CCXII. THE ASSAY OF *TRANS*DEHYDROANDROSTERONE AND ITS EFFECTS ON MALE AND FEMALE GONADECTOMIZED RATS.

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IN our previous papers [Korenchevsky, 1935-36; Korenchevsky *et al.* 1934-36] we have studied the effects of those sexual hormones which are known to be present in the organs or excreted in the urine, namely, androsterone, testosterone and oestrone.

In the present paper the series of male hormones is concluded by giving the results of our experiments with *transdehydroandrosterone*. It has not yet been proved in the case of human urine that the oestrogenic hormone in the urine of the male is oestrone nor has it been proved for any species that the male activity of female urine is due to androsterone, *transdehydroandrosterone* or testosterone. Häussler [1934] and Girard (personal communication), however, isolated a chemically pure preparation of oestrone from the urine of stallions. It is therefore almost certain that the oestrogenic activity of human urine and that of other mammals is also due to oestrone. In addition to oestrone Girard (personal communication) isolated from the urine of stallions compounds similar to, but in some respects different from, equilenin. We have therefore studied the effect of oestrone on males and, since at least some of the male hormones mentioned above are suspected of being the cause of the specific sexual activity of the urine and blood of females, the effect of these male hormones on females was investigated.

Recently Koch [1936] made a careful quantitative examination of the male and female sexual activities in the human urine of both sexes.

A. *Previous experiments with transdehydroandrosterone.*

transDehydroandrosterone (hereinafter referred to as T.D. androsterone) was isolated from male urine by Butenandt and his co-workers [1934; 1935]; by Schoeller *et al.* [1935] and by Oppenauer [1935], the quantity of this hormone in the urine being about the same as that of androsterone. These workers, as well as Ruzicka & Wettstein [1935, 1, 2], Ruzicka *et al.* [1935] prepared this hormone artificially. The biological properties of this hormone have been studied very little and some of the results obtained are contradictory. While Butenandt found that 1 capon unit was contained in about 600 γ , Tschopp [Ruzicka *et al.* 1935; Tschopp, 1936] stated that 1 c.u. was contained in about 150-200 γ , but in another paper [Ruzicka & Wettstein, 1935, 1] gave the figure as 500 γ . Deanesly & Parkes [1936, 1] found 1 c.u. in 300 γ .

Tschopp [Ruzicka & Wettstein, 1935, 2] found that up to 500 γ of T.D. androsterone injected daily for 10 days had no definite effect on the seminal vesicles and prostate of castrated rats. Deanesly & Parkes [1936, 1], also injecting for 10 days, found that doses of 1 and 2 mg. per day had a slight effect on these organs.

Butenandt *et al.* [1935] found no oestrogenic effect of 2 mg. of T.D. androsterone on the vagina of ovariectomized mice, but obtained positive results with normal infantile females. Tschopp [1936], working with ovariectomized rats, confirmed this statement by Butenandt of the lack of oestrogenic effect of T.D. androsterone. Deanesly & Parkes [1936, 2], in 4 of 5 rats, found, however, that total doses of 2–3 mg. had oestrogenic activity on the vagina in both normal and ovariectomized rats.

B. *Present experiments.*

The experiments were performed with T.D. androsterone artificially prepared by Ruzicka and his co-workers and kindly supplied by Messrs Ciba Ltd. The hormone was dissolved in sesame oil and was investigated by our usual technique which is fully described in our previous papers. The animals were injected in the assay experiments for 7 days and in the prolonged experiments for 21 days, the daily dose being injected in half-doses twice a day. The control rats, normal and gonadectomized, were injected with the sesame oil only.

I. *The assay of T.D. androsterone.*

Using Korenchevsky's method, the assay was performed on 38 rats belonging to 8 litters. The ages of the litters at dissection varied from 57 to 65 days. The results obtained are given in Table I. Although the effect on the sexual organs

Table I. *Assay experiment.*

The effect of 7 days' injection of testosterone on sexual organs, thymus and body weight of male castrated rats.

