LXXXI. THE ASSAY AND THE EFFECT OF TESTOSTERONE ON RATS COMPARED WITH THOSE OF OTHER SEXUAL HORMONES.

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LAQUEUR and his co-workers [David, 1935; David *et al.*, 1935; Laqueur *et al.*, 1935] isolated testosterone from the testes of the bull. Ruzicka and Wettstein [1935; Wettstein, 1935] and Butenandt and Hanisch [1935] prepared this compound artificially. The natural and artificial preparations were similar in all respects, one capon unit being contained in about 10 γ and the effect on the seminal vesicles of castrated rats being greater than that on the prostate. According to Tschopp [1935; Ruzicka and Wettstein, 1935, p. 1270] 10 γ of testosterone injected into castrated rats for 10 days brought about an increase in the size of the seminal vesicles of about 300 % and of the prostate of about 113 %.

Laqueur *et al.* found that the effect of testosterone on the seminal vesicles and prostate of castrated rats could be doubled or even more than doubled, if a physiologically indifferent, impure extract, prepared from the tissues of animals or plants were injected simultaneously. These observations were corroborated by Tschopp [Ruzicka, Wettstein and Kagi, 1935], who [Ruzicka, Goldberg and Rosenberg, 1935] also compared the various artificial male sexual hormones prepared by Ruzicka and his co-workers. In the same paper Ruzicka *et al.* pointed out the correlation between the chemical constitutions of the hormones and their selective effects in birds and rats.

The following experiments were performed with artificial testosterone prepared by Ruzicka and kindly supplied to us by Messrs Ciba Ltd. The testosterone was dissolved in olive oil and the daily dose was injected in half doses (0.1 ml. of oily solution) twice a day. Two series of experiments were made, one of 7 days' duration, in which the assay of testosterone was performed and a second of 23 days' duration, in which a study was made of the effects of prolonged injections. For economy of space Tables I-V showing the results have been much compressed.

I. THE ASSAY OF TESTOSTERONE.

Assay was performed on 80 rats belonging to 17 litters, the technique used being the same as that described in our previous papers with the following modification. We usually divide each litter into two or three groups, each containing 2 or more rats. A general average percentage change for each dose is then calculated from the percentage changes obtained in each of the groups injected with that particular dose. In the assay of gonadotropic hormone [Korenchevsky *et al.*, 1935, 2, p. 2523] some of the litters were used for an assay made in the way described above, while the rest of the litters were divided so that one rat of each litter was placed in each group and a general average of the weights of the organs obtained for each dose. The percentage changes were then

calculated from these general average weights. The results obtained by the two methods differed very little.

In this assay therefore we tried to simplify the technique still further, in order to economise the number of rats used in the control and injected groups. Two or three litters of the same age and weight were used as if they were one litter, the rats of these litters being distributed evenly in the various groups. Not less than 6 and generally 7 rats were used for each "dose-group". Since preliminary experiments showed that one rat unit was contained in about 8.3 γ , this amount was chosen as the smallest dose, most of the other doses being simple multiples thereof. There were more rats (13) in the control than in any other group, since the former contained 1 rat from most of the litters used in the experiment. The weights of the seminal vesicles and prostates of the uninjected control rats used in our previous assays of sexual hormones [*e.g.* Korenchevsky and Dennison, 1935, 1, 2; Korenchevsky *et al.*, 1935, 1] approximated closely to the averages obtained for these weights in the control group in the present

Table I. *The effect of 7 days' injection of testosterone on sexual organs, fat and on the gain in body weight of male castrated rats.*

Average actual weights of organs and the average percentage change in weight (actual and per unit of body weight) after injection.

Organ	Weights	Control rats	Rats injected with (γ)									
			8.3	16.7	33.3	67	125	167	334	500	1000	1410
			A. Average weights.									
Seminal vesicles (mg.)	Actual	10	16	21	26	28	35	41	57	54	68	87
Prostate (mg.)	Actual	54	76	86	97	102	123	137	187	173	196	230
Prostate with seminal vesicles (mg.)	Actual	64	92	107	123	130	158	178	244	227	264	217
Preputial glands (mg.)	Actual	54	53	57	63	64	82	82	89	96	109	127
Thymus (mg.)	Actual	680	723	710	632	481	569	511	433	558	569	575
Fat (g.)	Actual	8.1	7.4	7.9	7.3	7.5	7.3	6.4	6.9	8.7	6.9	7.7
B. Percentage change in weight.												
Seminal vesicles	Actual	—	+60	+110	+160	+180	+250	+310	+470	+440	+580	+770
	Per unit of body weight	—	+75	+100	+163	+187	+262	+312	+450	+412	+537	+700
Prostate	Actual	—	+41	+ 59	+ 80	+ 89	+128	+154	+246	+220	+263	+326
	Per unit of body weight	—	+49	+ 56	+ 77	+130	+123	+161	+244	+205	+247	+293
Prostate and seminal vesicles	Actual	—	+44	+ 67	+ 92	+103	+147	+181	+281	+255	+313	+239
	Per unit of body weight	—	+14	+ 63	+ 90	+139	+145	+184	+276	+237	+292	+357
Penis	Actual	—	+27	+ 58	+ 52	+ 51	+ 93	+ 92	+121	+118	+146	+149
	Per unit of body weight	—	+35	+ 51	+ 49	+ 54	+ 88	+ 97	+116	+107	+131	+128
Preputial glands	Actual	—	- 2	+ 6	+ 17	+ 19	+ 52	+ 52	+ 65	+ 78	+102	+135
	Per unit of body weight	—	+ 5	+ 2	+ 16	+ 23	+ 49	+ 58	+ 65	+ 74	+ 93	+121
Thymus	Actual	—	+ 6	+ 4	- 7	- 29	- 16	- 25	- 36	- 18	- 16	- 15
	Per unit of body weight	—	+13	$\pm$ 0	- 9	- 27	- 19	- 24	- 38	- 22	- 21	- 23
Fat	Actual	—	- 9	- 2	- 10	- 7	- 10	- 21	- 15	- 7	- 15	- 5
	Per unit of body weight	—	-10	- 10	- 10	- 2	- 10	- 22	- 16	+ 2	- 21	- 11
Gain in body weight		—	+ 7	+ 19	+ 22	+ 41	+ 30	+ 30	+ 11	+ 48	+ 26	+ 15

