CCLXXVI. THE ACTIVATION OF THE MALE SEX HORMONES. I.

BY KARL MIESCHER, ALBERT WETTSTEIN
AND ERNST TSCHOPP.

From the "Ciba" Research Laboratories.

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I. *The activation of testosterone by the addition of organic acids.*

It is well known that in the rat test testosterone exhibits a greater activity than androsterone, especially on the seminal vesicles of castrated rats. As we have recently shown [1936, 1, 2] this only applies to oily solutions of testosterone; testosterone is, strangely enough, hardly active when dissolved in paraffin oil or in aqueous solutions of glycerol. We found, however, that after the addition of certain carboxylic acids, especially of the fatty acid series such as ricinoleic acid, the activity reappeared. The effect of solutions in fatty oils, like sesame oil, is increased to a considerable degree by such saturated and unsaturated fatty acids.

Meanwhile the number of the acids investigated has been considerably augmented and the earlier particulars supplemented. The weights of the seminal vesicles, prostate, Cowper's glands and preputial glands attained after administration of testosterone in combination with the new acids tested and with those of which the examination has been extended are given in Table I.

Technique. The rats weighed 60–80 g. at the time of castration; the experiments were not made until 25–30 days after castration. The animals received once a day a dose of 50 γ testosterone during 10 days, subcutaneously injected together with the quantity of acid mentioned dissolved in 0.5 ml. sesame oil. The rats were killed on the 11th day and the accessory organs were weighed immediately. Fatty acids which are solid at room temperature, e.g. hydroxyacids and stearic acid, frequently had to be dissolved and injected at body temperature on account of their low solubility.

The acids are arranged in the table approximately according to decreasing activity. Some of them, e.g. suberic, mandelic, tropic, nicotinic, piperic and α -naphthoic acids, are only very slightly soluble and for this reason their solutions were almost inactive; thus the question whether the acids themselves are active or not was left open. Some proved to be toxic or produced pronounced local irritation, e.g. glycerophosphoric, undecylenic, phenylpropionic, tetrolic, phenylacetic, lactic, pyruvic, acetic and formic acids, with which therefore the values obtained varied considerably. The irritating effect of the latter acids is probably due to their high acidity. Saturated higher fatty acids such as palmitic acid produce practically no irritation.

As will easily be seen from Fig. 1 the increase of activity can attain a considerable value with increasing doses of the acid. Still higher doses generally caused technical difficulties due to the saturation limit being attained or to

Table I.

Acid used +50 γ testosterone daily in 0.5 ml. sesame oil	Daily quantity of acid in mg.	Weight of the glands in mg.				No. of rats per experi- ment
		Seminal vesicles	Prostate	Cowper's glands	Preputial glands	
ω -Hydroxytridecanoic acid, $C_{13}H_{26}O_3$	10	132	124	154	108	2
ω -Hydroxymargaric acid, $C_{17}H_{34}O_3$	20	184	194	155	98	2
ω -Hydroxypalmitic acid, $C_{16}H_{32}O_3$	25	150	158	175	91	2
Pyruvic acid, $C_3H_4O_3$	10	85	90	134	80	2
Glycerophosphoric acid, $C_3H_9O_6P$	10	80	110	120	92	2
Palmitic acid, $C_{16}H_{32}O_2$	50	235	210	205	91	6
	25	115	150	170	80	4
	10	52	101	130	64	4
	5	40	75	88	41	4
λ -Hydroxystearic acid, $C_{18}H_{36}O_3$	50	210	220	192	95	2
	25	170	190	175	97	4
	10	70	103	105	48	4
Ricino-elaidic acid, $C_{18}H_{34}O_3$	10	78	105	91	51	4
	5	47	88	88	52	2
ω -Hydroxypentadecanoic acid, $C_{15}H_{30}O_3$	25	140	159	168	108	2
ω -Hydroxypelargonic acid, $C_9H_{18}O_3$	10	70	114	107	58	2
Behenic acid, $C_{22}H_{44}O_2$	50	165	202	228	86	8
	25	141	160	156	78	4
Camphor-3-carboxylic acid, $C_{15}H_{16}O_3$	25	106	124	128	78	6
λ -Ketostearic acid, $C_{18}H_{34}O_3$	25	95	125	130	75	4
ω -Hydroxydecanoic acid, $C_{10}H_{20}O_3$	25	84	122	138	80	2
Elaidic acid, $C_{18}H_{34}O_2$	25	62	100	112	48	2
	5	34	58	78	55	2
Acetic acid, $C_2H_4O_2$	10	65	70	—	—	2
Lactic acid, $C_3H_6O_3$	10	60	80	78	66	4
Arachidic acid, $C_{20}H_{40}O_2$	50	135	182	174	96	4
	25	117	168	170	83	3
β -Phenylpropionic acid, $C_9H_{10}O_2$	25	75	78	86	—	2
Propionic acid, $C_3H_6O_2$	50	117	158	150	70	3
	25	74	131	—	72	2
Hexahydrobenzoic acid, $C_7H_{12}O_2$	25	70	75	80	—	2
Benzoic acid, $C_6H_6O_2$	50	102	123	128	76	4
<i>n</i> -Hexanoic acid, $C_6H_{12}O_2$	50	95	140	130	71	4
Undecylenic acid, $C_{11}H_{20}O_2$	25	65	95	112	54	2
Sulphoricinoleic acid, $C_{18}H_{34}O_6S$	50	80	121	117	73	2
Pelargonic acid, $C_9H_{18}O_2$	25	55	98	107	—	2
Cinnamic acid, $C_9H_8O_2$	50	60	75	77	58	2
ω -Hydroxyundecanoic acid, $C_{11}H_{22}O_3$	25	56	90	98	65	2
Phenylacetic acid, $C_8H_8O_2$	25	52	85	—	—	2
Phenylpropionic acid, $C_9H_8O_2$	25	46	88	95	41	2
Tetrolc acid, $C_4H_4O_2$	25	30	64	—	—	2
Testosterone alone	—	42	66	75	55	4
Sesame oil	—	14	41	49	57	3

