CCCI. THE PROLONGED TREATMENT OF MALE AND FEMALE RATS WITH ANDROSTERONE AND ITS DERIVATIVES, ALONE OR TOGETHER WITH OESTRONE.

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IN this paper the results are given of the prolonged administration of androsterone and its derivatives, prepared by Prof. Ruzicka. A short communication with regard to some of them has already been made [Korenchevsky, 1935, 1, 2]. The results of our assay experiments (7 days' injection of the hormones) have been given in previous papers in this Journal [Korenchevsky and Dennison, 1935, 1, 2; Korenchevsky *et al.*, 1935, 1].

Callow and Deanesly [1935, 1, 2] studied some effects of fat-soluble androsterone and of its diol derivative on the seminal vesicles and prostate of rodents, using the method of quantitative investigation, the principle of which we are advocating. They confirmed our first experiments [Korenchevsky, 1935, 1], in which androsterone injections failed to bring about a complete return to the normal condition of the already atrophied secondary sexual organs of castrated rats ("recovery" experiments). In contrast to this result Callow and Deanesly were able to maintain ("maintenance" experiments) the seminal vesicles and prostate of castrated rats in a normal condition if the androsterone injections were started from the day of castration. They only studied this effect for 14 days however.

They also confirmed the results given from Laqueur's laboratory and the observations made by us and by some other workers [see references, Callow and Deanesly, 1935, 1, p. 1443], that there might be a significant difference between the action on rats and that on capons of some urine concentrates of the hormone of the same "capon" titre. Callow and Deanesly found the same difference between androsterone and some urine concentrates.

These and similar facts were explained by various workers by the existence of more than one sexual hormone, which fact has now been proved by the isolation of a new hormone from urine (dehydroandrosterone) by Butenandt and Dannenbaum [1934] and another from testes (testosterone) by Laqueur and his co-workers [David, 1935; David *et al.*, 1935; Laqueur *et al.*, 1935]. Ruzicka and his co-workers also continue their most important work of preparing new "artificial male sexual hormones". Among these synthetic testosterone is an important addition to their collection [Ruzicka and Wettstein, 1935]; according to Tschopp [1935, 2; Ruzicka and Wettstein, 1935] it stimulates the development of seminal vesicles and penis to a greater extent than is done by the diol (i.c.v. = about 10%).

Callow and Deanesly [1935, 2], Tschopp [1935, 1, 2] and Laqueur *et al.* [1935] found also that the diol was more active than androsterone in its effect on the seminal vesicles. This same fact and the action on the penis and preputial glands were clearly demonstrated in our assay experiments and by the curves we gave of the action on the sexual organs of these fat-soluble and water-soluble hormone

preparations [Korenchevsky and Dennison, 1935, 1, 2; Korenchevsky *et al.*, 1935, 1]. We also showed in these papers other important points of similarity and dissimilarity between the effects of androsterone and those of the diol, estimated rat units of these substances and demonstrated a considerable difference between the rat and capon units of some preparations.

The present long-duration experiments were carried out with the following fat- (f.s.) or water-soluble (w.s.) preparations: (1) crystalline androsterone (hereinafter designated "f.s. androsterone"), (2) the lithium salt of androsterone hydrogen succinate (designated "w.s. androsterone"), (3) crystalline androsterone-diol (designated "f.s. diol"), and (4) the lithium salt of the androsterone-diol hydrogen succinate (designated "w.s. diol"). All the substances were received from Prof. Ruzicka for the investigation of their biological properties.

MALE RATS.

Technique.

Experiments were performed on 19 old normal rats belonging to 5 litters and on 112 castrated rats belonging to 20 litters, which latter were divided for 21 experiments as shown in Table I. Two or three rats of each litter were usually used in each group of the experiment.

Table I. *Effect on sexual organs of male rats.*

Average actual weights of organs of castrated rats injected with androsterone or its derivatives as compared with those of control normal litter-mates or normal rats of similar age.

No. of expts.	Preparation and daily dose injected mg.	Injections		Seminal vesicles mg.		Prostate (mg.)		Penis (mg.)		Preputial glands (mg.)		Final age days
		Duration in days	Started after castration	Normal rat	Injected	Normal rat	Injected	Normal rat	Injected	Normal rat	Injected	
f.s. androsterone:												
1	0.5	47	3	565	39	750	237	340	214	150	119	77
2	1.0	47	3	565	64	750	452	340	262	150	243	77
3	1.8-2.2	21	2	218	81	450	504	292	218	98	143	49
4	0.9-3.6	53	4	640	234	1049	826	363	339	153	135	83
5	0.45	22	84	(1118)	41	(975)	210	(441)	209	(175)	115	132
6	0.9	22	78	(1118)	66	(975)	359	(441)	258	(175)	127	149
7	1.8	22	38	775	73	845	450	363	295	153	135	88
8	3.6	17	32	495	300	710	643	330	277	140	233	74
9	3.6	21	20	590	250	654	849	308	280	121	126	64
w.s. androsterone:												
10	13.6-27.2	27	39	834	392	1007	806	363	274	153	177	91
w.s. diol:												
11	1.46	21	2	218	355	450	707	292	339	98	124	49
12	2.93	21	2	218	677	450	816	292	307	98	152	49
f.s. diol:												
13	0.18	45	4	495	55	710	198	330	290	140	129	75
14	0.18-0.7	59	4	775	423	845	614	363	426	153	209	89
15	0.35	21	2	218	{ 174 (229)	450	{ 445 (483)	292	{ 269 (289)	98	{ 154 (173)	49
16	0.18-0.35	53	4	640	237	1049	561	363	407	153	100	83
17	0.7	20	20	590	363	654	616	308	293	121	162	64
18	0.7	32	32	775	797	845	1081	363	369	153	211	88
w.s. diol:												
19	3.54	21	39	683	641	807	653	363	324	153	167	85
20	3.54-5.31	27	39	834	791	1007	984	363	357	153	216	91
21	5.31	27	39	834	862	1007	1094	363	380	153	201	91

Note. In Exps. 5 and 6 in brackets weight of organs of normal rats aged 123 days; in Exp. 15 in brackets a larger weight obtained in one of the litter-mates.

The general technique was the same as that described in our previous papers, with the difference that all the rats were killed by bleeding instead of by gas. For this reason we were unfortunately unable to use the values of the weights of the organs of normal rats given in our previous paper [Korenchevsky and Dennison, 1934, 1] since in that paper an investigation was made of the weights of the organs of rats killed by gas (so that blood remained in the organs). Since both normal and castrated rats were wanted in the control groups of each litter, it was possible to have both these two control groups only in the large litters. In most of the experiments therefore the weights of the organs of the normal un-injected rats were taken from the averages of the weights of the organs of rats of other litters killed at the same age. For the final ages 74, 75 and 77 days the weights of the sexual organs of normal rats were interpolated from the weights of the organs of normal rats of the nearest ages above and below these. In these cases the differences between the weights of normal organs and those of the injected rats are so large that incorrect conclusions are impossible. No normal rats of the ages 132 days (Exp. 5) and 149 days (Exp. 6) were available, so that the weights of the organs of the litters nearest in age, which were 123 days old, are given in brackets. These values in any case may be assumed to be less than those for rats 132 and 149 days old. Thus, with the exception of Exps. 5 and 6, all the values for the weights of normal seminal vesicles and prostates were calculated from the organs of normal rats of the same age.