Organ	Weights	Control rats	Rats injected with (mg.)			
			0.8	1	2	4
A. Average weights.						
Seminal vesicles (mg.)	Actual	9.4	15.1	15.4	18.0	25.7
Prostate (mg.)	Actual	54	72	77	85	104
Prostate with seminal vesicle (mg.)	Actual	63	87	92	103	130
Penis (mg.)	Actual	72	94	92	104	120
Preputial glands (mg.)	Actual	60	95	110	116	118
Thymus (mg.)	Actual	666	658	608	599	568
Final body weight (g.)	Actual	211	222	228	227	219
No. of rats in each group	—	7	9	9	7	6
B. Percentage changes in weight.						
Seminal vesicles	Actual	—	+61	+64	+92	+173
	Per unit of body weight	—	+53	+52	+79	+164
Prostate	Actual	—	+33	+43	+57	+93
	Per unit of body weight	—	+27	+33	+47	+86
Prostate with seminal vesicles	Actual	—	+38	+46	+64	+106
	Per unit of body weight	—	+30	+35	+52	+98
Penis	Actual	—	+31	+28	+44	+67
	Per unit of body weight	—	+25	+19	+35	+62
Preputial glands	Actual	—	+58	+83	+93	+97
	Per unit of body weight	—	+49	+70	+79	+90

and thymus was clear, it was small even with such a large dose as 4 mg. per day. One rat unit was found to be contained in about 940 γ , as measured by a 40% increase in the actual weight of the prostate.

II. *Prolonged injection of T.D. androsterone, alone or simultaneously with oestrone, into castrated male rats.*

Experiments were performed on 42 rats belonging to 9 litters, half the litters being killed at about 68 days old and the rest at about 86 days old. Therefore, in Table II, in which all the other details are also summarized, the data for these two age groups are given separately. As no normal control rats were available for the age group 86 days, the average weights of the organs of 5 normal rats (about 96 days old) from another experiment were used for comparison and inserted into the corresponding group of col. i, Table II.

Table II. *Effect of prolonged injection of sexual hormones on male rats.*

The average actual weights of the organs of castrated rats injected with sexual hormones as compared with those of the organs of normal or castrated control rats injected with oil only.

	i	ii	iii	iv	v	vi	vii
			Castrated rats injected with				
			T.D. androsterone			Oestrone 6γ + T.D. andro- sterone	Av. age of rats days
Organ	Normal control rats	Castrated control rats	1 mg.	2 mg.	3 mg.	1 mg.	
Seminal vesicles (mg.)	677	11	18	—	29	23	68
	999	14	18	31	—	—	86
Prostate (mg.)	799	57	81	—	105	97	68
	1250	64	91	127	—	—	86
Seminal vesicles + pro- state (mg.)	1476	68	99	—	134	120	68
	2249	78	109	158	—	—	86
Penis (mg.)	280	63	98	—	120	110	68
	328	85	110	132	—	—	86
Preputial glands (mg.)	137	49	101	—	160	124	68
	144	57	117	109	—	—	86
Adrenals (mg.)	56	79	63	—	55	64	68
	60	85	71	66	—	—	86
Hypophysis (mg.)	9.2	13.3	12.5	—	12.7	14.3	68
	10.8	14.5	14.7	14.5	—	—	86
Thymus (mg.)	462	579	584	—	549	504	68
	315	615	543	579	—	—	86
Liver (g.)	13.3	10.9	11.0	—	11.7	9.4	68
	13.6	11.6	12.6	13.4	—	—	86
Kidney (g.)	2.06	1.65	1.76	—	1.72	1.72	68
	2.31	1.91	1.93	1.96	—	—	86
Heart (mg.)	928	703	780	—	772	706	68
	960	931	919	973	—	—	86
Average final body weight (g.)	295	244	250	—	255	220	68
	367	314	319	327	—	—	86
No. of rats in group	2 (+5)	9	14	7	2	8	—

In order to economize space, the weights of the organs per unit of body weight are not given, but will be mentioned in the text in those cases where the character of the changes, as judged by these two data, differs.

