LXIV. THE EFFECTS OF Δ^4 -ANDROSTENEDIONE AND Δ^5 -ANDROSTENEDIOL ON CASTRATED AND OVARIECTOMIZED RATS

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THE "sexual hormones" named in the title, which have been prepared artificially, but have not so far been found in the organism, are included in our series of experiments, since they are significant (particularly androstenedione) as probable intermediate products in the biological formation of the male and female sexual hormones from cholesterol [Ruzicka & Wettstein, 1935, 1, 2; Butenandt & Kudzus, 1935].

The influence of Δ^4 -androstenedione and of Δ^5 -androstenediol on the sexual organs was examined in short-duration experiments both by Swiss and German workers [Ruzicka & Wettstein, 1935, 1, 2; Butenandt & Kudzus, 1935; Butenandt & Hanisch, 1935; Butenandt, 1936, 1, 2; Tschopp, 1936].

A moderate activity on the male sexual organs of castrated rats was obtained with androstenedione, whilst the effect with androstenediol was weaker. A positive Allen & Doisy test (keratinization of vaginal epithelium) was obtained in ovariectomized rats with androstenediol, but not with androstenedione. In normal infantile rats however keratinization of the vagina was also produced with androstenedione.

The progestational reaction was also examined (proliferation of the uterine mucosa in infantile normal rabbits) after the injection of androstenedione, but the results were negative even with doses as high as 10 and 30 mg. Ruzicka & Wettstein [1935, 1], however, regard Δ^4 -androstenedione as a lower homologue of the corpus luteum hormone (progesterone). Deanesly & Parkes [1936, 1, 2] in short-duration experiments confirmed the results which have been given above.

Butenandt & Dannenberg [1936] and Butenandt & Schmidt-Thomé [1936] prepared also two other Δ^1 - and Δ^5 -isomerides of androstenedione. The Δ^1 -isomeride has an effect which is opposite to that of the Δ^4 -isomeride, i.e. no effect on the male sexual organs (maximum dose 4 mg.), while the Allen & Doisy test was positive after the injection of 2 mg. into ovariectomized rats. The study of the physiological effects of the Δ^5 -isomeride was insufficient for definite conclusions to be made.

In the present paper on the effects of prolonged treatment with Δ^4 -androstenedione and Δ^5 -androstenediol (referred to in this paper as "androstenedione" and "androstenediol") the sexual and other organs of gonadectomized male and female rats were investigated. The keratinization test on females, more particularly in short-duration experiments, does not explain the character of the activity of the sexual hormones, especially when this activity is unconnected with the development of keratinization.

An accurate and satisfactory answer can be obtained only from prolonged experiments by investigation of the organs both by the method of weights as it is advocated and used by us and histologically.

TECHNIQUE

The general technique was the same as that used in our previous experiments. The penis was cut off just below the basal bulb after fixation of this organ. Androstenedione and androstenediol were prepared and kindly supplied by Messrs Ciba Ltd., and oestrone by Prof. Girard. The hormones were dissolved in sesame oil. The doses used depended on the solubility of the hormone in this solvent, which was much higher in the case of dione (about 1 : 100) than in that of diol (about 1 : 1000). In consequence, while it was possible to inject the smaller doses in 0.1 ml. of oil twice a day, it was necessary to inject 0.3 ml. of solution twice a day in order to inject 600 γ of diol, whilst 3000 γ of dione were given by injecting 0.2 ml. in the morning and 0.1 ml. in the evening (total 0.3 ml. per day). Each 0.1 ml. however was injected into a separate place in the subcutaneous tissue in order to facilitate absorption of the hormone.

6 γ of oestrone were injected 4 times per week and the other hormones were injected daily. The period of injection was 21 days. The tables have been shortened and in order to economise space the weights per unit of body weight are not given. In drawing our conclusions however these were always taken into consideration and are mentioned in the text, where the results given by the weights per unit of body weight differ from those given by the actual weights. The weight of uterus (horns + cervix) does not represent the complete weight, being less by the weight of the parts of the horns which were removed with the ovaries at ovariectomy in order to ensure that the ovariectomy was complete. Therefore comparison of the weights of the cervix of uterus gives more accurate data.