With regard to the other organs within a period of 8–10 days there was little difference between the averages, whilst the individual differences were larger than in the case of the prostate and seminal vesicles. Therefore two general averages were calculated for the weights of the normal organs (except the seminal vesicles and prostate), one for the ages 62–70 days and one for the ages 83–91 days. The total number of normal rats used for all these averages was 57.

The hormone preparations were dissolved in the same way as in our previous experiments [Korenchevsky and Dennison, 1935, 1, 2; Korenchevsky *et al.*, 1935, 1]. The daily dose of the fat-soluble preparations was injected subcutaneously in two half-doses, one at about 10 a.m. and the second at about 5.30 p.m. the controls being injected with the same amount of pure oil; the water-soluble preparations were injected in three equal portions at about 9.30 a.m., 3 p.m. and 9.30 p.m.

The daily dose, the duration of the period of injection, the number of days after castration that the injections were started and the final age of the rats are given in Table I.

As in all our previous experiments all the organs were weighed after fixation in Bouin's fixative, except the hypophysis, which was fixed in 4% formalin-saline solution. This latter was necessary for the successful histological staining of this gland by a special modification of Mallory's method [Crooke and Korenchevsky, 1935]. After weighing, all the organs were embedded in paraffin for histological investigation, the detailed results of which will be given elsewhere.

*The effect of the male hormone preparations on
the secondary sexual organs.*

The average data of the results of these experiments are summarised in Table I. In order to economise space the weights of the organs of the uninjected control castrated rats are not given since these have already been given in several of our previous papers. At the same time it is important to show how near it is possible

to bring the atrophied sexual organs to the normal condition by the prolonged injection of different doses of the hormone. Therefore in Table I only the weights of the organs of normal control rats or litter-mates are given.

Maintenance experiments on males. *Androsterone* was used for the injections in Exps. 1-4 in doses of 0.5 to 3.6 mg. per day (Table I) for a period of 21 days (Exp. 3) to 47-53 days (Exps. 1, 2 and 4). The injections were begun 2-4 days after castration. A comparatively long period of injection was chosen as experiments of too short duration (7-14 days) do not show the continuance of the "maintenance" action.

As can be seen from the table, the normal average weights of the seminal vesicles at the respective ages were not attained by this means nor were even the lowest individual weights of the vesicles for the respective ages reached. With the prostate, however, after the injection of large doses (1.8-3.6 mg. per day) the weight of this organ even exceeded the average weight in Exp. 3, but not in Exp. 4, which was of much longer duration and in which the average normal weight of the prostate was not reached, though the lowest variation of 675 mg. observed for this age was exceeded even in this experiment. It is probable that the difference between Exps. 3 and 4 is explained by the fact that in Exp. 3 large doses were used from the beginning of the experiment, whilst in Exp. 4, 0.9 mg. was injected for the first 26 days, 1.8 mg. for the following 19 days and 3.6 mg. for the last 8 days. The doses used failed to maintain the normal weight of the penis, whilst the preputial glands in Exps. 2 and 3 reached and even exceeded the normal averages.

Androsterone-diols, if used in large doses (Exps. 11 and 12, 1.46-2.93 mg. of water-soluble preparation), not only maintained the normal weight of the secondary organs but (Exp. 12) increased these by about three times the normal weight in the case of the seminal vesicles and by about twice the normal weight in the case of the prostate, though to a smaller degree in the case of the penis and preputial glands. When a dose of 0.35 mg. of f.s. diol was injected from the beginning of the experiment (Exp. 15) the normal weight of the sexual organs was maintained only in some of the injected rats. No "maintenance" experiments were made with larger doses of f.s. diol and smaller doses failed to maintain the normal weights of the sexual organs (Exps. 13 and 14).

"Recovery" experiments on males. *Androsterone* failed to cause a complete return of the seminal vesicles to the normal average weight, the highest doses used being 3.6 mg. of f.s. androsterone and 13.6-27.2 mg. of w.s. androsterone.

The recovery of the prostate occurred only in Exp. 9, in which even the normal average weight was exceeded and in Exps. 8 and 10, in which, in the rats injected with the high doses given above, the lower variation in the normal weight of the prostate was reached.

With regard to the penis the normal average weight was in no case reached, whilst that of the preputial glands became normal or even exceeded normal (Exps. 8, 9, 10).

f.s. Diol, when injected in daily doses of 0.7 mg. for the long period of 32 days (Exp. 18) but not when injected for only 20 days (Exp. 17) caused a return of all the sexual organs to the normal weight, the weights even slightly exceeding the normal average weight. This also occurred with the dose 5.3 mg. of the w.s. preparation (Exp. 21). With smaller doses of w.s. diol (Exps. 19 and 20), the weights of the prostate and seminal vesicles were less than the normal averages, but in Exp. 20, in which the dose was higher and the period of injection longer than in Exp. 19 (Exp. 20-27 days; Exp. 19-21 days), the weights of the seminal vesicles and prostate even exceeded the lower variation in the normal weights.

It is interesting to note that in all recovery experiments the weights of the preputial glands were greater than the normal average weights.

Unlike androsterone, the diol causes a return of all the secondary sexual organs (including seminal vesicles) to a normal condition and a normal ratio of their weights.

The effect on the body weight, fat deposition, the endocrine and some other organs of male rats.

In Table II a summary is given of the data referring to the organs which were found to show a change in weight.

Table II. *Influence on organs of male rats.*

Average actual weights of adrenals, hypophysis, thymus, liver, kidney and heart in the injected rats as compared with those of control castrated litter-mates or normal rats of similar age.