their irritating effect. The relationship between constitution and effect of fatty acids as described in the earlier papers [1936, 1, 2] can be summarized as follows:

Saturated fatty acids are more effective than unsaturated fatty acids.

Simply unsaturated fatty acids are more effective than unsaturated fatty acids with several double bonds.

Unsaturated fatty acids of the *trans*-series are more effective than unsaturated fatty acids of the *cis*-series.

Unsaturated hydroxylated fatty acids are more effective than unsaturated fatty acids without a hydroxyl group

From this we deduced that the hydroxylated saturated fatty acids should prove to have the greatest activity. This is confirmed, as will be seen from Fig. 2 in which the series of saturated fatty acids is compared with a number of saturated hydroxy-fatty acids¹ in respect of the produced weight of the seminal vesicles.

Fig. 1. Effect of the addition of some organic acids to testosterone on the weight of seminal vesicles. 1, ω -Hydroxytridecanoic acid; 2, ω -hydroxymargaric acid; 3, ω -hydroxypalmitic acid; 4, pyruvic acid; 6, palmitic acid; 8, ricino-elaidic acid; 10, stearic acid; 13, behenic acid; 14, behenolic acid; 19, brassicic acid; 49, testosterone alone; 50, sesame oil.

X, "natural" X-substance preparation from urine.

Fig. 2. Effect of hydroxylated and non-hydroxylated fatty acids on the activity of testosterone in relation to the number of C atoms of the acids.

—○— Daily 50 mg. saturated normal fatty acid
 × — × Daily 25 mg. saturated normal fatty acid
 Δ --- Δ Daily 25 mg. saturated normal hydroxylated fatty acid
 ▲ Only 20 mg. acid

The dependence of the effect of the fatty acids upon the number of their carbon atoms is striking. Near C_{10} a minimum action is attained whilst near C_{16} a pronounced maximum is observed. Whether with higher acids than C_{22} a still further increase in activity would result is problematical and requires further investigation.

A marked effect is observed with lactic acid and especially with pyruvic acid, an α -keto-acid, in contrast to λ -ketostearic acid. It may be worth while

¹ The ω -hydroxy-fatty acids were kindly supplied to us by Messrs Firmenich and Co., Geneva, through the courtesy of Prof. Ruzicka.

Table II.

