V. THE MANIFOLD ACTIVITY OF TESTOSTERONE DIPROPIONATE AS COMPARED WITH THAT OF TESTOSTERONE PROPIONATE IN GONADECTOMIZED RATS

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ENOL esters of male sex hormones were first prepared by Ruzicka & Fischer [1936]. Miescher *et al.* [1937] have prepared several new members of this group, and have studied the effects of some of the most important testosterone compounds on capon's comb growth and on the weight of the seminal vesicles and prostate of rats. In a 10-day test the enol esters of testosterone increased the weight of the seminal vesicles less than did testosterone propionate, but with some of the diesters, in particular the dipropionate, the effect lasted much longer.

From the clinical point of view, preparations with more prolonged activity have evident advantages. Therefore the activity of one of the most interesting of the enol esters (according to Miescher *et al.* [1937]), namely, testosterone dipropionate, on weight and histological structure of the organs has been studied in this paper and compared with that of the propionate which is at present used almost exclusively in clinical practice. Testosterone propionate has already been studied by us [Korenchevsky, 1937, 1; Korenchevsky & Dennison, 1937; Korenchevsky *et al.* 1937; Korenchevsky *et al.* 1937, 1 and 2; Korenchevsky & Hall, 1937].

Technique

The pure crystalline hormones were supplied by Messrs Ciba Ltd., and were dissolved in sesame oil. Each dose was contained in 0.2 ml. of oil, and was injected daily into male or female rats, gonadectomized at the age of 22–24 days, i.e. before sexual maturity. The injections were continued in males for 23 days and in females for 21 days. The injections into the rats of groups “+ 10 days rest” (see Tables I and III) were commenced 10 days earlier than in the other groups, and these animals were not injected for the last 10 days before killing. In all the other groups the rats were killed on the day following the last injection. In this way the final age of all the rats in any one litter was the same.

The final age of the *female* rats was about the same in all groups (average 69 days). A large number of litters of *male* rats was used for the experiments, the final age varying in the different litters from 74 to 106 days.

Since in castrated control rats the average weights of some of the organs vary significantly according to age, for the sake of a more accurate comparison two average weights are given for these organs in the group “control castrated rats” (Table I, col. II): (1) upper figure—average age 96 days, (2) lower figure—average age 78 days. For the other organs of rats in this group, the general average age was 88 days. The average age of the normal control rats was 95 days, there being no normal controls to the younger rats.

Table I. *Effect on actual weights of organs of castrated rats of testosterone dipropionate alone or in combination with oestradiol dipropionate*

	I	II	III	IV	V	VI	VII	VIII	IX	X	
	Control rats injected with oil			Castrated rats injected with							
				Testosterone dipropionate				Oestradiol dipropionate		Oestradiol dipropionate + testosterone dipropionate	
Organs	Normal	Castrated		50 µg.	150 µg.	500 µg.	500 µg. + 10 days' rest	1500 µg.	6 µg.	150 µg.	
Seminal vesicles (mg.)	952	12	163	528	333	1026	1022	1197	51	601	
Prostate (mg.)	853	65	330	624	557	963	1135	1028	86	677	
Penis (mg.)	348	81	218	282	280	327	356	316	71	271	
Preputial glands (mg.)	135	37	95	117	119	140	119	128	43	106	
Adrenals (mg.)	58	74	71	55	54	58	52	56	59	78	
Hypophysis (mg.)	9.5	10.6	12.7	11.6	11.3	12.3	11.3	9.3	20.7	21.8	
Thymus (mg.)	349	472	530	477	490	188	228	142	309	242	
Liver (g.)	12.37	10.48 9.22	11.74	13.06	12.24	11.24	12.07	10.05	9.16	9.43	
Kidneys (g.)	2.32	1.83 1.61	1.96	2.29	2.06	2.32	2.23	1.92	1.85	2.10	
Heart (mg.)	1047	920 747	984	966	956	1052	1018	855	756	896	
Retropitoneal fat (g.)	13.0	11.1	10.2	11.9	10.1	11.2	13.5	8.0	8.4	11.6	
Final body weight (g.)	342	310 275	313	310	305	329	336	254	245	290	
Gain in body weight (g.)	116	100 132	155	160	148	104	99	115	77	86	
No. of rats in group	6	7	5	8	8	4	4	4	4	4	
Final age (days)	95	96 78	78	80	80	104	99	77	96	82	

For economy of space, the data per unit of body weight have not been tabulated, but will where necessary be referred to in the text.

A detailed account of the results of histological investigation will be given elsewhere, only some preliminary statements being included here.