Organs	Group of rats	Recovery								Maintenance			
		Androsterone (mg.)					Diol (mg.)			Androst. (mg.) 1.8-3.6	Diol (mg.)		
		Fat-soluble					Water-soluble 13.6-27.2	Fat-soluble			Water-soluble 3.5-5.3	Fat-soluble 0.17-0.7	Water-soluble 1.5-2.9
		0.45	0.9	1.8	3.6	3.6		0.7	0.7				
Adrenals (mg.)	Castrated	65	76	107	94	59	88	107	—	88	70	81	55
	Castrated injected	55	56	65	52	40	44	55	43	56	46	52	42
	Normal	—	—	52	47	46	52	52	46	52	—	55	50
Hypophysis (mg.)	Castrated	15.0	14.2	12.4	12.4	—	14.6	12.4	—	14.6	16.4	18.0	9.0
	Castrated injected	15.2	17.1	11.9	11.6	8.6	11.0	10.4	9.4	10.5	14.1	13.5	6.9
	Normal	—	—	9.4	—	8.9	9.4	9.4	8.9	9.4	—	—	9.0
Thymus (mg.)	Castrated	319	332	558	558	—	605	558	690	605	554	612	726
	Castrated injected	291	238	442	319	408	297	291	350	237	444	378	352
	Normal	—	—	401	—	475	401	401	475	401	—	373	599
Liver (g.)	Castrated	10.4	9.4	9.9	9.9	—	10.1	9.9	—	10.1	8.2	9.8	7.5
	Castrated injected	11.4	11.0	12.5	11.9	9.9	10.8	12.4	8.9	10.4	9.8	11.2	8.5
	Normal	—	—	11.3	—	10.4	11.3	11.3	10.4	11.3	—	11.3	10.6
Kidney (g.)	Castrated	1.74	1.72	1.76	1.76	—	1.73	1.76	—	1.73	1.55	1.94	1.37
	Castrated injected	2.00	1.99	2.07	1.96	1.95	1.91	1.97	1.59	1.99	1.74	2.17	1.77
	Normal	—	—	2.00	—	1.83	2.00	2.00	1.83	2.00	—	2.29	1.66
Heart (mg.)	Castrated	815	774	671	671	—	789	671	—	789	566	969	618
	Castrated injected	868	877	848	759	684	861	830	650	858	585	1000	649
	Normal	—	—	823	—	761	823	823	761	823	—	988	708

In order to economise space, the weights of the organs of litters injected with different doses were averaged together in some cases (last 4 columns of Table), since there was little difference between the changes obtained irrespective of the dose in these cases. In this table, the weights of the organs of injected castrated rats are compared with those of uninjected castrated rats and with the average weights of those of normal rats. The dashes in the table indicate that no normal rats of the corresponding age (74, 77, 132 and 149 days old) were available (see technique).

Adrenals. The weights of the glands of injected castrated rats showed in all cases a considerable decrease when compared with those of uninjected castrated rats. A comparison with the normal values shows that with all doses the injections caused the hypertrophied adrenals of the castrated rats to return to the normal weight and size. If general averages are made from the figures given in

Table II and from the corresponding figures calculated per 200 g. of body weight, it will be seen that the weights of the organs of the injected rats were identical with the normal weights:

	Weights (mg.)	
	Actual	Per 200 g. body weight
Castrated uninjected rats	81	62
Castrated injected rats	51	37
Normal uninjected rats	50	38

Hypophysis. In contrast to the complete return to normal weight after the injection of the hormones in the case of the adrenals, only large doses of f.s. androsterone (1.8–3.6 mg.), of f.s. diol (0.7 mg.) and of w.s. preparations caused some degree of decrease in the hypertrophy of hypophysis in the majority of the rats. In three experiments however (one, in which 3.6 mg. of androsterone were injected, and two maintenance experiments in which w.s. diol was used) the weight and size of the hypophysis were reduced to the normal (or even less than normal) average figure.

Using the data in Table II and those calculated per unit of body weight, the general averages of the weights of the hypophysis for castrated, castrated injected and normal uninjected rats are 13.9, 11.7 and 9.2 mg. (actual weights); and 10.2, 8.5 and 7.1 mg. (per 200 g. of body weight) respectively.

The histological investigation of all hypophyses, which is being carried out jointly with Dr A. C. Crooke, has not yet been completed for the large doses, including those cases in which the weight of the hypophysis returned to normal.

In agreement with our preliminary communications [Korenchevsky, 1935, 1; Crooke and Korenchevsky, 1935] it is possible to say that doses lower than 1.8 mg. of androsterone produced no histological effect. However, doses larger than 1.8 mg. androsterone and 0.35 mg. of diol produced a partial return to the normal histological condition, namely a reduction in the number and size of the "signet cells", which are specific for the "castration" hypophysis. A detailed description will be given elsewhere.

Thymus. In castrated rats, the involution of the thymus is greatly delayed. As can be seen from Table II, the rate of involution of the thymus was increased after the injection of all doses of androsterone and diol, exceeding in most cases the normal average value for the respective age. Thus, making general averages from the data of Table II and calculating these per unit of body weight, general average weights are obtained for the weights of the thymus of castrated, castrated injected and normal uninjected rats of 556, 337 and 441 mg. (actual weights), and 411, 256 and 345 mg. (per 200 g. of body weight) respectively.

Liver. We have found that the liver of castrated rats is smaller than that of normal rats [Korenchevsky and Dennison, 1934, 1]. This is also clearly seen in Table II by comparing the weight of the liver in normal rats with that in castrated uninjected rats.

Injections of f.s. preparations of androsterone and diol caused a return of the weight of "castration" liver to or towards the normal weight. With w.s. preparations, however, this effect was not so clearly seen. This is due to the fact that w.s. preparations decreased the appetite of the rats thus causing a decrease in the weight of the liver, the weight of which is always influenced by the amount of food consumed. The general average weights of the livers of castrated, castrated injected and normal uninjected rats are: 9.5, 10.7 and 11.0 g. (actual weights) and 6.9, 8.0 and 8.2 g. (per 200 g. of body weight) respectively.

Kidney. This organ is also slightly smaller in castrated rats [Korenchevsky and Dennison, 1934, 1] which fact is seen again in the present experiments, when a comparison is made of the figures given in Table II for normal and castrated rats. As in the case of the liver, injections of androsterone or of diol restored this atrophic condition of the kidneys of castrated rats to normal (except in one litter). The general averages for the weights of the kidneys of castrated, castrated injected and normal uninjected rats were: 1.71, 1.93 and 1.95 g. (actual weights), and 1.26, 1.44 and 1.46 g. (per 200 g. of body weight) respectively.

Heart. There is also a slight decrease in the weight of the heart in castrated rats [Korenchevsky and Dennison, 1934, 1] which fact is confirmed by the data of the present experiments (Table II). Injections of androsterone and of diol in most cases restored the normal condition. The general averages of the weights of the hearts of castrated, castrated injected and normal uninjected rats are 733, 789 and 814 mg. (actual weights) and 548, 600 and 602 mg. (per 200 g. of body weight) respectively.

The gain in body weight and the deposition of fat. As compared with the uninjected control castrated litter-mates the gain in body weight was greater in the rats injected with f.s. preparations of androsterone and diol (by 11–74 %, on the average 40 %), whilst the deposition of fat did not seem to be influenced in any definite way. On the other hand, the increase in the gain in body weight was very much less with w.s. preparations (not more than 9 %) and in most cases was even less in the injected rats than in the uninjected castrated litter-mates (by 11–28 %). The deposition of fat was also less than in the control rats in most cases (by 4–49 %). These facts, as with the liver, are probably explained by the reduced appetite due to injections of the w.s. preparations in the large doses used, which seem to be toxic.

