> androstenediol	160	150	155	7
K. Testosterone	16	18	17	60
L. Methyltestosterone	70	90	80	12

*trans*androstenediol, there is no serious discrepancy in the duplicate tests. Several of the results, however, are significantly different from those obtained by Tschopp [1936]. This difference may be due to the fact that his comparisons were made at a level of response given by 70 γ androsterone daily, whereas ours were made at a level given by 200 γ daily.

The following observations may be made on the data given in Table II.

(a) The only compounds less active than androsterone are those having a *trans*-3-hydroxyl group. Such a group is therefore specially unfavourable to activity on capons. Oxidation to a 3-keto-group (*trans*dehydroandrosterone to androstenedione) or conversion into a *cis*-3-hydroxyl (*trans*androsterone to androsterone) increases the activity considerably.

(b) The fact that methylandrostanediol and methyl-3-keto-androstanol are of similar activity suggests that the oxidation of the *cis*-3-hydroxyl group to a 3-keto-group has little effect on activity, a conclusion in keeping with Tschopp's data for androsterone and androstanedione. It may be supposed therefore that the so far unknown *cis*dehydroandrosterone and *cis*androstenediol will have activities on capons similar to those of androstenedione and testosterone respectively.

(c) Reduction of the 17-keto-group to a hydroxyl group increases the activity, slightly (*trans*dehydroandrosterone to *trans*androstenediol) or greatly (androsterone to androstanediol, and androstenedione to testosterone).

(d) The effect of unsaturation on the activity on capons is inconstant. Comparison of *trans*androsterone with *trans*dehydroandrosterone suggests an increase of activity by unsaturation. Tschopp's corresponding data support this, but his figures for androstanedione and androstenedione and for *trans*androstanediol

and *trans*androstenediol do not imply any effect of unsaturation on activity on capons.

(e) The introduction of a 17-methyl group slightly increases activity on the capon comb, except for the anomaly of methyltestosterone which is much less active than testosterone.

Activity of testosterone benzoate. Androsterone benzoate has a delayed and prolonged action on the capon comb [Callow and Deanesly, 1935, 1; Callow, 1936], but the ultimate amount of growth produced may be even greater than that caused by a similar amount of free hormone. Testosterone benzoate however was found to have no appreciable activity. A total dose of 1 mg. to each of 5 capons gave no detectable comb growth within a month.

IV. Activity on the prostate and seminal vesicles of the castrated rat.

Nature of dose/response curves. The results of the rat experiments are summarised in Tables III and IV and shown graphically in Figs. 1-4. In these

Table III. Activity of non-methylated compounds on the castrated rat.

Compound	Total dose (mg.)	No. of rats	Average weight of prostate (mg.)	Average weight of seminal vesicles (mg.)
A. Androsterone	3.5	10	37	8
	6.0	11	54	12
	10.0	10	92	22
	15.0	10	135	23
	20.0	10	141	32
C. Androstanediol	1.5	5	54	16
	2.5	10	73	30
	5.0	10	96	34
	10.0	10	168	79
F. <i>trans</i> Dehydroandrosterone	10.0	5	19	6
	20.0	5	24	9
G. Androstenedione	2.5	5	58	22
	5.0	10	78	33
	10.0	10	150	58
H. <i>trans</i> Androstenediol	5.0	10	59	28
	10.0	10	104	82
K. Testosterone	0.6	5	33	10
	2.0	5	61	27
	4.0	5	96	47
	6.0	5	136	84
	8.0	5	156	99

Table IV. Activity of methylated compounds on the castrated rat.

Compound	Total dose (mg.)	No. of rats	Average weight of prostate (mg.)	Average weight of seminal vesicles (mg.)
D. Methylandrostanediol	2.5	5	40	27
	5.0	5	151	59
	7.5	5	201	78
E. Methyl-3-keto-androstanol	2.5	5	83	23
	7.5	5	230	136
J. Methyl <i>trans</i> androstenediol	2.5	5	30	9
	5.0	5	105	50
	7.5	5	116	65
L. Methyltestosterone	2.0	5	75	32

Fig. 1.

Fig. 3.