Sexual organs and thymus. In spite of the fact that in this prolonged experiment the period of injection of T.D. androsterone was three times longer than in the assay experiment, the hypertrophy of the sexual organs and the rate of

involution of the thymus (Table II, cols. iii, iv, v) were only slightly greater than those observed in the assay experiments (Table I).

The addition of oestrone (Table II, col. vi) caused only a slight increase in the effect of the similar dose (col. iii) of T.D. androsterone.

Adrenals and liver. In contrast to the effects described above, the injections brought about a return to the normal weight in the case of the hypertrophied "castration" adrenals and the decreased "castration" liver, the effect increasing with the larger doses. Whilst the addition of oestrone did not definitely influence the changes in weight produced in the adrenals, it appeared to prevent the hypertrophy of the liver, even in some cases causing a decrease in the actual weight. The data per unit of body weight however show that this failure of the liver to hypertrophy must be partly explained by the smaller liver observed in animals with a smaller body weight, the smaller body weight in this case being caused by the injection of oestrone.

Other organs, fat deposition and body weight. Castration produces hypertrophy of the hypophysis (col. ii) and a slight decrease in the weight of the kidneys and heart (col. ii). With perhaps the exception of some effect on the heart, the injections did not bring about a return to the normal weight in the case of these organs. The changes in the weight of the heart however were very small and are only mentioned because they are in agreement with the results obtained with the other male hormones [Korenchevsky *et al.* 1935; 1936].

No changes were observed in the thyroids, spleen, fat deposition and body weight after the injections; excepting in the rats injected simultaneously with oestrone and T.D. androsterone, in which the body weight, gain in body weight and fat deposition were less than in the rats injected with T.D. androsterone alone. This is a typical effect of oestrone as was shown in our previous papers.

III. *Prolonged injections of T.D. androsterone, alone or simultaneously with oestrone into female spayed rats.*

The experiment was performed on 61 rats belonging to 13 litters, ovariectomized before sexual maturity and killed at ages varying from 61 to 77 days. The injections were begun from 14 to 34 days after ovariectomy and continued daily for 21 days. The data obtained and other details of the experiments are summarized in Table III. In the normal females the weights of the uteri of only those rats which were in dioestrus were used for the average.

The sexual organs. While not large, the stimulating effect of T.D. androsterone on the uterus and vagina was quite definite (cols. iv, v, vi), though less than that of oestrone (col. iii). Only in some cases was the addition of oestrone (cols. vii-ix) followed by a slight increase in the degree of hypertrophy observed with oestrone alone (col. iii).

In contrast to this small effect of T.D. androsterone on the uterus and vagina is the high degree of hypertrophy of the female preputial glands which exceeded even the normal size whilst the addition of oestrone did not have any effect on the weight of these glands.

Thymus. The rate of involution of the thymus was definitely increased by the injection of T.D. androsterone but was less than in the rats injected with oestrone alone or simultaneously with T.D. androsterone.

Adrenals and liver. As in castrated males, injections of T.D. androsterone (cols. iv-vi) were followed by a decrease in the weight of the adrenals and an increase in the weight of the liver. When oestrone was injected simultaneously these effects decreased in degree or disappeared entirely.

Table III. *Effect of prolonged injection of sexual hormones on female rats.*

The average actual weights of the organs of spayed rats injected with sexual hormones as compared with those of the organs of normal or spayed control rats injected with oil only.