Thyroids and spleen. The changes in weight of these two organs were not constant. Until the histological investigation has been completed it is impossible to come to any definite conclusion with regard to the changes in the thyroid, though from our previous results [Korenchevsky and Dennison, 1934, 1] and our preliminary experiments with androsterone definite changes were expected.

The effect on the organs of males of simultaneous injections of male and female sexual hormones.

Experiments were performed on two litters, one containing 8 and the other 4 rats. In the first litter 2 rats were left uninjected as a control group, 3 rats were injected with androsterone alone and 3 rats were injected with androsterone and oestrone simultaneously. In the second litter 2 rats were injected with androsterone and 2 with androsterone and oestrone. The results obtained were so regular that a conclusion can be drawn. The rats were castrated at 27 days old. On the fourth day after castration daily injections with androsterone were started and three times a week oestrone was injected into those rats receiving both hormones. The injections were continued for 47 days and the rats were killed at 77 days old. The changes obtained in this "maintenance" experiment are summarised in Table III.

It can be seen from the table, that in the rats injected simultaneously with both hormones (1) the prostate and the seminal vesicles were much larger than in those injected with androsterone alone; (2) the percentage increase in the prostate was nearly equal to (Exp. 2) or larger than (Exp. 1) that of the seminal vesicles and (3) with the smaller dose of androsterone the stimulating action of oestrone was much more pronounced. This last effect may be explained by the fact that an increase in the dose of androsterone has a much greater stimulating

Table III. *Effect on actual weight of organs of simultaneous injection of androsterone and oestrone on male rats.*

Organs	Groups injected with:	Average weight of organs (mg.)		% change in weight of organs	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
Seminal vesicles	Androsterone	39	64	—	—
	Androsterone + oestrone	68	87	{ + 74 { (+106)	{ +36 { (+57)
Prostate	Androsterone	237	452	—	—
	Androsterone + oestrone	517	637	{ +118 { (+164)	{ +41 { (+62)
Adrenals	Androsterone	48	41	—	—
	Androsterone + oestrone	65	55	{ + 35 { (+ 72)	{ +36 { (+55)
Hypophysis	Androsterone	13.8	12.1	—	—
	Androsterone + oestrone	15.0	14.7	{ + 9 { (+ 29)	{ +21 { (+39)
Thymus	Androsterone	745	465	—	—
	Androsterone + oestrone	527	363	{ - 29 { (- 16)	{ -22 { (-12)
Gain in body weight	Androsterone	183	188	—	—
	Androsterone + oestrone	143	156	- 22	-17

Note. In Exp. 1 the hormones were injected in the daily doses: androsterone 0.5 mg., oestrone 60 i.u.; in Exp. 2, androsterone 1 mg., oestrone 60 i.u. In brackets percentage change in weight of organs per unit of body weight.

effect on the sexual organs than the simultaneous administration of oestrone. This is shown even in recovery Exps. 8 and 9 (Table I), in which large doses of androsterone after only 17–21 days' injection brought about an increase in the weight of the prostate about equal (Exp. 8) to that obtained in Exp. 2, Table III or above the normal weight (Exp. 9, Table I). By increasing the dose of androsterone in Exps. 8 and 9 much larger seminal vesicles also were obtained (250–300 mg.) than by addition of oestrone to androsterone in Exps. 1 and 2, Table III (68 and 87 mg.). The addition of oestrone to androsterone caused an increase in the weight of the adrenals, a slight increase in the weight of the hypophysis and a decrease in the weight of the thymus and the gain in body weight. The deposition of fat decreased slightly (by 26 % in one litter and by 8 % in the other). The changes in the adrenals and hypophysis and the gain in body weight must be considered pathological.

No considerable or constant changes were noted in the weights of the penis, preputial glands, thyroid, liver, kidneys, spleen or heart.

A striking difference is seen on comparing the results obtained in these experiments with those previously published [Korenchevsky and Dennison, 1934, 2] on the simultaneous injection of oestrone and testicular hormone preparations from urine. Whilst in the present experiments the effect of the addition of oestrone to androsterone was more marked in its action on the prostate, the addition of oestrone to the urinary hormone preparations had much more effect on the seminal vesicles than on the prostate. This is probably connected with the weaker reaction to androsterone shown by the seminal vesicles as compared with the much stronger reaction of the prostate. Points of similarity in the previous and present results are the decrease in the gain in body weight, the slight decrease in the deposition of fat, the stimulation of the rate of involution of the thymus and the slight increase in the weight of the adrenals and hypophysis (though the adrenals were slightly increased only in the experiments with the

urinary preparation "H" [Korenchevsky and Dennison, 1934, 2, Table V, p. 1491].

The variation in the "hypertrophic" effect of oestrone on the adrenals of castrated rats probably depends on the degree of conversion of the adrenals by castration into "castration" adrenals. As we have already shown [Korenchevsky and Dennison, 1935, 3] the effect of oestrone on the adrenals of normal rats is similar to, if not identical with that observed after castration. Therefore if castration has already produced the maximum effect on the adrenals, oestrone should have no further effect; but if the conversion of the adrenals by castration is not complete, the injection of oestrone will finish this change.

Does testicular hormone possess a so-called "rejuvenating" influence?

Androsterone was injected for 24 days into 12 approximately year-old normal male rats belonging to 5 litters, 7 litter-mates being left as the controls. These rats had already become lazy and obese with unclean fur and skin, not cleaning themselves properly from insects, and had lost the agility, restlessness and curiosity typical of healthy young and adult rats, whilst some had in addition chronic pneumonia which was revealed on dissection. Observation during life and examination of the organs after killing showed that androsterone in doses of 0.1 or 0.9 mg. had no favourable effect on these old injected rats as compared with their control uninjected litter-mates.

The changes obtained in the weights of the organs, the fat deposition and body weight were small and were not constant. The testes in most of the injected rats were on the average about 10% smaller. The results obtained were therefore similar to those previously obtained on normal adult rats injected with purified testicular hormone prepared from urine [Korenchevsky *et al.*, 1933].

The relation between the dose of androsterone and the effect on the sexual organs.