	Daily dose γ	Without palmitic acid Weight of the glands in mg.				No. of rats	With 50 mg. palmitic acid daily Weight of the glands in mg.				No. of rats
		Seminal vesicles	Prostate	Cowper's glands	Preputial glands		Seminal vesicles	Prostate	Cowper's glands	Preputial glands	
3- <i>cis</i> -Androsterone	2000	142	260	206	142	2	149	270	180	130	2
	500	68	131	120	107	2	51	158	90	70	2
3- <i>trans</i> -Androsterone	1000	14	39	40	45	2	14	38	52	55	2
17- <i>trans</i> -Testosterone (testis hormone)	500	127	173	165	88	2	354	350	241	150	2
	200	124	176	162	81	4	272	280	212	110	4
	100	62	86	140	58	4	263	263	206	90	4
	50	40	70	74	55	4	220	209	165	78	4
	25	—	—	—	—	—	117	200	120	61	4
	10	—	—	—	—	—	37	76	97	60	4
	5	—	—	—	—	—	32	75	88	40	4
17- <i>cis</i> -Testosterone	1000	15	41	50	80	3	15	43	60	85	3
17-Methyltestosterone	200	140	167	156	94	2	—	—	—	—	—
	100	80	98	130	80	3	—	—	—	—	—
	50	58	88	75	51	3	160*	210*	162*	88*	3*
17-Ethyltestosterone	500	107	129	161	82	2	—	—	—	—	—
	200	44	57	70	51	2	74	86	105	60	2
17-Ethylidihydrotestosterone	500	49	108	113	61	2	70	148	157	78	2
	200	19	55	60	48	2	40	60	107	80	2
	100	13	45	46	46	—	—	—	—	—	—
	50	14	36	44	30	—	—	—	—	—	—
Androstane-3- <i>cis</i> -17- <i>trans</i> -diol	200	74	140	145	89	4	239	308	210	161	4
	100	43	85	123	68	4	—	—	—	—	—
Androstene-3- <i>trans</i> -17- <i>trans</i> -diol	1000	48	71	80	83	2	114	136	156	153	2
	500	30	50	81	107	2	99	120	152	177	2
Androstanedione	500	30	108	89	73	4	28	122	120	83	2
Androstenedione	500	126	166	176	101	4	208	251	196	130	3
	200	90	130	150	80	4	—	—	—	—	—
Sesame oil alone	—	14	41	49	57	3	14*	38*	45*	40*	2*

* Values obtained with brassidic acid instead of palmitic acid.

to study still higher α -hydroxy- or α -keto-fatty acids. The apparently very high activity of ω -hydroxytridecanoic acid requires still further investigation; in fact the number of experiments made with most of the acids was insufficient to yield definite results. Glycerophosphoric acid showed very high activity.

II. The activation of other compounds with male hormone activity by the addition of organic acids.

Table II shows the effect of palmitic acid (or brassidic acid) on a number of other male hormone preparations. The results obtained with testosterone are also given for comparison. From this it is seen that the activities of *cis*- and *trans*-androsterone, *cis*-testosterone and androstenedione are not changed; a moderate effect is observable in the case of the 17-ethyl compounds (17-ethyl-testosterone and 17-ethyldihydrotestosterone) and a still greater effect in the case of androstenedione and *trans*-androstenediol. The most pronounced influence, however, is exerted in the cases of androstane-3-*cis*-17-*trans*-diol, 17-methyltestosterone and 17-*trans*-testosterone. It certainly seems that a hydroxyl group in the 17-*trans*-position in combination with one in the 3-*cis*-position or with an α , β -unsaturated keto-group in the 3-position contributes specially to the activating effect.

Deanesly & Parkes [1936] recently confirmed our findings as regards the possibility of increasing the effect of an oily solution of testosterone by addition of palmitic acid. Contrary to us they found that the effect of androsterone, given in a total dose of 10 mg., could also be increased to a certain degree.

III. The activation of testosterone by the addition of alcohols etc.

Since the hydroxy-acids exhibited marked activating properties it was interesting to study the effect of the monohydric alcohols. As seen from Table III the activating power of these alcohols is very considerable¹ and attains a

Table III.

Preparation used + 50 γ testosterone daily in 0.5 ml. sesame oil	Daily quantity of alcohol mg.	Weight of the glands in mg.				No. of rats per experiment
		Seminal vesicles	Prostate	Cowper's glands	Preputial glands	
Ethyl alcohol, C ₂	50	82	98	106	61	2
Propyl alcohol, C ₃	50	117	156	152	66	2
<i>iso</i> Propyl alcohol, C ₃	50	120	150	145	80	2
Butyl alcohol, C ₄	50	72	110	130	68	4
Hexyl alcohol, C ₆	50	95	125	120	70	4
Octyl alcohol, C ₈	50	77	115	125	50	2
Dodecyl alcohol, C ₁₂	50	95	98	120	72	2
Cetyl alcohol, C ₁₆	50	125	165	185	95	4
Stearyl alcohol, C ₁₈	50	200	220	210	71	4
Oleyl alcohol, C ₁₈	50	87	111	110	59	2
Cinnamyl alcohol, C ₉	50	80	81	70	45	2
Ethyl propionate	50	33	66	70	48	2
Methyl butyrate	50	44	65	80	48	2
Methyl valerate	50	56	80	78	55	2
Methyl stearate	50	43	89	75	54	2
Monostearin	50	40	76	72	56	4
Testosterone alone	—	42	66	75	53	—
Sesame oil	—	14	41	49	57	—

¹ It was established by titration that the activity of these alcohols is not due to admixture with the corresponding fatty acids.

strikingly high maximum with stearyl alcohol. (These values were obtained under the same experimental conditions as those used in previous experiments.) On the other hand, the corresponding unsaturated oleyl alcohol is much less effective. As already mentioned, testosterone in glycerol—a trihydric alcohol—is completely ineffective. It is still doubtful whether testosterone is also inactive in entirely pure triglycerides since one cannot exclude the possibility of the oils previously tested [Miescher *et al.* 1936, 2] containing some “natural” X-substances.

