CCLXXVII. THE ACTIVATION OF THE MALE SEX HORMONES. II.

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I. *The activation of testosterone by esterification.*

WE showed in an earlier paper [1936, 1] that on injecting testosterone and palmitic acid at different sites in the body of the rat an activating effect could no longer be observed when the sites of injection were too far apart. In our opinion this suggested that the carboxylic acids have no effect on the sex organs themselves, but rather a local effect at the site of injection, in creating more favourable conditions for the absorption of the administered solution. Deanesly & Parkes [1936, 1], who confirmed the fact itself, suggested that the increased effect was due to retardation of the absorption of testosterone. They point to the retarded effects of benzoates of oestrone and oestradiol and are of the opinion that one cannot exclude the possibility of esterification of testosterone by the acid added, even during the preparation of the solution. However, the possibility of activating testosterone by primary alcohols and especially the fact that we found testosterone palmitate and stearate to have practically no effect are in conflict with this theory.

We decided, however, to investigate thoroughly the problem of testosterone esters. Testosterone acetate and benzoate had already been tested. Tschopp [1936] found the former to be just as effective as testosterone, whilst according to Deanesly & Parkes [1936, 2] the latter is practically ineffective on the capon's comb. In order to compare these findings we prepared a new series of testosterone esters with aliphatic acids.¹ Information is given in Table I concerning the properties of the two already known and the nine new esters.

Table I.

	Number of C atoms of the acid residue	M.P. of the ester (corr.)	One international (capon's comb) unit is equivalent to γ	Number of international units contained in 1 mg.
Formate	1	127–129°	20	50
Acetate	2	140–142°	20	50
Propionate	3	121–123°	20	50
<i>n</i> -Butyrate	4	111–113°	60	17
<i>iso</i> Butyrate	4	134–136°	70	14
<i>n</i> -Valerate	5	109–111°	200	5
<i>iso</i> Valerate	5	138–140°	250	4
<i>n</i> -Decanoate	10	55–57°	350	3
Palmitate	16	72–74°	> 1000	< 1
Stearate	18	79–80°	> 1000	< 1
Benzoate	7	198–200°	> 1000	< 1

¹ See also our preliminary report [1936, 2]. The chemical part of this work will be published by Ruzicka & Wettstein [1936].

(a) *Effect of the esters on the capon's comb. Technique.* The daily dose of the compounds investigated was dissolved in 0.5 ml. sesame oil and injected for 6 days into healthy capons. The increase and decrease of the surface of the comb was controlled by measuring the shadow pictures by a planimeter.

The effects of the various esters are to be seen on Fig. 1. The graphs show the average values obtained on 3-4 animals. It will be seen that the actions of the formate, acetate and propionate are the most rapid and pronounced. The

Fig. 1. Effect of testosterone esters on the capon comb (daily injections during 6 days).
— With daily dose of 50 γ — — — With daily dose of 100 γ.

T = Testosterone.

Fig. 2. Time course of effect of approximately 1 international capon unit of different testosterone esters on the capon comb.

T = Testosterone.

duration of the effect is somewhat longer with the propionate. The intensity of action then decreases rapidly as the number of carbon atoms of the esterified acids increases. It disappears almost entirely with palmitate, stearate and benzoate.

To convert the results into international units the doses of the esters were reduced to such an extent that a maximum increase of the surface of the comb amounting to about 30% of the original surface was obtained, equal to the action of 100 γ androsterone under our test conditions.¹ From Fig. 2 it will be seen distinctly that the higher the mol. wt. of the esterified acid, the later the maximum increase of the surface of the comb is attained. For comparison it should be said that, conditions being equal, one international unit, i.e. 100 γ androsterone, corresponds to the activity of 15 γ testosterone. (Deanesly and Parkes found previously one unit in 17 γ testosterone and Tschopp [1936], under somewhat different conditions, 1 unit in 13 γ .)

The addition of palmitic acid does not effect either the activity of testosterone or its esters on the capon's comb.

(b) *The effect of esters on castrated rats.* The esters were also thoroughly tested on the castrated rat. All the animals used in the experiments were castrated at a weight of 60–80 g. and the tests not begun until 16–21 days after castration.² We employed three methods.