	i	ii	iii	iv	v	vi	vii	viii	ix
	Spayed rats injected with								
				T.D. androsterone			Oestrone	Oestrone	Oestrone
							6γ + T.D. andro- sterone	6γ + T.D. andro- sterone	6γ + T.D. andro- sterone
Organ	Normal control rats	Spayed control rats	Oestrone 6γ	1 mg.	2 mg.	4 mg.	1 mg.	2 mg.	4 mg.
Uterus (mg.) (dioestrus)	359	33	123	56	57	69	141	140	110
Cervix of uterus (mg.)	103	13	50	22	22	30	57	53	47
Vagina (mg.)	241	130	186	143	152	163	206	200	204
Female preputial glands (mg.)	116	76	78	190	176	146	141	158	216
Adrenals (mg.)	62	80	79	74	62	55	71	73	70
Thyroids (mg.)	16	19	18	21	22	22	20	18	18
Hypophysis (mg.)	13.3	12.3	12.9	12.2	12.2	11.8	13.6	12.4	12.1
Thymus (mg.)	413	687	499	519	564	549	477	465	471
Liver (g.)	8.2	9.0	7.4	10.0	10.5	11.1	8.5	8.6	9.8
Kidney (g.)	1.40	1.59	1.53	1.53	1.56	1.54	1.59	1.51	1.52
Heart (mg.)	628	694	619	677	687	713	635	644	647
Fat (g.)	7	7	6	7	7	8	5	5	6
Average final body weight (g.)	181	218	189	213	208	210	191	182	206
Gain in body weight (g.)	59	89	40	85	71	82	51	33	56
No. of rats in group	8	13	11	4	6	4	5	6	4

Thyroids. When calculated per unit of body weight, no increase in the weight of the thyroids was seen after ovariectomy, whilst after T.D. androsterone injections there was a slight increase both in the actual weight and in the weight per unit of body weight of these glands. The increase however was small and therefore until the histological examination is completed the significance of this increase cannot be ascertained.

Other organs, fat deposition and body weight. No definite changes in the weight of the hypophysis, kidneys and heart or in the fat deposition and body weight were observed after the injection of T.D. androsterone, whilst injections of oestrone alone (col. iii) or simultaneously with T.D. androsterone (cols. vii-ix) caused a decrease in the final body weight, the gain in body weight and the fat deposition as compared with spayed control rats (col. ii).

DISCUSSION.

Before the histological investigation is completed, it is not possible to make a final evaluation of the results obtained, more especially as regards the question whether the return to or towards the normal weight is accompanied by the restoration of the normal histological picture.

Several points however are clearly demonstrated in the results obtained.

Male rats.

The rat unit. The rat unit (as defined by Korenchevsky) is contained in 8 γ of testosterone, 21 γ of androsteronediol, 170 γ androsterone and 940 γ of *trans*-dehydroandrosterone. Thus from the point of view of the smallest active dose T.D. androsterone is a very weak sexual hormone.

The qualitative effect on the sexual organs. The prolonged experiments confirm the low activity of this hormone on the sexual organs. It was shown in our previous paper (see summarizing Table VII, Korenchevsky *et al.* 1936, p. 572) that the ratio ($\times 100$) of the percentage increase in the weight of the prostate to that of the seminal vesicles observed after injections of the hormone indicates how nearly the changes approximate to the normal development of the sexual organs. To show this, a comparison is made with the ratio obtained during the normal growth of the organs in normal rats and after the injection of gonadotropic hormone into normal infantile rats.

It is seen from Table IV that in the short-period injections (cols. i, ii) this ratio for T.D. androsterone was on the average 59 (col. ii), which figure is close to those obtained with androsteronediol (64) and testosterone (51), but considerably different from that obtained with androsterone (106). The normal ratio

Table IV. *Ratio ($\times 100$) of percentage increase in actual weight of prostate to that of seminal vesicles after injection of transdehydroandrosterone compared with this ratio obtained with other hormones or observed during natural growth of the organs in normal rats.*

	i	ii	iii	iv
	Short-period injections		Long-period injections	
	Daily dose γ	Ratio prostate to seminal vesicle	Daily dose γ	Ratio prostate to seminal vesicle
<i>trans</i> Dehydroandrosterone	800	54	1000	70
	1000	67	2000	81
	2000	62	3000	51
	4000	54	—	—
<i>trans</i> Dehydroandrosterone	Average	59	1000–2000	76
	—	—	3000	51
Androsterone	Average	106	450–2200	126
	—	—	3600 or more	55
Androsteronediol	Average	64	175–350	54
	—	—	700 or more	24
Testosterone	Average	51	33–167	37
	—	—	500–1000	29
Gonadotropic hormone	Average	54	—	—
Normal development in normal rats	—	—	—	25

for comparison in these short-duration experiments is given by the gonadotropic hormone which in a few days forces the development of the prostate and seminal vesicles of normal infantile animals. This ratio is 54 (col. ii). In short-duration experiments therefore T.D. androsterone as well as androsteronediol and testosterone (but not androsterone) give a ratio approximating to the normal.

