ACTIVATION OF TESTOSTERONE BY HIGHER FATTY ACIDS AND THEIR ACID SODIUM SALTS*

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The influence of male sex hormones on the development of the accessory sex organs in the rat can be enhanced: (*a*) by esterification with certain lower fatty acids (1, 2); (*b*) by administering the hormone together with either certain higher fatty acids (3) or high molecular weight saturated primary alcohols ((3) p. 1974); the injection of the hormone and the second component must be made at the same time and site to obtain the desired effect. It has further been found that the free hormone only can be activated, and that a combination of hormone esters with fatty acids appears to bring about no further increase of the physiological effect ((1) p. 1986, (2)).

Laqueur and his collaborators found that an activating factor, the so called *X* substance, is present in various tissues (4). Tschopp and coworkers succeeded in isolating palmitic acid from the *X* substance fraction of testes by means of the chromatographic adsorption technique (5). They also investigated the activating effect of a number of naturally occurring fatty acids and found palmitic acid to be the most effective (3).

In an earlier paper Ehrenstein and Britton (6) describe the isolation of an acid sodium salt of palmitic acid, $C_{15}H_{31}COOH \cdot C_{15}H_{31}COONa$, from adrenal glands. Its possible action as an activator of male sex hormones is there discussed. Evidence has now been obtained that this salt is not originally present in the adrenal tissues. It is apparently formed by the permutit treatment employed during the preparation of corticoadrenal

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extract. Moreover, it was observed that permutit can generally be used for the isolation of higher fatty acids from various kinds of tissue. Thus we obtained the acid sodium salt and, by acidification, palmitic acid from testes and liver. Only traces could be isolated from ovarian tissue. This indicates that the occurrence of free palmitic acid, like that of the *X* substance, is not restricted to the tissues of the endocrine glands.

The present paper deals especially with the activating effect of palmitic and stearic acids and their corresponding acid sodium salts on the male sex hormone testosterone.

EXPERIMENTAL

Chemical Procedures

Isolation of Palmitic Acid from Various Tissues—All tissues were treated according to the modified method used in this laboratory for the preparation of corticoadrenal extract. Before the treatment with permutit the alcoholic solution was brought to dryness. On addition of pure acetone to the residue, a sticky precipitate was obtained, apparently consisting mainly of those last traces of phospholipids which had escaped the first precipitation. On acidification of this resinous material, which contained but traces of sodium, no free fatty acid could be isolated. The acetone solution was brought to dryness, the residue dissolved in 70 per cent alcohol, and this alcoholic solution treated with permutit in the usual manner. This solution was then brought to dryness and the residue treated with acetone. In all experiments (beef adrenals, testes, ovaries, liver) a more or less powdery, light brown precipitate was obtained which, on being treated with water, yielded a white precipitate of microscopically fine flat needles. These needles represented the acid sodium salt of palmitic acid, $C_{15}H_{31}COOH \cdot C_{15}H_{31}COONa$, in a more or less pure form. On acidification, the free acid was obtained and could be readily identified as palmitic acid. A satisfactory yield was obtained from adrenal glands; testes and liver yielded a little less and not quite pure palmitic acid. Only traces of rather impure palmitic acid could be secured from ovarian tissue.

Preparation of Sodium Bipalmitate, $C_{15}H_{31}COOH \cdot C_{15}H_{31}COONa$, and Sodium Bistearate, $C_{17}H_{35}COOH \cdot C_{17}H_{35}COONa$—0.02 mole of pure acid (Kahlbaum) was dissolved in 80 cc. of warm 95 per

cent alcohol. To this solution was added rather quickly a warm solution of 0.01 mole of sodium hydroxide in 57 cc. of alcohol (the NaOH is first dissolved in 2 cc. of water, then 55 cc. of 95 per cent alcohol are added). On standing, the salt crystallized in long flat spears. The melting point of the bipalmitate was 122–124° (sintering between 80–90°) and that of the bistearate 121–124° (sintering at about 100°). It should be noted that the molten salts were not quite transparent.

Animal Experiments

Method of Assay—Young, male white rats weighing 50 to 80 gm. were castrated. They were used for assaying the various preparations not before the 25th day and not later than the 30th day after operation. Subcutaneous injections were carried out for 9 successive days, and sacrifice of the animals and dissections were made on the 10th day. The daily dose was always dissolved in 0.5 cc. of sesame oil. In those cases in which testosterone was combined with either a free acid or an acid sodium salt, the two constituents were dissolved in 0.25 cc. of sesame oil each. The syringe was filled by drawing in the solution of testosterone first and that of the activator thereafter. Since palmitic acid and stearic acid as well as their corresponding acid sodium salts are only slightly soluble in sesame oil at room temperature, these preparations were heated in a paraffin bath to about 130° (*i.e.* a little higher than the melting points of the salts). Although the free acids yield a homogeneous solution at a considerably lower temperature, they were heated as high as the salts in order to insure comparable conditions. It should be stated that with the higher concentrations of acid, and especially of salt, a formation of lumps under the skin was observed. The health of the rats was not, however, impaired; all experimental animals appeared to be in good condition at the time of sacrifice.