Other details of the experimental technique can be found in our previous papers.

Castrated male rats

The experiments were performed on 59 rats, divided into 11 groups as shown in Table I (groups I-X) and Table II (group XI). Groups XII and XIII in Table II contain additional data taken from our previous experiments on 8 rats [Korenchevsky *et al.* 1937, Table I].

Table II. *Effect on actual weights of organs of castrated rats of testosterone propionate in doses comparable with those of testosterone dipropionate in Table I*

Organs	XI	XII	XIII
	Rats injected with testosterone propionate		
	50 μ g.	500 μ g.	500 μ g. + 10 days
Seminal vesicles (mg.)	417	1228	284
Prostate (mg.)	507	1248	614
Penis (mg.)	256	301	276
Preputial glands (mg.)	103	154	118
Adrenals (mg.)	60	59	59
Hypophysis (mg.)	11.4	12.5	13.3
Thymus (mg.)	502	219	291
Liver (g.)	10.49	12.1	11.52
Kidneys (g.)	1.79	2.03	1.84
Heart (mg.)	864	932	927
Retroperitoneal fat (g.)	9.8	12.2	13.1
Final body weight (g.)	288	316	318
Gain in body weight (g.)	144	122	116
No. of rats in group	5	4	4
Final age	74	89	89

Sex organs

Injections of 500 μ g. of testosterone dipropionate daily (Table I, group VI) cause complete return to normal size, weight and histological structure of the seminal vesicles, prostate, penis and preputial glands, and this effect remains unchanged, or is even increased, 10 days after the last injection (group VII). Injections of 150 μ g. are unable to restore the size and weight of these glands completely to normal, and the restorative effect obtained in the seminal vesicles, and less so in the prostate, begins to decrease during the period without injections, the weights of the penis and preputial glands remaining unchanged. Increasing the daily dose to 1500 μ g. (group VIII) does not produce a significantly greater effect than that obtained with 500 μ g. (groups VI and VII). Simultaneous injections with oestradiol dipropionate (group X) show only a very weak co-operative effect (as judged by weight) on the seminal vesicles and prostate.

Comparison with testosterone propionate. While with the small dose of 50 μ g. (Table I, group III and Table II, group XI) the activity of the dipropionate is considerably less than that of the propionate, this difference is much less pronounced with the medium dose of 500 μ g. (Table I, group VI and Table II, group XII).

Supernormal seminal vesicles (1441 mg. average) are produced by 1400 μ g. of the propionate [Korenchevsky *et al.* 1937, Table I, col. IV] but not by 1500 μ g. of the dipropionate (this paper, Table I, group VIII). Thus the dipropionate is weaker than the propionate, although the difference is considerable only with the small doses.

A more important difference is the fact that the effect of 500 μ g. of the dipropionate is maintained or even increased 10 days after the injections have ceased (Table I, group VII), and even with 150 μ g. there is a considerable decrease only in the seminal vesicles (group V) while the effect of all doses of the propionate is very considerably decreased by the tenth day [Korenchevsky *et al.* 1937; see also Table II of this paper, groups XII and XIII].

The dipropionate appears to have a slightly better co-operative activity with oestradiol, since with the propionate [Korenchevsky & Dennison, 1937] an effect was recorded only on the seminal vesicles.

Other organs

The changes produced by the dipropionate on the weights of other organs are similar to, or even greater than, those caused by the propionate. The effects are maintained for at least 10 days with both hormones.

Adrenals. The hypertrophied "castration" adrenals return to normal size and weight (except with the lowest dose) this restoration being explained by a decrease in the width of the cortex. Only the largest dose of 1500 μ g. causes considerable depletion of lipid granules in the cortex.

Thymus. Involution, delayed in castrated animals, returns to or exceeds the normal level (as shown by weight and histologically) with medium and large doses of the hormone.

Liver, kidney and heart. The weights (actual and per unit body weight) of these organs, decreased after castration, return to or towards normal. Since histologically nothing abnormal (liver, heart) or a slight increase of some tubules or their cells (kidneys) was found, the enlargement obtained should be considered as a stimulation of these organs by the injections.

Hypophysis. It is difficult to decide how much significance should be attached to the slight enlargement of the hypophysis observed in most rats injected with all but the largest dose of the hormone. A certain amount of variation was present in all groups and the results obtained with the largest dose were rather indefinite. Oestradiol dipropionate, as usual, produced hyperplasia, which the simultaneous injection of testosterone dipropionate, in the dose used, did not prevent (Table I, groups IX and X).