EFFECTS ON CASTRATED MALES OF ANDROSTENEDIONE ALONE OR IN COMBINATION WITH OESTRONE OR TESTOSTERONE

The experiment was performed on 51 rats belonging to 10 litters, the average age of which was 79 days. The rats were distributed into 9 groups as shown in Table I, one rat of each litter being placed in a different group and each group containing approximately the same number of rats of the same age.

Sexual organs. As can be seen from Table I (cols. III–V) and from a comparison with the normal controls (col. I) complete recovery of the sexual organs, atrophied after castration, was not obtained even with 3 mg. per day (the highest dose used), except in the case of the preputial glands, the weight of which even exceeded the normal weight. While in combination with oestrone no co-operative effect was obtained (compare col. III with VI and col. V with VII), in combination with testosterone this effect was clearly seen (cols. III, VIII and IX) especially in the case of the seminal vesicles, on which the effect was greater than would be given by pure summation.

Adrenals. The hypertrophied “castration” adrenals (col. II) were reduced to the normal size and weight by all the doses of androstenedione used. Combination with testosterone did not influence this effect (col. IX). It has been previously found [Korenchevsky & Dennison, 1934] that oestrone produces hypertrophy of the adrenals and checks the restorative effect of androsterone on “castration” adrenals particularly in the case of small doses of androsterone [Korenchevsky *et al.* 1935, Table III, p. 2541]. In the present experiments this same depressing effect of oestrone on the action of androstenedione on the adrenals was again observed and again was seen more clearly with the smaller dose (col. VI).

Table I. *Effect on male rats of androstenedione alone or in combination with oestrone or testosterone*

The average actual weights of organs of castrated rats injected with the hormones compared with those of the organs of normal and castrated uninjected litter-mates

Organ	I	II	III	IV	V	VI	VII	VIII	IX
	Castrated rats injected with								
	Control rats		Androstenedione			Oestrone 6 γ +		Testosterone 500 γ	Testosterone 500 γ + androstenedione 500 γ
	Normal	Castrated	500 γ	1000 γ	3000 γ	Androstenedione 500 γ	Androstenedione 3000 γ		
Seminal vesicles (mg.)	615	12	86	170	367	90	375	146	346
Prostate (mg.)	809	60	307	484	713	320	703	304	630
Penis (mg.)	285	77	206	232	254	202	275	194	248
Preputial glands (mg.)	130	52	116	144	200	123	170	104	165
Adrenals (mg.)	52	78	56	56	52	67	61	60	54
Hypophysis (mg.)	11.0	15.2	14.3	15.4	16.0	16.3	15.9	13.4	13.6
Thymus (mg.)	461	565	527	463	326	395	346	525	409
Liver (g.)	11.9	9.5	10.5	10.9	10.7	10.3	11.1	11.2	11.3
Kidneys (g.)	2.05	1.71	1.77	1.81	1.89	1.94	1.99	1.80	1.87
Heart (mg.)	878	784	846	831	830	818	884	801	835
Fat (g.)	9.2	10.0	7.3	9.2	7.3	5.6	7.2	6.9	7.9
Final body weight (g.)	292	277	259	281	275	238	279	270	275
Gain in body weight (g.)	93	82	109	92	92	81	86	105	97
No. of rats in group	7	9	5	7	6	4	7	3	3

Thymus, liver and heart. The usual effects of the male sexual hormones on the thymus (increase in the rate of involution) and liver (increase in weight) of castrated rats were also seen after androstenedione injections. The weight of the liver however did not reach normal. The very small changes in the weight of the heart were within the physiological fluctuations and are mentioned, since with the male hormones this change usually occurs to a more pronounced degree: in the present experiment the weight of the heart, which decreased slightly after castration (col. II), increased after the injection of the hormones (cols. III–IX).

Gain in body weight and fat deposition. The changes obtained were also typical of the effects of the male hormones: the gain in body weight, slightly decreased by castration (col. II), was restored to normal or was even greater than normal after the injections in spite of a decrease in the deposition of fat (cols. III–V). This suggests the stimulation of the anabolic processes in the building of new tissues. While the addition of testosterone (col. IX) had no influence on this metabolic effect, the addition of oestrone had a depressing effect (cols. VI and VII). A similar checking of the gain in body weight was seen when oestrone was injected in combination with androsterone [Korenchevsky *et al.* 1935, Table III, p. 2541], with testosterone [Korenchevsky *et al.* 1936, Table IV, p. 565] or with *transdehydroandrosterone* [Korenchevsky & Dennison, 1936, p. 1517].