Fig. 2.

Fig. 4.

Fig. 1. Response of the prostate of the castrated rat to male hormone compounds (non-methylated).

Fig. 2. Response of the seminal vesicles of the castrated rat to male hormone compounds (non-methylated).

Fig. 3. Response of the prostate of the castrated rat to male hormone compounds (methylated).

Fig. 4. Response of the seminal vesicles of the castrated rat to male hormone compounds (methylated).

graphs the ordinates are logarithmic, and in 3 of the 4 experiments for which there are most data (androsterone and testosterone, both prostate and seminal vesicles) the results suggest strongly that the response bears not a linear but a sigmoid relationship to the logarithm of the dose. The curves given by androstanediol and androstenedione can be considered as the middle parts of sigmoid curves, and the same applies to methylandrostanediol and methyltransandrostanediol. For the remaining four compounds the data are inadequate to show the nature of the dose/response curve. Further analysis of these curves and of the variability of the response will be made elsewhere.

Comparative activities of compounds. Testosterone is the most active of any of the non-methylated compounds. From our results, this superiority is well marked on the seminal vesicles, but insignificant on the prostate. On both it is much less than shown by Tschopp's results. Further, we find that methyl-3-keto-androstanol is more active than testosterone on both prostate and seminal vesicles, and methylandrostanediol is more active on the prostate, though both were found by Tschopp to be less active. On both the prostate and seminal vesicles the non-methylated compounds have the following order of descending activity, androstanediol, androstenedione, transandrostanediol, androsterone, transdehydroandrosterone, except that the curve for the response of the seminal vesicles to transandrostanediol intersects that for the response to androstenedione. Relative to the other compounds however androsterone is much less active on the seminal vesicles than on the prostate, whilst transandrostanediol is more active on the seminal vesicles than on the prostate. This point will be dealt with more fully below, but meanwhile it may be noted:

(a) That taking androsterone as the standard substance, comparisons of activity on the seminal vesicles will give very different results from those obtained by comparisons on the prostate. Thus (Figs. 1 and 2) testosterone is about 10 times more active than androsterone when tested on the seminal vesicles, but only 2-5 times when tested on the prostate. transAndrostenediol is an extreme case; it is only slightly more active on the prostate than androsterone, but it is 4-5 times more active on the seminal vesicles.

(b) Differences in the slope of the dose/response curves for the different substances make it, in some cases, almost impossible to give a definite ratio for their relative activities. This applies whether the relative amounts of two substances required to produce the same degree of response are compared or the relative responses produced by the same amount of two substances. Thus on the prostate, 1 mg. of testosterone $\equiv$ 4 mg. of androsterone, but 5 mg. $\equiv$ 12 mg. and 6 mg. $\equiv$ 20 mg.

Similarly, from the above results, the relative activities of androstanediol, transandrostanediol and androstenedione depend very much on the points at which the comparison is made. In these circumstances it is difficult to see how one substance can be equated with another and in particular to see how androsterone can serve in a rat test as a standard for substances other than androsterone or androsterone-containing extracts. For these reasons we refrain from trying to construct any table of relative activities of the various compounds on rats.

This difference in slope of the dose/response curves for different substances does not seem hitherto to have been specifically noted, probably because such an extensive group of hormones as the androsterone-testosterone series possessing similar biological activities has not hitherto been available. A comparative investigation of oestrone, oestradiol and oestriol from the point of view of slope of dose/response curves seems to be indicated. Hill *et al.* [1934]

observed a difference in slope of the dose/response curves for the ovulation-producing activity of hypophyseal and urine extracts, but such extracts are so complex as to be scarcely comparable with crystalline substances.

The following tentative conclusions may be drawn about the effect of molecular configuration on male hormone activity in rats.

(a) The *trans*-configuration of the 3-hydroxyl group is obviously unfavourable to activity in *trans*androsterone, but is not incompatible with good activity in *trans*androstenediol, especially on the seminal vesicles. The *cis*-3-hydroxyl and the 3-keto-group confer about equal activities on the compound (methyl-androstenediol and methyl-3-keto-androstanol). Tschopp's data on androsterone and androstenedione suggest a similar conclusion.