The number of experiments available is too small for drawing a definite curve. Moreover, the ages of the rats injected and the period of time after castration that the injections were started differed. However, for comparison Exps. 5, 6, 7 and 9 were chosen, since in these androsterone was injected for about the same period (21 or 22 days). From Table IV it can be seen that there

Table IV. *Relation between the dose of androsterone and percentage increase in weight (actual and per unit of body weight) of sexual organs of castrated male rats.*

Androsterone (mg.)	% increase in weight of organs							
	Seminal vesicles		Prostate		Penis		Preputial glands	
	Actual	Per unit of body weight	Actual	Per unit of body weight	Actual	Per unit of body weight	Actual	Per unit of body weight
0.45	228	221	222	223	106	130	114	116
0.9	404	403	476	506	124	111	166	179
1.8	461	400	800	724	204	183	181	160
3.6	1823	2060	1598	1887	226	281	163	214

appears to be a direct proportional relationship between the dose and the percentage increase in the case of the prostate, which is in agreement with our assay experiments [Korenchevsky and Dennison, 1935, 1]. Whilst the seminal vesicles show this same proportional relationship with the two smaller doses of 0.45 and 0.9 mg. and the highest dose of 3.6 mg., a lag occurred with the middle

dose of 1.8 mg. which produced an effect equal only to the half dose of 0.9 mg. We draw attention to this fact, since we found a similar flattening of the curve of seminal vesicles with the middle dose in our assay experiments of 7 days' duration [see Korenchevsky and Dennison, 1935, 1; Table VI, p. 1726].

The same approximate direct proportion between the dose and the effect is also seen (Table I) in the present Exp. 1 (0.5 mg. androsterone) and Exp. 2 (1.0 mg. androsterone). Injections were made for 47 days and the following percentage increases in the weights of the organs were obtained:

Androsterone (mg.)	Seminal vesicles		Prostate	
	Actual	Per 200 g.	Actual	Per 200 g.
0.5	179	175	248	234
1.0	357	309	565	500

FEMALE RATS.

Technique.

Injection experiments were performed on 18 litters containing 103 spayed rats and the effects of ovariectomy were studied in 4 litters containing 19 rats. Ovariectomy was performed at ages varying from 23 to 27 days, *i.e.* before sexual maturity. The number of rats used in each experiment, the final age, the number of days after ovariectomy that the injections were started and the period of injection in days are given in the last 4 lines of Tables V and VI. The dose of the hormone injected is given in the top line.

Table V. *Effect of sexual hormones on female rats.*

Average actual weights of organs of control rats and their litter-mates, injected with f.s. or w.s. preparations of androsterone, androsterone-diol and oestrone.

Organ	Group	Androsterone (mg.)			Diol (mg.)				Oestrone (i.u.)		
		f.s.			f.s.		w.s.				
		0.9	1.8	3.6	0.49	0.62	2.29	3.54	20	45	180
Uterus (mg.)	Control	33	33	34	40	33	33	33	36	34	26
	Injected	32	39	47	59	99	56	123	155	131	135
Vagina (mg.)	Control	115	125	94	125	81	81	81	119	88	131
	Injected	130	124	124	180	155	135	172	201	157	205
F. preputial glands (mg.)	Control	—	—	65	83	85	85	85	83	75	75
	Injected	—	—	274	244	198	191	148	68	56	50
Adrenals (mg.)	Control	84	82	60	73	88	88	88	73	74	78
	Injected	66	61	37	57	42	42	38	66	62	75
Hypophysis (mg.)	Control	13.4	13.3	12.0	14.7	13.5	12.0	12.0	13.2	12.4	12.1
	Injected	12.7	13.0	9.7	15.2	10.6	9.0	10.2	13.9	12.8	15.4
Thymus (mg.)	Control	411	409	551	474	607	607	607	382	579	372
	Injected	330	253	378	366	519	483	312	330	447	300
Liver (g.)	Control	8.06	8.27	8.42	6.68	8.74	8.74	8.74	7.91	8.58	8.48
	Injected	9.54	9.83	9.92	8.41	10.73	9.41	8.99	7.07	6.53	8.62
Kidney (g.)	Control	1.75	1.80	1.34	1.33	1.79	1.79	1.79	1.46	1.56	1.57
	Injected	1.94	1.93	1.83	1.60	2.00	2.11	1.84	1.43	1.36	1.72
Gain in body weight (g.)	Control	13	17	90	27	131	131	131	33	111	56
	Injected	28	38	106	45	127	125	102	10	73	26(−14)
Total no. of rats	...	8	4	4	9	5	4	4	20	9	10
Final age, days	...	118	131	63	105	63	63	63	118	62	118
Days after spaying	...	69	82	11	55	10	10	10	68	11	67
Period of injections, days	...	22	22	27	23	27	27	27	28	28	27

Table VI. *Effect on female rats of addition of testicular hormones to oestrone injections.*

Average actual weight of organs of oestrone-injected rats and their litter-mates injected simultaneously with oestrone and androsterone or androsterone-diol.

		Daily doses of oestrone (i.u.) or testicular hormone preparations (mg.)				
		Oestrone 20 Andro- sterone 1.88	Oestrone 100 Andro- sterone 3.6	Oestrone 20 Diol 0.36	Oestrone 100 Diol 0.57	Oestrone 180 Diol 0.35
Organs	Group of rats injected with:					
Uterus (mg.)	Oestrone	171	155	146	109	128
	Oestrone + testicular hormone	185	251	208	237	300
Vagina (mg.)	Oestrone	181	146	256	154	171
	Oestrone + testicular hormone	236	202	275	250	214
F. preputial gland (mg.)	Oestrone	65	56	68	62	—
	Oestrone + testicular hormone	275	149	184	134	—
Adrenals (mg.)	Oestrone	82	61	70	59	64
	Oestrone + testicular hormone	71	45	66	39	53
Hypophysis (mg.)	Oestrone	16.1	13.0	15.1	13.2	12.1
	Oestrone + testicular hormone	16.1	9.9	14.9	13.8	10.1
Thymus (mg.)	Oestrone	383	486	416	351	505
	Oestrone + testicular hormone	448	278	307	215	365
Liver (g.)	Oestrone	6.50	6.34	6.43	6.58	6.67
	Oestrone + testicular hormone	7.25	8.45	7.96	6.50	7.73
Kidney (g.)	Oestrone	1.26	1.26	1.35	1.37	1.44
	Oestrone + testicular hormone	1.51	1.72	1.48	1.57	1.70
Gain in body weight (g.)	Oestrone	45	50	8	74	83
	Oestrone + testicular hormone	61	90	60	71	92
Total no. of rats	...	6	5	4	6	5
Final age, days	...	75	64	103	61	62
Days after spaying	...	28	11	55	10	11
Period of injections, days	...	22	28	22	27	28

The technique of the experiments and the preparations of the hormones injected were the same as those used in the experiments on male rats.

The weights of the organs of normal female rats were not investigated. In two litters only, 2 litter-mates of each litter were kept as normal controls. In the tables therefore control rats mean uninjected spayed rats as compared with injected spayed rats.

In normal female rats the condition of several organs changes to some extent with the sexual cycle. It would therefore be necessary to make a preliminary detailed investigation of the effect of the sexual cycle on the weight of the organs of normal female rats, before experimenting on normal females.