Thyroids, hypophysis and kidneys did not show any definite changes in weight as the result of the injections. However, the usual effect of castration, hypertrophy of the hypophysis and slight decrease in the weight of the kidneys was evident.

EFFECT ON OVARIECTOMIZED FEMALES OF ANDROSTENEDIONE
ALONE OR IN COMBINATION WITH OESTRONE

Experiments were performed on 58 rats belonging to 11 litters. The rats were arranged in 8 groups (Table II) in the same way as in the previous experiments with males.

Table II. *Effect on female rats of androstenedione alone
or in combination with oestrone*

The average actual weights of organs of ovariectomized rats injected with the hormones compared with those of the organs of normal and ovariectomized uninjected litter-mates

	I	II	III	IV	V	VI	VII	VIII	IX
	Ovariectomized rats injected with								
	Control rats		Oestrone 6 γ	Androstenedione			Oestrone 6 γ +		Age
Organ	Normal	Ovari- ectomized		500 γ	1000 γ	3000 γ	Andro- stene- dione 500 γ	Andro- stene- dione 3000 γ	
Uterus (horns + cervix) (mg.)	372 361	35 30	155 125	50 54	— 53	101 77	248 177	229 260	79 68
Cervix of uterus (mg.)	123 113	18 10	68 55	23 21	— 19	49 27	101 72	116 95	79 68
Vagina (mg.)	240 225	138 132	189 202	209 181	— 196	235 213	254 247	276 273	79 68
Female preputial glands (mg.)	101 106	67 75	67 78	157 161	— 189	204 260	120 193	228 257	79 68
Adrenals (mg.)	71 71	91 85	79 86	68 71	— 64	52 47	70 72	63 51	79 68
Thymus (mg.)	254 341	494 696	407 508	369 488	— 402	336 417	325 444	233 334	79 68
Liver (g.)	7.1 8.2	7.8 8.9	6.9 7.5	9.5 9.8	— 9.1	9.4 9.7	7.7 8.4	8.9 9.1	79 68
Fat (g.)	17.2 9.2	12.8 8.5	12.9 7.5	18.2 11.1	— 9.0	10.7 9.0	14.1 6.5	9.3 6.0	79 68
Final body weight (g.)	226 197	260 222	217 198	280 233	— 221	256 224	221 195	237 201	79 68
Gain in body weight (g.)	51 57	66 85	29 45	73 86	— 73	65 87	27 53	30 57	79 68
No. of rats in group	9	10	12	5	4	5	6	7	79 68

Sexual organs. It is clear from Table II that androstenedione has a considerable stimulating effect not only on the male sexual organs but also on those of females. As in the case of the other male sexual hormones, co-operative activity with oestrone and overstimulation of the female preputial glands were observed. With the largest daily dose used (3 mg.) the weight of the vagina became nearly normal, though that of the uterus was much less than normal (col. VI). In combination with oestrone while the vagina attained a weight greater than normal, the weight of the uterus was still less than normal (cols. VII and VIII).

On the development of a "clitoris-like" organ in females. In female rats there are three orifices in the hind part of the body; one under the tail, the orifice of the rectum; a second more ventrally, the orifice of the vagina; and a third still more ventrally, a funnel-like depression in

the skin into which opens in the middle the urethra and on each side the orifices of the female preputial glands. In normal female rats the ventral and side walls of about 2–3 mm. of the urethra are slightly thickened and have a slightly redder colour than the remaining part of the urethra. In ovariectomized rats this thickening of the urethra becomes very atrophic and remains so in ovariectomized rats injected with oestrone. Androstenedione injections, however, develop this thickening into a structure 5–6 mm. long and 2–3 mm. wide protruding over the orifice of the urethral canal into the funnel-like depression in the skin. The colour of this structure is also much deeper than it is normally. The appearance is that of a clitoris or short rudimentary penis with a preputium-like folding of the skin over it.

Before the histological investigation is completed, no definite conclusion can be made as to the nature of this structure.

Adrenals, thymus and liver. The action of androstenedione on these organs in ovariectomized females was similar to that on the corresponding organs in castrated males, injection bringing about a decrease in the weight of the adrenals and thymus (cols. IV–VI) (which organs are larger in spayed than in normal rats (cols. I and II)) and an increase in the weight of the liver as compared with that of control ovariectomized rats.