(b) Reduction of the 17-keto-group to hydroxyl greatly enhances activity, as shown by three pairs of compounds, androsterone and androstenediol, *trans*-dehydroandrosterone and *trans*androstenediol, and androstenedione and testosterone.

(c) The effect of unsaturation is rather uncertain. The data are inadequate for a comparison of *trans*androsterone with *trans*dehydroandrosterone. According to Tschopp, *trans*androstenediol is more active than *trans*androstenediol and androstenedione is more active than androstenedione. On the other hand, in our experience, methyl-3-keto-androstanol is of the same order of activity as methyltestosterone.

(d) In the three pairs of compounds available for comparison, the introduction of a methyl group increases the activity appreciably.

Ratio of prostate/seminal vesicle growth. Callow and Deanesly [1935, 1] showed that androsterone caused an abnormal ratio of growth between prostate and seminal vesicles, the latter being small for the size of prostate. They also showed [1935, 2] that androstenediol produced a normal growth relation between the two organs. These results have been confirmed by Korenchevsky and Dennison [1935, 1, 2]. Of the compounds dealt with above, the data for *trans*dehydroandrosterone are inadequate, and with methyl-3-keto-androstanol and androstenedione the ratio is fairly normal. From Fig. 5 it will be seen that testosterone given for 10 days seems to produce larger seminal vesicles in relation to the size of prostate than are found in the normal rat, whilst with *trans*androstenediol the abnormality of the ratio is very marked. This may be evidence that testosterone is not the only male hormone produced by the testis. It should be noted that these new data about the prostate/seminal vesicle ratio in experimental rats relate only to glands in the early stages of growth. Methylation, judging from the results on methyl-androstenediol and methyl-*trans*androstenediol (Fig. 6) seems slightly to decrease activity on seminal vesicles relative to activity on the prostate.

With the available information few conclusions can be reached about the effect of molecular configuration on the prostate/seminal vesicle ratio. In two pairs of compounds, reduction of the 17-keto-group to hydroxyl increases activity on the seminal vesicles relative to that on the prostate (androsterone-androstenediol, androstenedione-testosterone), but oxidation of the 3-hydroxyl to a keto-group does not affect the ratio.

Oestrone given over short periods is known to have a stimulating action on the fibro-muscular tissue of the seminal vesicles, with little effect on the prostate, so that it might have been anticipated that the capacity of a male hormone compound to produce abnormally large seminal vesicles relative to the prostate would be correlated with the degree of oestrogenic activity of the compound (see Deanesly and Parkes [1936]). It is impossible however to detect any obvious

correlation between these two properties; testosterone, for instance, is only very weakly oestrogenic.

Fig. 5. Relation between growth of the prostate and of the seminal vesicles.
 — Normal growth of seminal vesicles in relation to the prostate.
 Δ *H. transAndrostenediol*. $\square$ *K. Testosterone*.

Fig. 6. Effect of methylation of a compound on relation between growth of prostate and of seminal vesicles.

$\circ$ *C. Androstanediol*. $\odot$ *D. Methylandrostanediol*.
 Δ *H. transAndrostenediol*. $\odot$ *J. Methyl-transandrostanediol*.

V. Activity on rats in relation to activity on capons.

It has been mentioned above that extracts of male urine were found to be much less active on rats, per capon unit, than testicular extracts, and that the same applied to androsterone. Callow and Deanesly [1935, 2] found that androstanediol also had the same low activity on rats per capon unit as androsterone and thus lacked at least one characteristic property of testis extracts [cf. Korenchevsky *et al.* 1935]. It was anticipated that testosterone would be similar in this respect to testicular extracts, but our evidence, so far, points to the contrary. In Figs. 7 and 8 are shown the dose/response curves, of prostate and seminal vesicles respectively, for the various compounds, the abscissae being logarithmic scales of capon units (see Table I). From Fig. 7 it

Fig. 7. Activities of various compounds on the prostate of the castrated rat in relation to their activities on the capon comb.

Fig. 8. Activities of various compounds on the seminal vesicles of the castrated rat in relation to their activities on the capon comb.