Female preputial glands.

Under the skin at the exit of the urethra of the female rat are situated two flat club-shaped glands, which in their form and histological structure are homologues of the preputial glands of the male. We were unable to find a description of these glands in the previous literature and we have therefore named them "female preputial glands".

A detailed histological description will be given elsewhere.

The effects of ovariectomy.

For the reason just given we shall mention only briefly some definite results, which we obtained in experiments on 4 litters of females containing 19 rats, in which half the rats in each litter were spayed, the other half being left normal.

The litters were killed 33–88 days after ovariectomy at ages varying from 63 to 114 days. That the sexual organs atrophy after ovariectomy is already well known. In our rats the respective average percentage decreases in the weights of the uterus and of the vagina were 87 and 55 % (actual weights) and 89 and 56 % (per unit of body weight) as compared with those of their normal litter-mates. The female preputial glands which are homologous with the preputial glands of the male, were smaller in most rats by about 20–40 %. However, as in the case of the male glands, the individual weights of the female glands vary considerably. The involution of the thymus was delayed, so that in ovariectomised rats this organ was heavier than in the normal litter-mates (by about 40–70 %). The deposition of fat was increased in most of the rats (by about 24–77 %). Changes in the other organs were small.

The effect of sexual hormones on the organs of spayed females.

In the following series of experiments only one of the hormones (male or female) was injected into any rat. The results are summarised in Table V, in which, in order to economise space, only the actual weights of the organs are given and mention will be made of those cases, in which the percentage changes per unit of body weight differ considerably from these.

The sexual organs. Oestrone as is well known and as is shown in our experiments (Table V) causes a considerable return to the normal condition of the atrophied uterus and vagina. Our experiments showed that the female preputial glands remain unaffected or were even slightly decreased.

Whilst *androsterone* was found to have no considerable effect on the uterus and vagina, it caused a considerable hypertrophy of the preputial glands.

Diol, especially in the larger doses, had a considerable stimulating effect on all the sexual organs of spayed rats, uterus, vagina and preputial glands. With the largest dose (3.54 mg. w.s. diol, Table V) the effect approached that of oestrone, the condition of the sexual organs however being far from normal as is shown by the following average figures for the weights (mg.) of the uterus and vagina in one litter of rats:

	Uterus	Vagina
Normal uninjected rats	355	233
Spayed uninjected	33	81
Spayed injected with diol	123	172
Spayed injected with oestrone	155	146

In addition in some of the rats injected with diol a "female prostate" developed from the microscopic rudimentary periurethral glands. This reaction has already been mentioned [Korenchevsky, 1935, 2] and will be described in detail elsewhere.

Adrenals. Whilst the effect of oestrone on adrenals of spayed rats was slight or negligible, both androsterone and the diol caused a considerable, sometimes a very considerable decrease in the weight of these organs. Thus the effect of the male-hormone on the weight of the adrenals was the same in both castrated males and spayed females.

Hypophysis. Whilst oestrone had no effect on the weight of the hypophysis of spayed females, large doses of androsterone and diol were followed by a decrease in the weight of this organ.

Thymus. It is well known that involution of the thymus is delayed in spayed rats. The figures in Table V show that the female and male hormones caused a return to normal in the speed of involution of the thymus to about the same degree.

Liver and kidneys. The effects of androsterone and diol on the liver and kidneys of spayed females were similar to those observed on these organs in the case of castrated males. There was a definite increase both in the actual weight and in the weight per unit of body weight except with the highest dose of w.s. diol (3.54 mg. per day) with which dose the appetite decreased. As has already been mentioned, loss of appetite with this dose was also observed in males and resulted in a smaller liver weight. These increases in weight of liver and kidneys did not occur with oestrone injections, after which in the majority of rats the actual weight of the liver decreased, though this was less noticeable or even absent when the weights were calculated per unit of body weight. This is probably explained by the lower body weight of the oestrone-injected rats.

The gain in body weight and in the deposition of fat. The gain in body weight and the deposition of fat were increased slightly by the injection of androsterone and of small doses of diol. Large doses of diol did not improve the gain in weight (Table V) and with the largest doses there was even a small decrease in the gain in weight. The deposition of fat was decreased, on the average by about 50 % by the use of large doses of f.s. and w.s. diol.

Oestrone, in the doses used, in all cases slightly decreased the gain in body weight (Table V) and the deposition of fat (by about 17 %).

Thus, speaking generally, small doses of male hormones had a favourable effect, whilst large doses and oestrone injections had a depressing effect on the nutrition of female rats as far as can be judged from their gain in weight and fat deposition.

The effect on females of the addition of male hormones to oestrone injections.

This is an important question, since it is now established that both "male" and "female" hormones are normally excreted in the urine, probably in normal circumstances in a definite ratio.

The experiments for the elucidation of this question are summarised in Table VI, in which the following data are given: the weights of the organs in which changes were found, the number of rats used in the experiments, the age of rats, the number of days after spaying that the injections were started and the period of the injections.

The control rats in these experiments were oestrone-injected spayed females, *i.e.* rats already supplemented by the female hormone which might be expected to bring about a complete recovery of the spayed rats to the normal condition. These rats were compared with their litter-mates injected with the same dose of oestrone with the addition of androsterone or diol.

The sexual organs. If there were any doubt as to the male hormones alone having a stimulating effect on the sexual organs of spayed rats, the experiments on the simultaneous administration of these hormones with oestrone proves conclusively their co-operative effect.

It can be seen from the respective figures of Table VI that the addition of androsterone or diol increases the "recovery" effect of oestrone seen in the uterus. The largest doses of androsterone and diol increased this effect of oestrone by about 60 and 130 % respectively and in the case of the vagina increased the effect by about 40 and 60 % respectively. Thus the addition of male hormones to

oestrone produced a considerably larger effect on the uterus and vagina of spayed rats than is obtained by the use of either male or female hormone alone.

Both in the experiments which have already been mentioned (Table V) and in the experiments summarised in Table VI the female preputial glands of the rats injected with oestrone alone were small, varying on the average from 50 to 68 mg. This shows once more that oestrone has no effect on these glands. The addition of male hormone to oestrone injections increased the weight of these glands up to 275 mg. (Table VI) which weight can be obtained for these glands by the injection of androsterone or diol alone (Table V).

Therefore the addition of androsterone or of diol to oestrone injections was followed by the co-operation of these hormones in bringing about an improvement in the recovery to the normal weight in the case of the atrophied uterus and to a smaller degree of the atrophied vagina of spayed rats.

On the other hand this co-operation was absent in the case of the female preputial glands, for the greatest development of which the male and not the female hormones are responsible.