Table III likewise gives information as to the addition of fatty acid esters to oily solutions of testosterone; methyl valerate alone shows a slight effect.

It was natural to suspect a certain connexion between activation and surface activity. In fact it has been found that some of the known wetting agents are likewise more or less effective, but very irritating.

IV. *The activation of testosterone by the addition of “X-substances”.*

David *et al.* [1935] described for the first time the activating action of certain acid fractions of extracts derived from testes and other organs, or from body fluids like urine, in combination with testosterone. From Fig. 1 it will be observed that 3 mg. of an X-substance preparation, obtained by Dr Locher in our laboratories from urine, together with 50 γ testosterone will increase the weight of the seminal vesicles from 14 to 95 mg., that is to say about treble the increase produced by testosterone alone. This effect is approximated only by that produced by some hydroxy- and keto-acids.

The Amsterdam investigators [Polak *et al.* 1936, 1, 2] believe that there exists a specific difference between the “natural” X-substance¹ and the carboxylic acids found effective by us. However, this appears to us hardly probable. As Deanesly & Parkes have already reported, the difference is more quantitative than qualitative. Some time ago [1936, 1, 2] we drew attention to the fact that large quantities of free palmitic acid are present in the testes. Besides this, however, there exists in them an apparently very complex mixture of other, not yet identified, but also effective acids. It is quite probable that all the acids present participate in the general activating action according to the quantity in which they are present and the degree of their activity. Possibly the attainment of an optimum effect depends on the proportion in which the acids are mixed. If we understand correctly the investigators of the Amsterdam group, the difference between our carboxylic acids and the X-substances lies in the fact that the latter not only improve the absorption of the hormones at the site of injection but also enhance the effect of the hormones on the test organs. This, however, has not been proved. It is also doubtful if any acids, e.g. those found in the testes, or even those found in the urine, are connected with the activation of the testicular hormone under natural conditions [see also Miescher *et al.* 1936, 3].

The assumption of Polak *et al.* [1936, 1, 2] that all our effective acids could contain small amounts of the natural X-substance does not seem probable since our list also includes synthetically prepared acids of high activity.

¹ These authors specially point out that one of their X-substance preparations isolated from urine is effective in a daily dose of 0.3 mg. together with 70 γ androstanediol. The weights of the seminal vesicles and of the prostata amounted to only 25 mg. each in comparison with 14 and 25 mg. respectively with androstanediol alone; therefore it is difficult to compare their findings with ours. We preferred for our experiments greater differences which are far beyond the limits of error.

Finally it may be mentioned that the X-substance, as Freud *et al.* [1935] have shown, activates testosterone and androstanediol, but not androsterone. This fact agrees with our findings regarding the use of palmitic acid and points likewise to an effect of similar nature.

SUMMARY.

1. The activating effects of about 40 carboxylic acids on the effect of testosterone have been tested on castrated rats. These experiments supplemented earlier tests. It was observed that the saturated normal fatty acids exhibit certain regularities. In this series, acids with about 10 carbon atoms showed a minimum action while those with about 16 carbon atoms showed a decided maximum. Hydroxylated saturated or unsaturated fatty acids are as a rule more effective than saturated or unsaturated fatty acids without a hydroxyl group.

2. *cis*- and *trans*-Androsterone as well as androstanedione are not activated by palmitic acid. The most pronounced effect is observed with those male hormone preparations which, like testosterone, methyltestosterone and androstane-3-*cis*-17-*trans*-diol, possess a hydroxyl group in the 17-*trans*-position in combination with a hydroxyl group or with an α , β -unsaturated keto-group in the 3-position.

3. In the rat test monohydric alcohols also increase the effect of testosterone. Of those tested, the saturated stearyl alcohol is the most effective whilst the unsaturated oleyl alcohol is less effective. This is in agreement with our findings in the series of fatty acids where also the saturated are more active than the unsaturated. Certain wetting agents also exhibit an activating effect.

4. Acid fractions obtained from testes contain, in addition to palmitic acid, other more effective components. Probably the so-called natural activator represents a mixture of acids which differ only quantitatively as regards their activating effects. The question is left open to what extent, if any, such acids actually influence the activity of the genuine male hormones of the testes.

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