Gain in body weight and fat deposition. As is usual the ovariectomized rats were heavier and their gain in weight greater than that of the normal rats in spite of the absence (at this age) of an increase in the deposition of fat. While oestrone alone, or in combination with androstenedione, decreased the gain in body weight, with androstenedione alone no definite changes were seen.

EFFECT ON CASTRATED MALES OF ANDROSTENEDIOL ALONE OR IN COMBINATION WITH OESTRONE

The experiments were performed on 15 rats belonging to 3 litters 69 days old. The rats were divided into 5 groups as shown in Table III. With the doses used the effect on the sexual organs (except the preputial glands) was small and there was no definite co-operative effect with oestrone. The "castration" adrenals

Table III. *Effect on male rats of androstenediol alone
or in combination with oestrone*

The average actual weights of organs of castrated rats injected with the hormones compared with those of the organs of uninjected castrated litter-mates

	Control castrated rats	Castrated rats injected with			
		Androstenediol		Oestrone 6γ +	
		200γ	600γ	Androstene- diol 200γ	Androstene- diol 600γ
Seminal vesicles (mg.)	14	17	35	25	33
Prostate (mg.)	58	90	130	92	106
Penis (mg.)	72	97	134	103	121
Preputial glands (mg.)	60	81	160	121	167
Adrenals (mg.)	68.5	58.8	54.4	78.3	76.1
Thyroids (mg.)	20.4	20.7	24.9	20.7	23.5
Hypophysis (mg.)	14.3	14.1	15.3	19.1	18.8
Thymus (mg.)	659	646	600	697	602
Fat (g.)	8.7	9.3	6.7	7.0	5.9
Final body weight (g.)	267	265	229	241	247
Gain in body weight (g.)	116	111	76	90	76
No. of rats in group	3	3	3	3	3

decreased in weight after the injection of androstenediol, but this effect was completely suppressed by the addition of oestrone to the injections. A second noteworthy effect was the action on the hypophysis of a combination of oestrone and androstenediol, the "castration" hypertrophy of this gland being increased in all the rats injected. There was no definite change in the weight of the other organs investigated. The very weak effect or complete absence of effect on the thymus was unexpected with a "sexual" compound.

The gain in body weight and the deposition of fat decreased after the injection of a large dose of androstenediol alone and after either dose in combination with oestrone.

Ratio ($\times 100$) of percentage increase in actual weight of prostate to that of seminal vesicles after injection of androstenedione and androstenediol compared with this ratio observed during natural growth of the organs in normal rats

The relation of the growth of the prostate to that of the seminal vesicles is given in Table IV. We have previously discussed [e.g. Korenchevsky & Dennison, 1936, p. 1519] the significance of this ratio in estimating the qualitative effects of the sexual hormones, this ratio indicating how nearly the changes observed after the injections approximate to the normal relative developments of the prostate and seminal vesicles in normal rats (=25).

Table IV

Hormone	Daily dose γ	Ratio
Androstenedione	500	67
	1000	54
	3000	37
Androstenediol	200	257
	600	83
Normal development in normal rats	—	25

As was found with the other male hormones, the larger the doses of dione and diol injected, the closer to the normal ratio is the ratio obtained. It is also clear that the development of the sexual organs is more nearly normal with dione than with diol. However, even with the highest dose (3000 γ) the normal ratio is not attained (37 instead of 25).

EFFECT ON OVARECTOMIZED FEMALES OF ANDROSTENEDIOL ALONE OR IN COMBINATION WITH OESTRONE

The experiments were performed on 13 spayed rats belonging to 3 litters, the average age of which was 65 days. The results are summarized in Table V.

Sexual organs. The data given in Table II (cols. I and III, second lines) for normal control and spayed oestrone-injected rats might also be used for comparison in this experiment, since the ages of the two groups of rats were not very different (68 days in the previous experiment, 65 days in the present experiment).