Thymus. Oestrone slightly increases the rate of involution of the thymus in spayed rats (Table V). Therefore the weights of the thymus in the rats injected with oestrone alone (Table VI) are smaller than those given for this organ in uninjected spayed rats (Table V). By the addition of androsterone or of diol to oestrone in most cases the rate of this process of involution of the thymus, already stimulated by oestrone, was still further increased (Table VI). Therefore there appears also to be a co-operative action between androsterone and oestrone in the case of the action on the thymus, which is however much less than in the case of the uterus.

Adrenals. Not only was it seen that there was no "co-operative" action in the case of the adrenals, but in some cases oestrone appeared to have an antagonistic effect on the action of the male hormones. The following experiment on 6 spayed litter-mates may be given as an example. The actual weights of the adrenals of 2 rats injected with oestrone alone were 87 and 77 mg.; of 2 injected with androsterone 42 and 51 mg.; and of 2 injected with the same dose of androsterone and oestrone 70 and 73 mg. However, the typical effect (*i.e.* reduction of weight) of androsterone on the adrenals of spayed rats (although probably decreased) was still seen in all the experiments, in which male and female hormones were injected simultaneously (Table VI).

Hypophysis, liver and kidneys. In all these organs the typical action of androsterone or diol was obtained in most cases (Table VI), but, since the effect was no greater than that obtained by the injection of the male hormone alone, oestrone had no co-operative effect.

The gain in body weight and the deposition of fat. In the same way the beneficial stimulating effects of moderate doses of androsterone and of diol on the gain in body weight and on the deposition of fat predominated over the depressing effect of oestrone. The data in Table VI show this in the case of the gain in body weight. The fat deposition, which decreased slightly in most oestrone-injected rats (on the average by about 17%), increased as compared with these rats (on the average by about 55%) in rats injected simultaneously with oestrone and androsterone or diol. The following experiment on 6 spayed litter-mates will serve as an illustration. The actual weight and the weight per unit of body weight of the retroperitoneal fat were respectively in:

	g.	g.
2 oestrone-injected rats	3.1	2.8
2 androsterone-injected rats	4.5	5.2
2 androsterone- and oestrone-injected rats	6.8	4.6

DISCUSSION.

Before the histological investigation has been completed, we cannot say how far the changes obtained in the weights of the organs after the injections represent a true normal recovery.

The difference between the effects of fat-soluble and water-soluble preparations.

In general, it can be said that no important differences in specific action were found between fat-soluble and water-soluble preparations of the male hormones. The large doses of water-soluble preparations used in our experiments, however, had a slightly toxic effect, depressing the appetite, the gain in body weight, the deposition of fat and the stimulating action on the liver.

The difference between the effects of androsterone and diol preparations.

In *males* the most important difference lies in the difference in the actions on the sexual organs. Whilst androsterone, even in the largest doses used, was only able to bring about complete recovery to the normal condition in the case of the prostate and preputial glands, diol caused a complete return to the normal weight, in some cases even exceeding this, in the case of all the sexual organs. In Exp. 12 (Table I) this overstimulation by diol of the prostate and seminal vesicles reached a remarkably high degree and overstimulation of the preputial glands was seen in about half of the experiments.

In our previous papers [Korenchevsky and Dennison, 1935, 2; Korenchevsky *et al.*, 1935, 1, 2] from the results of 7-day experiments and from the comparison of the action of diol on the sexual organs of castrated male rats with that of gonadotropic hormone on the sex organs of immature male rats, we came to the conclusion that diol and not androsterone appeared to have the properties of a complete male sex hormone. How complete are these properties however it is impossible to say before completing the histological investigation and carrying out metabolism experiments. In addition it is necessary here to emphasise that diol has not yet been shown to be present either in the organism or in the urine, nor quantitatively is it so effective as testosterone.

In *females* it was also found that the stimulating action of diol was stronger than that of androsterone in the case of the recovery of the atrophic uterus and vagina. We did not find any other significant differences between these two substances.

The co-operative and antagonistic actions of male and female hormones.

We propose to use the term "co-operative action" in those cases where the action on some organs or functions of two or more substances (whether hormones or not) is greater when they are administered simultaneously than when they are used separately.

In our previous experiments with urinary concentrates of the hormone [Korenchevsky and Dennison, 1934, 2] and in the present experiments with androsterone a co-operative effect of male and female hormones on the reaction of the sexual organs (and to a less degree of the thymus) was seen in *males*.

The addition of oestrone to urinary hormone preparations, however, causes a much greater stimulation of the seminal vesicles than of the prostate, whilst, when androsterone is used with oestrone, the degree of stimulation of the seminal vesicles is less than or equal to that of the prostate. This fact also indicates that in the urinary concentrates of the male sexual hormone besides androsterone another male hormone is present.

This co-operative action was also seen in *females* in the effect on the female sexual organs (and to a smaller degree on the thymus) of androsterone or diol used with oestrone.

The *antagonistic effect* was not so clear as the co-operative effect, but was seen to some extent in two cases, in the action on the adrenals and on the gain in body weight. The addition of oestrone to androsterone was followed by a lessening of the action of androsterone in (1) decreasing the hypertrophied adrenals to the normal weight; and (2) in increasing the gain in body weight in castrated males and spayed females.

The importance of the co-operative action in the recovery to the normal condition of the sexual organs of castrated and spayed rats.

We have found that in *males* an increase in the dose of androsterone or of diol always gave a quicker and greater recovery of the sexual organs of castrated rats than was given by the addition of oestrone. The co-operative action in males therefore seems to be of little importance, since it is replaceable.

With *females* however this does not seem to be the case, though this conclusion was based on only one experiment of 7 litter-mates (final age 62 days; period of injection 28 days), since normal controls were present only in this litter. However the regularity of the results obtained in the individual rats of the groups and the clear-cut results between the groups are convincing as is shown below by the average weights of the sexual organs in the groups:

	Uterus (mg.)		Vagina (mg.)		Female preputial glands (mg.)	
	Actual	Per 200 g. body weight	Actual	Per 200 g. body weight	Actual	Per 200 g. body weight
2 normal litter-mates	277	335	229	279	94	118
2 spayed injected 180 i.u. oestrone	128	155	171	206	50	60
3 spayed injected 180 i.u. oestrone + 0.35 mg. diol	300	343	214	241	150	169

It follows from these figures that a large dose of oestrone injected alone failed to bring about a complete return of the sexual organs of spayed females to the normal condition, whilst complete recovery was obtained by the addition of diol.

The degree of change obtained in the other experiments given in Tables V and VI also suggests that the co-operative action of male and female hormones is important for the recovery of the sexual organs of spayed females to the normal condition. In contradiction to this is the fact that very large doses of androsterone (the hormone found in the organism) are required to produce this effect and even then the reaction is much less than that obtained with diol (the artificial hormone not found in the organism). There is reason however to expect that the action of testosterone on females will probably be similar to that of diol, since the effects of these substances on males are in many respects similar.