For comparison of the results of the long-period injections (cols. iii, iv) the normal ratio should be taken as that which is obtained from the normal development of the prostate and seminal vesicles in normal rats (= 25, see col. iv).

Usually injections of the higher doses of the hormones give a closer approximation to this normal standard [Korenchevsky *et al.* 1936]. It can be seen from Table IV (cols. iii, iv) that this ratio for the high doses of T.D. androsterone (51) and androsterone (55) is very different from the normal ratio (25), whilst for androsteronediol (24) and for testosterone (29) it approximates closely to the normal ratio. In general, however, taking into consideration both the short and long experiments, the development of the prostate and seminal vesicles obtained is qualitatively closer to the normal with T.D. androsterone than with androsterone, though this latter hormone is quantitatively the more potent of the two.

Although even the largest doses of T.D. androsterone used did not bring about any considerable increase in the weights of the seminal vesicles, prostate or penis, the weight of the preputial glands became normal or nearly normal.

The effect on other organs. The weights of the thymus, adrenals, liver, kidneys and heart, which change after castration, were restored to or towards the normal weight by androsterone, by androsteronediol and by testosterone. The decreased gain in body weight of castrated rats was also improved by each of these three hormones [Korenchevsky *et al.* 1935; 1936]. T.D. androsterone produced a similar effect in the case of the adrenals and liver, but the action on the thymus and heart was much weaker and in some cases absent altogether. The weight of the kidneys and the gain in body weight also showed no definite change with T.D. androsterone.

Female rats.

The sexual organs. The effect of T.D. androsterone on the uterus and vagina was greater than that of androsterone [Korenchevsky *et al.* 1935, Table V, p. 2543], but less than that of androsteronediol (*ibid.*) or of testosterone [Korenchevsky *et al.* 1936, p. 567]. Each of the four hormones brought about hypertrophy of the female preputial glands to a weight and size greater than normal. All these four male hormones, therefore, undoubtedly have some of the properties typical of the female hormones.

The possible significance of the preputial glands in a differential assay of the male and female hormones. In spite of the sensitivity to male hormones of the preputial glands of both sexes, they are not suitable for the assay of male hormones since the variation in the weight of these glands both in uninjected and in injected rats is high, and can only be compensated by using a large number of animals. However, since the weight of these glands in both sexes is unaffected by oestrone or oestradiol [Korenchevsky *et al.* 1936], this difference may be helpful as an extra indication of the degree or absence of contamination of the female hormone preparations with male hormones.

Adrenals. Owing to the greater irregularity in the weights of the adrenals in female than in male rats, we are still unable to say definitely how frequently after ovariectomy these glands hypertrophy. While we intend in our next experiments to collect more data and to investigate this question histologically, at present we can only say that in our experiments ovariectomy was often followed by an increase in the weight of the adrenals, as can be seen, for example, in Table III (cols. i, ii). The injection of T.D. androsterone (cols. iv-vi) decreased this hypertrophy and with the large dose the normal weight was restored. This decrease in the size and weight of the adrenals was also seen after the injection of androsterone, of androsteronediol and (taking into consideration our hitherto unpublished results with testosterone) of testosterone. With respect to this effect of testosterone, the statement in our previous paper as to the absence of effect of this hormone on the weight of the adrenals [Korenchevsky *et al.* 1936, p. 573] made provisionally (as only a few rats were used) must be corrected. Thus, in

both gonadectomized males and females, the weight and size of the adrenals are decreased by male hormones.

Liver. In both our published [Korenchevsky *et al.* 1936] and unpublished experiments with testosterone, no effect was seen on the weight of the liver, while with androsterone, androsteronediol and with T.D. androsterone (present experiments) there was a marked increase in the weight of the liver of ovariectomized rats.