First method using ten injections. The castrated animals received the indicated doses, dissolved in 0.5 ml. sesame oil, by subcutaneous injection daily during 10 days. On the 11th day the animals were killed and the sex organs weighed. Table II gives the results and Fig. 3 demonstrates graphically the

Fig. 3. Effect of testosterone esters on the weight of seminal vesicles (method using ten injections).

T = Testosterone.

effect especially on the seminal vesicles. The surprising fact emerges that the lower esters³ are far more effective than testosterone. The effects of some of these esters exceed even those produced by testosterone plus 50 mg. palmitic acid. On

¹ We observed in all cases the same maximum increase independent of the day on which it appeared; thus a simultaneous comparison of the effects was not made.

² However, in all the experiments using acids as activators this interval was 25–30 days.

³ In a private communication Dr Girard of Paris informs us that he also saw more favourable effects on the general condition of old capons following administration of testosterone acetate in high doses.

Table II.

	Daily dose γ	Weight of the glands in mg.				Number of rats
		Seminal vesicles	Prostate	Cowper's glands	Preputial glands	
Testosterone formate	500	460	360	240	146	2
	200	400	363	283	136	4
	100	281	290	217	111	6
	50	214	225	215	96	4
	25	153	171	171	70	4
	10	37	60	90	55	2
	5	32	64	88	36	2
Testosterone acetate	500	380	356	220	137	4
	200	383	357	228	136	2
	100	317	298	227	104	5
	50	218	241	195	86	8
	25	142	180	175	75	4
	10	30	62	83	49	2
Testosterone propionate	500	430	380	266	145	2
	200	444	375	244	131	4
	100	334	299	190	101	4
	50	225	270	190	89	4
	25	150	155	155	65	4
	10	30	50	50	36	2
	5	20	47	62	33	2
Testosterone <i>n</i> -butyrate	1000	466	422	258	202	2
	500	458	395	255	155	4
	200	332	383	233	104	4
	100	223	290	231	86	4
	50	181	185	190	73	4
	25	64	102	152	70	4
	10	22	58	81	45	2
	5	16	48	59	55	2
Testosterone <i>isobutyrate</i>	50	64	94	105	60	4
Testosterone <i>n</i> -valerate	50	122	152	146	60	4
Testosterone <i>isovalerate</i>	50	34	55	77	50	4
Testosterone <i>n</i> -decanoate	500	246	185	183	92	2
	200	100	95	116	61	3
	100	19	47	58	35	4
	50	15	38	50	45	4
	25	12	34	43	35	4
	10	13	35	40	30	2
	5	11	28	42	28	2
Testosterone benzoate	500	247	254	205	103	2
	200	130	142	136	80	4
	100	47	64	84	46	4
	50	17	42	50	39	8
Testosterone stearate	1000	30	50	50	32	2
	500	19	39	45	37	2
Testosterone palmitate	500	22	45	47	36	2
	200	17	43	45	37	2
Testosterone	500	127	173	165	88	2
	200	124	176	162	81	4
	100	62	86	140	58	4
	50	42	66	75	55	4
Testosterone + daily 50 mg. palmitic acid	500	354	350	241	150	2
	200	272	280	212	110	4
	100	263	263	206	90	4
	50	220	209	165	78	4

the other hand palmitic acid does not change the effect of, for example, testosterone acetate (see section III). Whilst the formate, acetate, propionate and butyrate show practically the same effect, the latter decreases rapidly when we pass to higher esters and to those containing a branched instead of a straight chain acid residue. Palmitate and stearate are almost ineffective, whilst benzoate and *n*-decanoate in higher doses show pronounced activities in this test.

Fig. 4. Effect of testosterone esters on the weight of seminal vesicles (method using two injections on the 1st and 6th day).

T = Testosterone.

Fig. 5. Effect of testosterone esters on the weight of prostate (method using two injections on the 1st and 6th day).

T = Testosterone.

It is worth mentioning that the maximum effect is obtained already with small doses of the lower esters; higher doses do not materially increase this effect. In no way can it be attained with testosterone alone, even in high doses.

The former findings of Tschopp [1936] that there was no difference between the effect of testosterone and that of its acetate is explained by the fact that at that time an older oil, probably rich in activators, was used. As a result of this, a wrong impression was obtained of the activity of testosterone, whilst on the other hand the effect of the acetate was not influenced. It would be desirable to employ inert solvents such as paraffin oil for elaborating these tests; however, we generally refrained from using such inert oils since in clinical practice only fatty oils are used.