Pathological effects.

The female preputial glands in females are homologous with the preputial glands in males. Whilst oestrone appears to have no effect on either of these glands, both androsterone and diol cause their overstimulation beyond the "age-weight in" both cases. It seems that the overstimulation of the female preputial glands and the development of the "female prostate" are both abnormal features

produced in spayed females by large doses of male hormones. Simultaneous administration of oestrone failed to counteract this abnormal effect of the male hormones. These abnormal actions of the male hormones must be regarded as being relatively unimportant since they have no harmful effect on the organism as compared with the dangerous action of oestrone on some of the organs in males [Lacassague, 1933; Burrows and Kennaway, 1934; Korenchevsky and Dennison, 1935, 3].

The manifold effects of the sexual hormones.

In our previous papers [Korenchevsky *et al.*, 1933; Korenchevsky and Dennison, 1934, 1; 1935, 4] we demonstrated the manifold effects of castration in male rats and the reversal by purified male hormone preparations prepared from urine of most of the changes produced.

The present experiments with pure androsterone and its derivatives confirm the manifold effects which, in addition to those on the sexual organs, are seen in the changes in gain in body weight, deposition of fat, adrenals, thymus, liver, kidneys and heart.

We wish particularly to emphasise the stimulating effect of these hormones on the heart, liver and kidney, with regard to the clinical possibilities. If this effect should also be found in human beings, the male hormones may become a valuable acquisition in the treatment of suitable diseases in male patients, since these hormones are natural stimulants and seem to be mild but effective in action. We therefore suggest that a clinical trial of these substances should be made in suitable diseases, since no harmful results can be expected to follow their application, whilst the advantages may be considerable.

SUMMARY.

1. Experiments were performed on 188 normal and castrated male rats and 122 normal and ovariectomised female rats in order to study the effects of the prolonged administration of Ruzicka's artificially prepared androsterone and its fat- and water-soluble derivatives, injected alone or simultaneously with oestrone.

2. *Castrated male rats.* From the comparison of the results of the previous short-duration experiments on these substances, the present long-duration experiments and our previous experiments with the gonadotropic hormone of pregnancy urine, diol was found to have the qualitative properties of a true male hormone: the secondary sexual organs (including the seminal vesicles) of castrated males were maintained (by suitable doses) in a normal condition or even reached more than the normal weight and the atrophied organs were restored to the normal weight.

3. Androsterone (even in the largest daily dose used of 3.6 mg.) had not all these properties, being mainly deficient in restoring the atrophied seminal vesicles and penis in castrated males, though prostates and preputial glands of normal or more than normal weight were obtained in both maintenance and recovery experiments.

4. The injection of very large doses of water-soluble androsterone (about 13.6–27.2 mg. per day) and water-soluble diol (about 2.9–5.3 mg. per day) had a depressing effect on the gain in body weight, the deposition of fat and the stimulation of the liver.

5. The effects of androsterone and diol and their water-soluble esters were manifold, for not only were the sexual organs of castrated rats restored to or

towards the normal condition, but also the following organs or functions: gain in body weight, adrenals, hypophysis (with large doses), thymus, liver, kidneys and heart.

6. A co-operative action on the seminal vesicles and prostate (and to a smaller degree on the thymus) was seen between androsterone and oestrone, though a quicker and greater effect could be obtained by increasing the dose of the male hormone preparations. An antagonistic action on the adrenals appeared to occur between the male hormones and oestrone.

7. In the present experiments of long duration, as in the previous experiments of short duration, a direct proportional relationship was found within the range observed between dose and effect on the prostate and seminal vesicles (with an apparent lag however in the effect on the seminal vesicles with the middle dose used).

8. *Normal male rats.* No "rejuvenating" or other favourable effect on old male rats was seen with the doses used.

9. *Female rats.* A description was given of the "female preputial glands", homologues of the preputial glands of the male rat.

10. *Ovariectomised female rats.* The injection of large doses of diol caused a partial recovery of the atrophied uterus and vagina of ovariectomised females. This effect was slight even with large doses of androsterone.

11. These injections also caused a hypertrophy of the female preputial glands to a weight which was above the normal for the respective age and in some rats the development of a "female prostate" from the rudimentary periurethral glands.

12. Large doses of diol depressed the appetite and gain in body weight, whilst with medium and small doses and with fat-soluble androsterone these were improved. The injection of androsterone and of diol also increased the rate of involution of the thymus, decreased the weight of the adrenals and (with large doses) of the hypophysis and slightly increased the weights of the kidneys and liver, thus in general producing results similar to those obtained in castrated male rats.

13. In ovariectomised female rats injections of oestrone brought about a return towards the normal weight of the atrophied uterus and vagina, increased the speed of involution of the thymus and decreased the deposition of fat and the gain in body weight, but seemed to have no effect on the weight of the adrenals. In most cases there was a slight decrease in the weights of the liver and kidneys.

14. Simultaneous injections of oestrone and large doses of androsterone or diol showed a "co-operative recovery effect" on the weights of the uterus and vagina and to a smaller degree on the thymus, whilst there seemed to be a slightly antagonistic effect between the actions of oestrone and the male hormones on the adrenals.

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REFERENCES.

- Butenandt and Dannenbaum (1934). *Z. physiol. Chem.* **229**, 192.
Burrows and Kennaway (1934). *Amer. J. Cancer*, **20**, 48.
Callow and Deanesly (1935, 1). *Biochem. J.* **29**, 1424.
—— (1935, 2). *Lancet*, ii, 77.
Crooke and Korenchevsky (1935). *Proc. Roy. Soc. Med.* **28**, 72.
David (1935). *Acta Brev. Neerland.* **5**, 85.
—— Dingemanse, Freud and Laqueur (1935). *Z. physiol. Chem.* **233**, 281.
Korenchevsky (1935, 1). *Nature*, **135**, 434.
—— (1935, 2). *Nature*, **136**, 185.
—— and Dennison (1934, 1). *J. Path. Bact.* **38**, 231.
—— (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. Roy. Soc. Med.* **28**, 71.
—— and Kohn-Speyer (1933). *Biochem. J.* **27**, 1506.
—— ——— Levy Simpson (1935, 1). *Biochem. J.* **29**, 2131.
—— ——— (1935, 2). *Biochem. J.* **29**, 2522.
Lacassagne (1933). *Compt. Rend. Soc. Biol.* **113**, 590.
Laqueur, David, Dingemanse and Freud (1935). *Acta Brev. Neerland.* **5**, 84.
Ruzicka and Wettstein (1935). *Helv. Chim. Acta*, **18**, 986, 1264.
Tschopp (1935, 1). *Klin. Woch.* **14**, 1064.
—— (1935, 2). *Nature*, **136**, 258.