Explanation of symbols in Figs. 7 and 8:

• A. Androsterone.	× G. Androstenedione.
○ C. Androstanediol.	△ H. <i>trans</i> Androstenediol.
⊙ D. Methylandrostanediol.	⊕ J. Methyl- <i>trans</i> androstenediol.
+ E. Methyl-3-keto-androstanol.	□ K. Testosterone.
□ F. <i>trans</i> Dehydroandrosterone.	⊙ L. Methyltestosterone.

will be seen that, per capon unit, only four of the compounds have higher activities on the prostate than androsterone and that testosterone has appreciably less activity. Of the four more active ones, the two *trans*androstenediols occupy the position because of their low activities on capons (due to the *trans*-3-hydroxyl group), not because of marked activities on rats, and it is possible that the high activities per capon unit of these two compounds are not of much significance. Methyltestosterone is anomalous owing to the curious effect of methylation of testosterone on activity on capons.

All four of the compounds however are unsaturated and it is possible to infer that high activity per capon unit on the prostate is restricted to unsaturated compounds. Certainly Tschopp's figures for androstenedione and androstenediol, for which the capon activities are similar, provide a striking demonstration of the greater activity per capon unit of the unsaturated compound on rats. The four compounds whose activities per capon unit on the prostate are no greater than that of androsterone have no single feature in common and it is impossible at this stage to say what determines low activity per capon unit.

As regards the seminal vesicles, only *trans*dehydroandrosterone, of the compounds examined, had as low an activity per capon unit as androsterone. Testosterone, over the range at which comparison could be made, was very little better than androsterone, but androstenediol and its methylated derivative were slightly more active. As with the prostate however the highest activities per capon unit were shown by the four unsaturated compounds: androstenedione, *trans*androstenediol, methyl*trans*androstenediol, and methyltestosterone. The second of these appears to be ten times as active per capon unit as androsterone. The same general remarks on this varying activity per capon unit apply to the seminal vesicles as well as to the prostate.

SUMMARY.

1. The male hormone activities on capons and castrated rats of eleven compounds of the androsterone-testosterone series have been examined in relation to their molecular configurations and have been compared with the known activities of testis and urine extracts.

2. Testosterone, on both capons and rats, is far the most active of the three substances so far prepared from natural sources. It is also more active than any of the non-methylated "artificial" compounds. Differences in the slope of the dose/response curves on rats for the different substances make any definite figures for the relative activities of the various compounds unsatisfactory, but testosterone may be said to be about 6 times as active on capons, 2-5 times as active on the prostate and 10 times as active on the seminal vesicles of castrated rats as androsterone. Most of the other compounds show intermediate degrees of activity. The most active of all the compounds we have examined was methyl-3-keto-androstanol, *i.e.* methyl-dihydrotestosterone.

3. The following conclusions may be drawn with reference to the effect of molecular structure on male hormone activity:

(a) A *trans* configuration of the 3-hydroxyl group is especially unfavourable to activity on the capon comb (compare compounds A and B, Table II). It may be unfavourable to activity on the prostate and seminal vesicles of the castrated rat (as with *trans*androsterone), but is compatible with high activity (*trans*-androstenediol). Oxidation of the *cis*-3-hydroxyl group to a keto-group has little effect on activity, either on rats or capons (compare D and E, Tables II and IV, and Figs. 3-4).

(b) Reduction of a 17-keto-group to hydroxyl increases activities on both capons and rats (compare compounds A and C, F and H, and G and K, Tables II and III and Figs. 1 and 2), especially on the seminal vesicles of the latter.

(c) Unsaturation (Δ^4 or $\Delta^{5,6}$) may increase activity on capons (compare compounds B and F, Table II) but this does not apply to all compounds, for instance androstenedione and androstenedione [Tschopp, 1936]. The effect on activity on the rat is also inconstant.

(d) The introduction of a 17-methyl group increases activity on capons slightly in two compounds (compare C and D, H and J, Table II). In the case of testosterone it much decreases activity. Methylation enhances activity on rats, especially activity on the prostate, in all three pairs of compounds available for comparison.