Second method using two injections with an interval of 5 days. The above experiments show the effect of the esters only on the 11th day following the beginning of the test, but do not give any idea as to the time course of the action. In order to ascertain this, relatively high doses of 1 mg. each, dissolved in 1 ml. sesame oil, were injected into castrated rats on the 1st and 6th days of the experiment. Some of the animals were killed and examined on the 11th day, others at further constant intervals of 5 days.

The effects on the seminal vesicles and prostates are shown in Figs. 4 and 5. The course of the effect on the other organs weighed (preputial glands, Cowper's glands) is quite similar. The general picture of the results obtained with the ten-injections method is changed to a marked degree by the new method. Now on the 11th day the effect of testosterone has already disappeared whilst that of the esters is more or less prolonged. There is a striking difference between the formate and acetate on the one hand, whose effects are already very small on the 11th day, and on the other hand the neighbouring higher esters, beginning with the propionate, which show very high activities. Propionate attains its optimum effect at the 11th day, or earlier, butyrate at the 16th and *n*-valerate only at the 21st day. In the last case especially the increase of the effect is retarded; on the other hand it is the curve of this ester which attains the highest level. The *isocompounds*, such as *isobutyrate* and especially *isovalerate*, have distinctly weaker effects than the corresponding normal esters. The activities of *n*-decanoate and benzoate are considerably retarded and diminished but are therefore of relatively long duration. In this test stearate and palmitate showed no effect.

Third method using a single injection. In order to obtain a still clearer picture of the development of the effect with time we administered in another series of experiments on the 1st day a single dose of 2 mg., dissolved in 2 ml. of sesame oil. We began with examination of the rats already about the 4th day, using at least three animals for the determination of each value. The results regarding the seminal vesicles and prostates are given in Figs. 6 and 7. The effect of testosterone compared with that of the esters is again remarkably slight. As in the foregoing test, the formate and acetate are as effective as testosterone plus 200 mg. of palmitic acid. Their maximum effects are observed already on the 6th day. With propionate, butyrate and valerate, the maximum attains a much higher level than with the lower esters. The whole activity lasts at least 2–4 weeks. In spite of this more intensive and protracted action of these highly active esters, the rate of increase of the effect compared with formate and acetate is not diminished. This is, however, the case with the less effective esters of testosterone, like *n*-decanoate and benzoate.

We found that the testosterone esters act not only on the sex organs of the castrated rats but also on those of the uncastrated rats; we shall return to this question at a later date.

Besides the testosterone esters we examined under the new test conditions on the rat (and also on the capon's comb) a considerable number of esters of

other compounds with male hormone activity, for instance the esters of dihydrotestosterone (androsterone-3-one-17-*trans*-ol) and androstanediol. These results will also be published in due course.

Fig. 6. Effect of testosterone esters on the weight of seminal vesicles (method using a single injection).

T = Testosterone.

Fig. 7. Effect of testosterone esters on the weight of prostate (method using a single injection).

T = Testosterone.

II. The ratio between the activities of testosterone esters on the seminal vesicles and on the prostate and between those on capons and rats.

In Fig. 8 the ratio between the activities of testosterone esters, of free testosterone and of testosterone + palmitic acid on the seminal vesicles and

Fig. 8. The ratio between the activity of testosterone esters on the seminal vesicles and on the prostate.

× <i>T</i> .-formate.	△ <i>T</i> .-isobutyrate.	⊙ <i>T</i> .-benzoate.
⊗ <i>T</i> .-acetate.	□ <i>T</i> .- <i>n</i> -valerate.	● Testosterone (= <i>T</i> .).
○ <i>T</i> .-propionate.	▣ <i>T</i> .-isovalerate.	✕ <i>T</i> . + daily 50 mg. palmitic acid.
△ <i>T</i> .- <i>n</i> -butyrate.	⊗ <i>T</i> .- <i>n</i> -decanoate.	

Fig. 9. The ratio between the activities of testosterone esters on the seminal vesicles of the rat and on the capon comb.

T = Testosterone.

Table III.