Other organs and gain in weight. Except for a slight increase in the rate of involution of the thymus no definite influence of T.D. androsterone was observed on the other organs, fat deposition or gain in body weight. In contradistinction to this, androsterone, androsteronediol and testosterone caused a greater increase in the rate of involution of the thymus, slightly decreased the weight of the hypophysis and (androsterone and androsteronediol) increased the weight of the kidneys and gain in body weight [Korenchevsky *et al.* 1935; 1936].

The co-operative activity of T.D. androsterone and oestrone on males and females. In the case of males this activity was found to be very slight and in the females, unless shown on histological investigation, is doubtful. With androsterone, androsteronediol or testosterone on the other hand this co-operation with oestrone was definite [Korenchevsky *et al.* 1935; 1936].

SUMMARY.

1. Experiments were performed on 7 normal and 78 castrated males and 8 normal and 61 ovariectomized females, in order to investigate the effect on the weight of various organs of Ruzicka's artificial *trans*dehydroandrosterone, when injected alone or simultaneously with oestrone.

2. Korenchevsky's rat unit of T.D. androsterone was found to be contained in about 940 γ .

3. The restorative effect of T.D. androsterone on the atrophied sexual organs of male and female gonadectomized rats was definite, but (except in the case of the preputial glands) small. The co-operative effect with oestrone was only slight (males) or doubtful (females).

4. Thus T.D. androsterone showed some of the properties typical of the female hormones.

5. The response of the preputial glands in both sexes to all the male hormones (so far investigated by us) and the lack of such a response to oestrone or oestradiol can be used as an additional check in the differential assay of male and female sexual hormone preparations.

6. T.D. androsterone caused a decrease in the weight of the adrenals, slightly increased the rate of involution of the thymus and increased the weight of the liver in both male and female gonadectomized rats.

7. The similarities and differences in the biological properties of T.D. androsterone and the other male hormones as investigated by us were discussed.

8. The ratio of the percentage increase in the weight of the prostate to that of the seminal vesicles after the injection of T.D. androsterone was found to be similar to that obtained with gonadotropic hormone, but different from that observed during the normal development of normal rats.

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REFERENCES.

- Butenandt & Dannenbaum (1934). *Hoppe-Seyl. Z.* **229**, 192.
—— Hanisch & Kudzusz (1935). *Hoppe-Seyl. Z.* **237**, 57.
Deanesly & Parkes (1936, 1). *Biochem. J.* **30**, 291.
—— — (1936, 2). *Brit. med. J.* **i**, 257.
Häussler (1934). *Helv. chim. Acta*, **17**, 531.
Koch (1936). *J. Urol.* **35**, 382.
Korenchevsky (1935, 1). *Nature, Lond.*, **135**, 434.
—— (1935, 2). *Nature, Lond.*, **136**, 185.
—— (1936). *Nature, Lond.*, **137**, 494.
—— & Dennison (1934, 1). *Biochem. J.* **28**, 1474.
—— — (1934, 2). *Biochem. J.* **28**, 1486.
—— — (1935, 1). *Biochem. J.* **29**, 1720.
—— — (1935, 2). *Biochem. J.* **29**, 2122.
—— — (1935, 3). *J. Path. Bact.* **41**, 323.
—— — (1935, 4). *Proc. R. Soc. Med.* **28**, 71.
—— — (1936). *J. Path. Bact.* **42**, 91.
—— & Levy Simpson (1935, 1). *Biochem. J.* **29**, 2131.
—— — (1935, 2). *Biochem. J.* **29**, 2522.
—— — (1935, 3). *Biochem. J.* **29**, 2534.
—— & Brovsin (1936). *Biochem. J.* **30**, 558.
Oppenauer (1935). *Nature, Lond.*, **135**, 1039.
Ruzicka, Fischer & Meyer (1935). *Helv. chim. Acta*, **18**, 1483.
—— & Wettstein (1935, 1). *Helv. chim. Acta*, **18**, 986.
—— — (1935, 2). *Helv. chim. Acta*, **18**, 1264.
Schoeller, Serini & Gehrke (1935). *Naturwissenschaften*, **23**, 337.
Tschopp (1936). *Arch. int. Pharmacodyn.* **52**, 381.