Thyroids, parathyroids, pancreas and spleen did not show any changes.

Fat deposition and gain in body weight. There appeared to be a more pronounced gain in body weight with small doses of the dipropionate (groups III-V), but no definite changes in fat deposition, so that the increase cannot be explained by accumulation of fat in the body. 500 μ g. produced no definite change (groups VI and VII), while with 1500 μ g. (group VIII) both fat deposition and also gain in body weight were slightly decreased. The dipropionate, therefore, appears to have some effect on metabolism.

Simultaneous injections of oestradiol dipropionate (group X) neutralize the effects of the male hormone on the adrenals and also prevent more or less its stimulating activity on liver, heart and gain in body weight, but not the enlargement of the kidney (groups IX and X). Co-operative activity of the two hor-

mones is shown in the acceleration of the involution of the thymus. Similar results have been obtained previously after the addition of oestradiol to testosterone propionate [Korenchevsky & Dennison, 1937].

Ovariectomized females

Experiments were performed on 36 rats belonging to 8 litters and divided into 8 groups as shown in Table III.

Table III. *Effect on actual weights of organs of spayed female rats of testosterone dipropionate alone or in combination with oestradiol dipropionate (and compared with the effect of testosterone propionate)*

	I	II	III	IV	V	VI	VII	VIII
	Spayed rats injected with							
	Control rats		Testosterone dipropionate			Oestra- diol dipro- pionate		
			150 μ g. + 10 days rest			6 μ g. + testosterone dipropionate 150 μ g.		
	Normal	Spayed	150 μ g.	1500 μ g.		6 μ g.	150 μ g.	1500 μ g.
Uterus (cervix + horns, mg.)	359	31	113	63	246	212	245	242
Cervix of uterus (mg.)	103	13	48	29	111	92	109	114
Vagina (with clitoris, mg.)	241	168	280	259	410	240	337	338*
Preputial glands (mg.)	97	66	126	95	176	66	109	255
Adrenals (mg.)	58	68	48	42	50	69	68	62
Hypophysis (mg.)	11.8	11.0	9.6	7.7	7.8	21.6	18.5	11.8
Thymus (mg.)	382	590	445	487	147	257	257	87
Liver (g.)	8.03	8.45	9.68	10.32	8.69	8.58	8.55	8.73
Kidneys (g.)	1.42	1.51	1.69	1.71	1.74	1.77	1.89	1.76
Heart (mg.)	665	708	765	750	744	689	681	764
Retroperitoneal fat (g.)	9	13	13	12	9	8	9	5
Final body weight (g.)	191	236	243	240	225	188	205	213
Gain in body weight (g.)	70	95	108	95	82	56	69	53
No. of rats in the group	9	8	4	3	3	3	4	2

* In group VIII the vaginal weight is given without clitoris.

The data in group V (testosterone dipropionate) are compared with those given in group VIII, from rats injected with testosterone propionate (1500 μ g.). The testosterone compounds were injected daily and the oestradiol dipropionate three times per week in the doses given in the table.

Sex organs. The restorative effect on the sex organs of the spayed rats was very pronounced, and similar to that of the propionate (compare groups V and VIII). The weight of the vagina after injections of both 150 and 1500 μ g., especially the latter, exceeded that of the normal control, but complete recovery of the uterus was not obtained even with the large dose. The duration of the effect of the injections was not studied, except in the case of the small dose (150 μ g., group IV), when the effect, especially on the uterus, decreased considerably during the 10 days after the injections were discontinued.

There was definite co-operative activity with oestradiol dipropionate on the uterus and especially on the vagina, but not on the preputial glands (group VII).

Histological changes in the sex organs were similar in character but not in degree to those obtained with testosterone propionate [Korenchevsky & Hall,

1937]. With 150 $\mu\text{g.}$ a dioestrus uterus and metaplasia of the vaginal epithelium into small mucous cells were obtained, but with 1500 $\mu\text{g.}$ the progestational changes both in the uterus and vagina approached much more closely than with the propionate to those seen during pregnancy. Thus the dipropionate possesses, even more strongly than the propionate, this remarkable property of producing progestational changes in the sex organs of ovariectomized animals, without any previous sensitization with oestrogens. The histological changes produced by the injections were maintained during the 10 days' "rest" even less than were the weight changes: the uterus became atrophic, and the mucous metaplasia in the vagina partly, or in some rats entirely, disappeared.

Co-operative and antagonistic activities of testosterone dipropionate and oestradiol dipropionate

These two hormones when injected simultaneously show both co-operative and antagonistic activities.