	Daily dose (γ) dissolved in 0.5 ml. sesame oil	Without palmitic acid Weight of the organs in mg.				Number of rats	With 50 mg. palmitic acid daily Weight of the organs in mg.				Number of rats
		Seminal vesicles	Prostate	Cowper's glands	Preputial glands		Seminal vesicles	Prostate	Cowper's glands	Preputial glands	
<i>cis</i> -Androsterone acetate	500	14	32	—	37	2	12	40	50	46	2
<i>cis</i> -Androsterone benzoate	500	13	39	40	45	2	12	38	42	45	2
<i>trans</i> -Dehydroandrosterone acetate	3000	56	92	117	178	2	—	—	—	—	—
	2000	36	72	75	86	2	43	70	81	90	2
	500	13	43	42	78	2	13	40	46	96	2
Testosterone acetate	200	383	357	228	136	3	302	334	220	149	3
	100	286	234	227	98	3	250	322	210	111	3
	50	240	241	195	86	4	210	279	215	96	4
	25	142	180	175	75	4	134	179	131	64	4
	10	30	62	83	49	4	33	70	119	46	4
Testosterone propionate	50	225	270	190	89	4	207	253	251	119	2
Testosterone <i>n</i> -butyrate	50	181	185	190	73	4	215	214	180	86	2
Testosterone <i>n</i> -decanoate	50	15	38	50	45	4	19	43	54	60	2
Testosterone benzoate	200	140	142	136	80	2	210*	150*	148*	80*	2*
Androstane-3- <i>cis</i> -17- <i>trans</i> -diol 3-benzoate	1000	77	90	98	60	2	—	—	—	—	—
	500	26	51	56	47	2	45	85	80	80	2
	200	—	—	—	—	—	30	61	72	55	2
Androstane-3- <i>cis</i> -17- <i>trans</i> -diol 17-benzoate	500	49	83	71	72	2	—	—	—	—	—
	200	—	—	—	—	—	25	47	63	76	2

* Values obtained with brassidic acid.

prostate, according to the 10-days test, is shown graphically. With the exception of the benzoate all the active esters in higher doses produce seminal vesicles which are heavier than the prostate, as is the case in the normal adult rat. With testosterone + palmitic acid equality of weights is reached; with testosterone alone in all doses the seminal vesicles are lighter than the prostate, as is the case in the infantile or castrated rat. When comparing the maximum weights of seminal vesicles and prostates of rats obtained according to the second and third test methods (repeated and single injection), it is remarkable that only by using highly active esters, such as propionate etc., can a weight of the seminal vesicles surpassing that of the prostate be obtained.

It was recently shown by Deanesly & Parkes [1936, 2] that testosterone is about as active on the seminal vesicles and prostates as androsterone when given in doses containing the same number of capon units; thus there is still a considerable difference between the qualitative actions of testosterone and testicular extracts. As will be seen from Figs. 9 and 10 (rat test using ten injections) the esters of testosterone and also testosterone + palmitic acid appear to act much more favourably in this respect, so that similar conditions are attained as by using testicular extracts.

III. *The influence of acids on the activity of esters.*

Table III gives particulars of the results of our experiments to determine the influence of palmitic or brassidic acid on the effects of the esters of testosterone, androsterone, *trans*-dehydroandrosterone and androstanediol in the 10-days test. Pronounced increases of activity were only observed with testosterone benzoate and androstanediol benzoate (but not with androsterone benzoate).

Table IV contains results of experiments made to determine the influence of the solvent on the effects of testosterone and testosterone acetate, with and without an activator, on the seminal vesicles and prostate. Testosterone in 50 %

Table IV.

	Daily dose γ	Weight of glands in mg.		Number of rats
		Seminal vesicles	Prostate	
Testosterone in sesame oil	50	42	66	4
Testosterone in glycerol 50 %	500	20	—	2
	50	16	46	2
Testosterone in glycerol + daily 50 mg. ricinoleic acid	50	221	205	2
Testosterone in paraffin oil	50	15	44	2
Testosterone in paraffin oil + daily 50 mg. palmitic acid	50	126	145	2
*Testosterone acetate in sesame oil	200	383	357	2
	50	218	241	8
*Testosterone acetate in sesame oil + daily 50 mg. palmitic acid	200	302	334	2
	50	210	279	2
Testosterone acetate in paraffin oil	200	252	293	2
	50	65	105	2
Testosterone in glycerol 50 %	125	45	72	2
	50	12	36	2
Testosterone acetate in glycerol + 50 mg. ricinoleic acid	125	215	283	2

* See also Table III.

glycerol, as well as in paraffin oil, is almost ineffective; the addition of acids and particularly of 50 mg. ricinoleic acid to the glycerol solution activates it to a striking degree. On the other hand we had formerly found that an oily solution of testosterone is only slightly influenced by the addition of ricinoleic acid. In the experiment with glycerol an unsaturated hydroxy-acid was chosen instead of palmitic acid since the latter is only slightly soluble in glycerol.