4. *trans*Androstenediol and to a less extent testosterone produce abnormally large seminal vesicles relative to the prostate (Fig. 5). The relative growths caused by compounds other than androsterone are fairly normal. Methylation somewhat decreases the growth of the seminal vesicles relative to that of the prostate (Fig. 6).

5. It is known that the rat activity of androsterone per capon unit is less than that of testicular extracts, so that it is curious that testosterone should have even less activity on the prostate per capon unit than androsterone (Fig. 7) and only slightly more on the seminal vesicles over the range where comparison is possible (Fig. 8). High activity per capon unit on rats is restricted to four other unsaturated compounds.

6. In view of the above conclusions, and also since testosterone is only very slightly oestrogenic [Deanesly and Parkes, 1936], it seems likely that this compound alone cannot account for the whole endocrine activity of the testis.

It is possible that an accessory substance as postulated by Laqueur and his co-workers [David *et al.*, 1935] is necessary to increase the activity of testosterone or that a further hormone is involved.

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REFERENCES.

- Brambell and Parkes (1929). *Proc. Roy. Soc. Lond. B* **105**, 36.
Butenandt (1931). *Z. angew. Chem.* **44**, 905.
—— (1932). *Z. angew. Chem.* **45**, 655.
—— (1933). *Naturwiss.* **21**, 49.
—— and Dannenbaum (1934). *Z. physiol. Chem.* **229**, 192.
—— and Hanisch (1935, 1). *Ber. deutsch. chem. Ges.* **68**, 1859.
—— (1935, 2). *Z. physiol. Chem.* **237**, 89.
—— and Tscherning (1934, 1). *Z. physiol. Chem.* **229**, 167.
—— (1934, 2). *Z. physiol. Chem.* **229**, 185.
—— (1935). *Z. physiol. Chem.* **234**, 224.
Callow (1936). *J. Physiol.* In the press.
—— and Deanesly (1935, 1). *Biochem. J.* **29**, 1424.
—— (1935, 2). *Lancet*, **229**, 77.
—— and Parkes (1935). *Biochem. J.* **24**, 1414.
David (1935). *Acta Brev. Neerl.* **5**, 85.
—— Dingemanse, Freud and Laqueur (1935). *Z. physiol. Chem.* **233**, 281.
—— and Freud (1935). *Acta Brev. Neerl.* **5**, 31.
Deanesly and Parkes (1936). *Brit. Med. J.* In the press.
Dingemanse, Freud and Laqueur (1935). *Nature*, **135**, 184.
Greenwood, Blyth and Callow (1935). *Biochem. J.* **29**, 1935.
Hill, Parkes and White (1934). *J. Physiol.* **81**, 335.
Korenchevsky (1935). *Nature*, **135**, 434.
—— and Dennison (1935, 1). *Biochem. J.* **29**, 1720.
—— (1935, 2). *Biochem. J.* **29**, 2122.
—— and Simpson (1935). *Biochem. J.* **29**, 2535.
Parkes (1935). *Chem. and Ind.* **54**, 928.
Ruzicka Goldberg and Meyer (1935, 1). *Helv. chim. Acta*, **18**, 210.
—— (1935, 2). *Helv. chim. Acta*, **18**, 994.
—— Brüngger and Eichenberger (1934). *Helv. chim. Acta*, **17**, 1395.
—— and Rosenberg (1935). *Helv. chim. Acta*, **18**, 1487.
—— and Wettstein (1935, 1). *Helv. chim. Acta*, **18**, 986.
—— (1935, 2). *Helv. chim. Acta*, **18**, 1264.
—— and Kägi (1935). *Helv. chim. Acta*, **18**, 1478.
Tscherning (1933). *Ergebn. Physiol.* **35**, 301.
—— (1934). Quoted by Butenandt and Schmidt, *Ber. deutsch. chem. Ges.* **67**, 2092.
Tschopp (1935, 1). *Nature*, **136**, 258.
—— (1935, 2). *Klin. Woch.* **14**, 1064.
—— (1936). *Arch. int. Pharmacodyn.* In the press.
Wettstein (1935). *Schweiz. med. Woch.* **65**, 912.