From a comparison of the results obtained after injection with the data for control uninjected normal and spayed rats it may be concluded that androstenediol produced some degree of development of the sexual organs. A comparison of the weights of the sexual organs of rats injected with similar doses of androstenediol (600 γ , Table V) and androstenedione (500 γ , Table II, col. IV) shows that the diol had a stronger action on the uterus than had the dione, while on the

Table V. *Effect on female rats of androstenediol alone or in combination with oestrone*

The average actual weights of organs of spayed rats injected with the hormones compared with those of the organs of uninjected spayed litter-mates

	Control spayed rats	Spayed rats injected with			
		Androstenediol		Oestrone 6 γ +	
		200 γ	600 γ	Androstene- diol 200 γ	Androstene- diol 600 γ
Uterus (horns + cervix) (mg.)	28	51	80	134	127
Cervix of uterus (mg.)	10	19	28	44	52
Vagina (mg.)	127	141	174	211	186
Female preputial glands (mg.)	59	178	145	112	176
Adrenals (mg.)	71.5	55.6	57.0	71.8	65.7
Hypophysis (mg.)	12.8	12.5	14.7	14.9	15.7
Thymus (mg.)	501	412	332	422	242
Fat (g.)	7.2	4.9	4.7	5.8	3.7
Final body weight (g.)	201	187	188	172	182
Gain in body weight (g.)	79	67	56	45	51
No. of rats in group	3	2	3	2	3

vagina the activities of these hormones were similar. Whilst, however, diol (Table V) showed no co-operative activity with oestrone (since the weights of the organs were about equal to those obtained after the injection of oestrone alone, Table II, col. III), in the case of dione co-operation with oestrone was clearly seen (Table II, cols. VII and VIII). As with all the other hormones, which have definite male activity, the weight and size of the female preputial glands also increased abnormally after androstenediol injections.

Other organs. The action of androstenediol on the adrenals was typical of that of the male hormones, the weight decreasing when diol was injected alone. This effect however was suppressed or decreased when androstenediol was injected in combination with oestrone. Whilst the rate of involution of the thymus increased after the injection of diol, no effect or even an increase was obtained in the weight of the "castration" hypophysis.

Both alone and in combination with oestrone androstenediol decreased the gain in body weight and the deposition of fat.

Note on the preliminary histological investigation of the female sexual organs. Histologically both androstenedione and androstenediol produced various degrees of hypertrophy of the uterine mucosa and myometrium.

Under the influence of androstenedione the vaginal epithelium became slightly hypertrophic and with high doses slight mucification of the cells occurred, while with androstenediol the mucification of the hypertrophied cells was complete.

Combination of dione or diol with oestrone caused hypertrophy of the uterus, the microscopical appearance of which was that seen in dioestrus.

On the vagina however the addition of oestrone to these two hormones had different effects, for with dione slight or medium pregnancy-like mucification of the vaginal epithelium was produced, whilst with diol oestrone completely suppressed the mucification, which was obtained with diol alone.

SUMMARY

1. Experiments were performed on 7 normal and 59 castrated male rats, and on 9 normal female and 62 ovariectomized rats, belonging to 27 litters for the purpose of investigating the actions of Δ^4 -androstenedione and Δ^5 -androstenediol.

2. Both these artificial sexual hormones undoubtedly have both male and female sexual activities, the weight, size and histological structures of the organs atrophied after gonadectomy being restored towards but not up to the normal condition.

3. The weight and size of both the male and female preputial glands however become normal or supranormal after the injections.

4. The hypertrophying effect on the female preputial glands places both these hormones biologically (our definition) in the group of male hormones (e.g. testosterone) and not in that of female hormones (e.g. oestrone).

5. In the doses used, androstenedione shows a co-operative activity with oestrone on the female sexual organs only, but not on the male organs, while androstenediol shows no co-operative activity with oestrone either on the male or female organs.

6. On the contrary there is histological evidence of an antagonistic effect of oestrone on the action of androstenediol on some of the female sexual organs.

7. There is co-operative activity on the male sexual organs between androstenedione and testosterone, especially on the seminal vesicles.

8. In female rats the thickened peripheral end of the urethra atrophies very considerably after ovariectomy, but after the injection of androstenedione into spayed rats it hypertrophies into a structure which is much larger than that seen in normal rats and is reminiscent of a clitoris. Oestrone does not have this effect.

9. Both hormones cause a definite decrease in the weight of the adrenals and an increase in the rate of involution of the thymus in both castrated and ovariectomized rats (except that in males the effect of androstenediol on the thymus was indefinite). The addition of oestrone seems to depress this action on the adrenals, especially in the case of androstenediol.

10. The effects of these hormones on the other organs, on the fat deposition and on the gain in body weight were also discussed.

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