Testosterone acetate dissolved in paraffin oil is less effective than in sesame oil. It dissolves badly in aqueous glycerol and its effect is only very slight therein. In this instance also the effect can be increased almost to the normal level by the addition of 50 mg. ricinoleic acid. Thus the remarkable fact emerges that the effect of testosterone acetate, when dissolved in aqueous glycerol, is increased by the addition of an activator, but that such an addition does not increase the effect when testosterone acetate is dissolved in sesame oil. Further investigations will be required in order to elucidate this result.

Fig. 10. The ratio between the activities of testosterone esters on the prostate of the rat and on the capon comb.

T = Testosterone.

IV. The activation of testosterone by other hormones.

For the sake of completeness it should be mentioned that the effect of testosterone can also be enhanced by addition of other hormones. The reciprocal influence of the anterior lobe of the pituitary gland is well known. After the Amsterdam investigators had described the "paradoxical" influence of the female hormone on the male sexual organs, Korenchevsky [1935; Korenchevsky & Dennison, 1935; Korenchevsky *et al.* 1935, 2], Tschopp [1936] and Steinach *et al.* [1936; see also Clauberg, 1936; Petterson, 1936] recently demonstrated in rats the reinforcing influence of oestrone and its derivatives on the effect of the male hormones.

V. *The question of the standardization of the male sex hormones.*

At the "Second conference on the standardization of sex hormones"¹ appointed by the League of Nations Health Organization, and held during the summer of 1935, a standard was fixed for male sex hormone preparations. According to this the unit of activity was defined as the activity of 0.1 mg. of the standard androsterone preparation as measured by a specific biological reaction. In the opinion of the members of the conference, the capon's comb test was at that time the only specific test which allowed comparison with the standard. Attempts were recommended to improve the standardization by using mammals.

The reference to the specificity of the capon's comb test is, according to our investigations, warranted, since it is less susceptible to influences of the medium than the rat test; in particular no activating effect has so far been observed on the comb. Even comparison of the standard with esters having protracted action is possible, provided that only the optimum effect is considered and that the demand for simultaneous reading of the standard effect and that of the preparation is abandoned as in our experiments. In this manner it is possible to avoid the trouble of having to choose a new standard for every ester.

It ought to be possible to standardize not only the intensity but also the duration of the effect, for example, by determining the ratio of the number of days in which the maximum effect is attained, on the one hand by the stipulated quantity of the standard and on the other by the compound to be tested. The notion that two preparations, showing the same activity when expressed in international units, must agree in all their pharmacological properties is not justified, since it is known that the relation between the effects on the capon's comb and on the rat can also be quite different when various preparations are tested. Even the relationship between the effects on the seminal vesicles and on the prostate can vary to a considerable extent [see also Deanesly & Parkes, 1936, 2]. Therefore, evaluation in units always relates only to a narrowly limited property and no indication is given as regards other actions; if it is desired to standardize these, other tests and new units will be needed.

The question, however, whether the capon test is sufficient for the characterization of male sex hormone compounds, must be answered in the negative; testosterone benzoate for example is practically ineffective in this test, but effective in the rat.

In view of the above, the following conclusions may be drawn in connexion with the testing of male sex hormone preparations:

- (1) In addition to capon units, rat units should be introduced [see e.g. Korenchevsky *et al.* 1935, 1; 1936].
- (2) The comparison can only refer to a narrowly limited property.
- (3) For this purpose, as the basis for comparing the intensities of effect, only the maximum effects produced by the standard and the preparation should be selected, without regard to the time when the maxima appear. The duration of the effect affords a special characterization.
- (4) The methods used for the tests must be exactly standardized.