Results

By comparison of the results in Tables I and II, it will be seen that under the experimental conditions described, the effect of testosterone on the development of the seminal vesicles and prostate gland can be considerably enhanced by combining the hormone either with free higher fatty acids (palmitic acid, stearic

acid) or their corresponding acid sodium salts ($C_{15}H_{31}COOH$·-$C_{15}H_{31}COONa$, $C_{17}H_{35}COOH \cdot C_{17}H_{35}COONa$). Rather low doses (up to 25 mg. daily) of the salts proved to be much more effec-

TABLE I

Activation of Testosterone

50 micrograms of testosterone and various concentrations of activator were administered daily to castrated rats.

No. of rats	Concentration and kind of activator	Average body weight at killing	Average weight		Seminal vesicles	Prostate
			Seminal vesicles	Prostate		
		Palmitic acid				
	mg.	*gm.*	*mg.*	*mg.*	*per cent of body weight*	*per cent of body weight*
4	25 Palmitic acid	145	51	46	0.034	0.031
4	50 " "	156	154	86	0.100	0.055
4	10 Na bipalmitate	146	92	66	0.060	0.045
6	25 " "	135	139	75	0.104	0.055
6	50 " "	126	185	96	0.147	0.075
	Standard deviation*..	12.1	36.5	14.6	0.0293	0.0112
		Stearic acid				
4	25 Stearic acid	158	70	45	0.044	0.028
4	50 " "	166	175	100	0.106	0.061
4	75 " "	177	311	121	0.176	0.068
4	10 Na bistearate	176	110	49	0.062	0.028
4	25 " "	156	157	89	0.102	0.057
4	50 " "	153	144	87	0.095	0.057
4	75 " "	152	135	91	0.099	0.060
	Standard deviation*..	9.2	41.0	11.4	0.0263	0.0072

The figures contained in the table confirmed preliminary experiments carried out with fewer animals.

* Pooled standard deviation of observations within groups from their respective means.

tive than the same doses of the free acids. With medium doses (50 mg. daily) the effect was about the same with either the free acid or the acid sodium salt. Higher doses of free acid (75 mg. daily), as shown in the stearic acid series, brought about a further

increase of the activating effect. Activation with the acid sodium salt, however, could not be increased beyond that obtained with the 50 mg. daily dosage.

TABLE II

Effectiveness of Testosterone, Testosterone Acetate, and Testosterone Propionate†*

Castrated rats were given 50 micrograms of the various preparations daily, no activator being added.

In the case of control animals which were given only 0.5 cc. of pure sesame oil daily, the average weight of the seminal vesicles was 5 mg. and of the prostate 3.9 mg. (2 animals).

No. of rats	Concentration of male hormone in 0.5 cc. sesame oil	Average body weight at killing	Average weight		Seminal vesicles	Prostate
			Seminal vesicles	Prostate		
		gm.	*mg.*	*mg.*	*per cent of body weight*	*per cent of body weight*
5	Normal animals; no injection given	150	205	110	0.134	0.072
	Standard deviation‡		67.1	34.7	0.0347	0.0182
4	50 micrograms testosterone	140	25	21	0.018	0.016
3	50 " " acetate	164	83	54	0.056	0.037
4	50 micrograms testosterone propionate	158	62	34	0.041	0.021
	Standard deviation‡	20.4	21.2	9.0	0.0197	0.0066

* Perandren, a preparation of the Ciba Company, Inc., New York. By courtesy of Dr. Haskell.

† Testoviron, a preparation of the Schering-Kahlbaum, A. G., Berlin. By courtesy of Professor W. Schoeller.

‡ Pooled standard deviation of observations within groups from their respective means.

A satisfactory explanation of the action of the salts cannot be given at this time. Possibly the suspension of the salt in sesame oil, which represents a mass of vaseline-like consistency, acted as a colloidal system such that if 25 mg. or more of the salt was administered daily all of the testosterone was adsorbed by it and slowly liberated at a more or less constant rate.

The activity of highly effective esters of testosterone (such as the acetate and propionate) was considerably surpassed in these experiments. Furthermore, the weights of the accessory sex organs of normal animals of approximately the same body weight were exceeded in the experiments in which 50 micrograms of testosterone were combined with 75 mg. of free acid.

It is known that testosterone palmitate and stearate ((1) p. 1980) are almost inactive. Thus the suggestion of an ester theory for the explanation of the activating effect of free higher fatty acids is not justified.

SUMMARY

The increased effect of testosterone upon the accessory sex organs of the male rat induced by palmitic or stearic acid or by their acid sodium salts is demonstrated by comparison with the effect of testosterone alone or with that of two esters of testosterone.

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BIBLIOGRAPHY

1. Miescher, K., Wettstein, A., and Tschopp, E., *Biochem. J.*, **30**, 1977 (1936).
2. Parkes, A. S., *Lancet*, **2**, 674 (1936).
3. Miescher, K., Wettstein, A., and Tschopp, E., *Biochem. J.*, **30**, 1970 (1936).
4. See Koch, F. C., *Physiol. Rev.*, **17**, 202 (1937).
5. Miescher, K., Wettstein, A., and Tschopp, E., *Schweiz. med. Woch.*, **66**, 310 (1936).
6. Ehrenstein, M., and Britton, S. W., *Am. J. Physiol.*, **120**, 213 (1937).