Miescher *et al.* [1938] have shown that oestradiol dipropionate has a greater and more prolonged activity than pure oestradiol. This is also shown in the present experiments, when 6 $\mu\text{g.}$ of the dipropionate produced about the same effect by weight and histologically as 30 $\mu\text{g.}$ of pure oestradiol [Korenchevsky & Hall, 1937]. In the uterus, 6 $\mu\text{g.}$ of the dipropionate produced the same high cylindrical epithelium, transformed in places into squamous epithelium (metaplasia), as [Korenchevsky & Hall, 1938] that with 30 $\mu\text{g.}$ of oestradiol. This metaplastic epithelial change is important as indicating the first step in the "carcinogenic" activity of oestrogens.

Simultaneous injections of testosterone dipropionate and oestradiol dipropionate gave a picture typical of the female hormone. Thus oestradiol dipropionate is *antagonistic* to and overrules the activity of testosterone dipropionate. It is important that the doses used of testosterone dipropionate did not prevent the metaplastic changes produced by oestrogens in the uterus, while these changes were prevented by progesterone [Korenchevsky & Hall, 1938].

A *co-operative* activity of the dipropionates of testosterone and oestradiol was shown in a better recovery in weight and size of the uterus and vagina.

Effect on female prostate and clitoris, and classification of the hormone. The injections caused a great development of the female prostate and clitoris, similar to that described previously for the other male hormones [Korenchevsky, 1937, 2; Hall, 1938].

The female prostate, unlike the uterus and vagina, did not regress during the 10 days after the cessation of the injections (group IV). Moreover, 1500 $\mu\text{g.}$ and even 500 $\mu\text{g.}$ of testosterone dipropionate overstimulate the male organs, but 1500 $\mu\text{g.}$ are unable to restore the uterus to normal. These facts place testosterone dipropionate (using the classification suggested by Korenchevsky [1937, 1]) in the group of "bisexual hormones possessing chiefly male properties."

Other organs, fat deposition and gain in body weight

Thymus and adrenals. Testosterone dipropionate had a similar effect on the weights and histological structure of these organs in females as in males.

Liver, kidneys, heart, fat deposition and gain in body weight. The general effect on these organs appears to be similar in character to that in males, but weaker in degree and less constant. More numerous experiments, and especially of longer duration, are needed in order to come to a definite conclusion. No pathological changes were found histologically in the liver and kidneys.

The similarity of these changes in the two sexes is rather remarkable, as the effects of gonadectomy on most of the organs investigated were different in males from those in females. This will be discussed in more detail elsewhere.

When injected simultaneously (group VII) oestradiol dipropionate was found to neutralize more or less completely the activity of testosterone dipropionate on adrenals, hypophysis (increased weight), liver, heart, fat deposition and gain in body weight (decrease), while stimulation of kidneys was evident both in actual weight and per unit of body weight.

SUMMARY

1. In gonadectomized male and female rats the manifold effects of testosterone dipropionate are in general similar to those of testosterone propionate, with, however, some important differences.

On male sex organs

2. The effect of small doses of the dipropionate on these organs is weaker (e.g. on the prostate, by about 1.5 times) than that of similar doses of the propionate. With larger doses this difference is much less.

3. In contrast to the propionate, however, the effect of the dipropionate on the male sex organs is maintained (completely with 500 μ g., partially with 150 μ g.) for at least 10 days after the last injection.

On female sex organs

4. Testosterone dipropionate alone, without previous stimulation with oestrogens, can produce progestational changes in the uterus and vagina, much more similar to those found during pregnancy than the changes produced by the propionate.

5. After the cessation of the injections (small doses) the development of the vagina is maintained better than that of the uterus.

6. Testosterone dipropionate has very pronounced bisexual properties (including strong stimulation of the female prostate and clitoris), but, from a comparison of its "male" and "female" activities, it can be classified as a "bisexual hormone possessing chiefly male properties".

7. Testosterone dipropionate, in the dose used, did not prevent the metaplastic changes produced in the uterine epithelium by oestradiol dipropionate.

Non-sexual organs of both sexes

8. Testosterone dipropionate influenced the thymus, adrenals, liver, kidneys, heart, fat deposition and gain in body weight in both sexes, but in most cases, with the doses used, the effects in females were less pronounced and less constant than in males.

9. Testosterone dipropionate and oestradiol dipropionate show both co-operative and antagonistic activities on sexual and non-sexual organs.

10. The experimental evidence obtained indicates a possible advantage of testosterone dipropionate over the propionate for clinical use.

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