The characterization of the effect of a preparation in units is only a temporary expedient. Every substance is really only comparable with itself. Nevertheless the definition of generally accepted standards is of great practical importance because it facilitates a quick evaluation of the most varied preparations in an accepted measure.

¹ This conference was attended by one of us (K.M.).

SUMMARY.

1. Eleven testosterone esters (nine of them new), especially with acids of the aliphatic series, have been prepared and examined as regards their effects.

(a) The lowest esters (formate, acetate and propionate) are the most effective on the capon's comb. The longer the carbon chain of the fatty acid residue, the more protracted is the effect, but meanwhile the intensity decreases rapidly. Palmitate and stearate, as well as benzoate, are practically ineffective.

(b) In the rat test (seminal vesicles, prostate etc.) the lower esters of testosterone are generally many times more effective than testosterone alone. For the first time in addition to the intensity the duration of activity of male hormone preparations was also tested on rats; in this way it was shown that in both respects testosterone formate and acetate give results approximately similar to those obtained with testosterone plus an activator. However, higher esters, from propionate upwards, proved to be much more effective. According to the test the activity reaches maximum intensity and duration in the cases of the *n*- and *iso*-butyrates or *n*-valerate. When we pass to still higher esters, especially to *n*-decanoate and benzoate, the rise in effect is already greatly retarded and its maximum diminished. Testosterone palmitate and stearate are ineffective.

2. When the capon and rat tests are considered simultaneously testosterone propionate shows an especially favourable total action. Comparing testosterone, testosterone + palmitic acid (or testosterone acetate) and testosterone propionate by the method using one injection of 2 mg., the maximum increases in the weight of the seminal vesicles of castrated rats obtainable are in the proportion of approximately 1:4:12.

3. According to the different test methods on the rat, only by using esters of testosterone, like the propionate etc. is it possible to produce in the castrated infantile rat seminal vesicles heavier than the prostate, as is the case in the normal adult rat. Using free testosterone the prostate remains always heavier than the seminal vesicles, as is the case in castrated and infantile rats.

4. Testosterone esters are relatively more effective on rats than on capons, as is also the case with natural testicular extracts, and differ in this respect from androsterone and even from testosterone. In the case of the lower esters, up to the propionate, this favourable relation is due to a high activity on rats, not to a low effect on capons.

5. The effects of the esters of the testosterone and androsterone series are in general not increased by addition of acids when dissolved in fatty oils. The specially low activity of testosterone acetate in 50 % glycerol however is nearly brought to the normal by the addition of ricinoleic acid.

6. The recommendation to introduce besides capon units also rat units is again put forward. The methods of standardization should be accurately defined. Instead of simultaneous comparison between the standard and the preparation to be tested, comparison of the maximum intensities of the effects independently of their time of occurrence is recommended. Intensity and duration of the effect cannot be fixed by one unit alone. The duration of the effect should be characterized separately.

REFERENCES.

- Clauberg (1936). *Zbl. Gynäk.*, No. 25, p. 1457.
Deanesly & Parkes (1936, 1). *Lancet*, i, 837.
—— — (1936, 2). *Biochem. J.* **30**, 291.
Korenchevsky (1935). *Nature, Lond.*, **137**, 494.
—— & Dennison (1935). *J. Path. Bact.* **41**, 323.
—— — & Brovsin (1936). *Biochem. J.* **30**, 558.
—— — & Simpson (1935, 1). *Biochem. J.* **29**, 2142.
—— — (1935, 2). *Biochem. J.* **29**, 2534.
Miescher, Wettstein & Tschopp (1936, 1). *Schweiz. med. Wschr.* **66**, 310.
—— — (1936, 2). *Schweiz. med. Wschr.* **66**, 763.
Petterson (1936). *Dtsch. med. Wschr.* Nos. 14 and 23.
Ruzicka & Wettstein (1936). *Helv. Chim. Acta*, **19**, 1141.
Tschopp (1936). *Arch. int. Pharmacody.* **52**, 381.
Steinach, Kun & Peczenik (1936). *Wien. klin. Wschr.* **49**, 899.

Note added 9 October 1936. Our results regarding the activities of the testosterone acetate and propionate have been fully confirmed in the meantime by Parkes [1936] *Lancet*, ii, 674. This author has also made other valuable observations with regard to these esters.