experiment. This indicates that in future assays on castrated rats the control group may be dispensed with, provided that the experiments are carried out with the same stock of rats, bred and fed under the same conditions and that the "standard" weights of the sexual organs of these rats at special periods of time after castration are well established.

Sexual organs. The results of the present experiments are summarised in Table I and represented by the curves (Figs. 1, 2, 3 and 4). In spite of the variation between the individual figures, deviations from the average weight

Fig. 1. Relation between dose of testosterone and percentage increase in actual weight of seminal vesicles and prostate.

exceed 25 % only in 3 cases out of 80 in the actual weight of the prostate and in 6 out of 80 in the weight of the prostate per 200 g. of body weight. In these nine variations, the deviation is <27 % in 4 cases and <35 % in another 4; in 1 case only is there a deviation of 57 %. In the seminal vesicles the number of deviations greater than 25 % is 8 out of 160 (80 actual and 80 per unit of body weight). Of these 3 do not exceed 27 %, 4 do not exceed 32 % and in 1 case only the deviation is 40 %.

The percentage increase in the weights of the seminal vesicles and prostate shows a sharp rise with the small doses, followed by a flattening of the curves with the larger doses, which is typical of this hormone. The larger response of the seminal vesicles as compared with the prostate is also characteristic. There is no direct proportion between dose and effect such as was seen within a certain range, for example, with androsterone and androsterone-diols.

One rat unit (minimum dose producing 40 % increase in the weight of the prostate) is contained in 8 γ , calculated from the actual weights; and in about 7 γ calculated from the weight per unit of body weight. The percentage increases in the actual weights of the seminal vesicles and penis corresponding to these

Fig. 2. Relation between dose of testosterone and percentage increase in weight of seminal vesicles and prostate calculated per unit of body weight.

Fig. 3. Relation between dose of testosterone and percentage increase in actual weight of penis.

Fig. 4. Relation between dose of testosterone and percentage increase in weight of penis calculated per unit of body weight.

doses are about 60 and 27 % and per unit of body weight 61 and 29 % respectively.

As we have stated before, the weight of the preputial glands in both normal and castrated uninjected rats always shows greater variation than is shown by other sexual organs. As can be seen from Table I doses of 8–16 γ have no definite effect, the changes being too small to be considered. The dose 33 γ has a small definite effect, after which the curve rises more slowly than in the case of the penis but with the highest dose reaches nearly the same height as that reached by the penis.

Thymus, deposition of fat and gain in weight. As can be seen from Table I, even after only 7 days of injection the normal response of the thymus is obtained, i.e. the rate of involution of the thymus, delayed by castration, returns towards normal after the injection. The effect however is comparatively small though definite with doses of 67 γ and more.

The gain in weight (Table I), though only slightly greater in the injected rats, occurs in most cases and with all the doses used. This cannot be explained by an increased deposition of fat, which is in most cases less than that of the control uninjected rats (Table I).

Statistical discussion of the data.

By J. M. C. Scott.

Curves. Curves were fitted to the data by least squares. The system of curves employed was a modification of that described previously [Korenchevsky and Dennison, 1935, 2, p. 2128].

Probable errors. It was clear from the data that the chance fluctuations of the weight of a given organ are not independent of the dose but become greater as the dose is increased. The hypothesis that the probable error of the weight of

Table II. *Statistical summary of results of the assay experiments.*

J. M. SCOTT.			
I	II	III	
Organ and basis of calculation	Probable error for weight of organ (mean of 6 rats)	Coefficient for probable error for percentage increase in weight of organs	
Seminal vesicles (actual weight)	± 4.8	5.8	
Seminal vesicles (per unit of body weight)	± 4.2	5.0	
Prostate (actual weight)	± 3.8	4.6	
Prostate (per unit of body weight)	± 4.1	5.0	
Penis (actual weight)	± 3.6	4.3	
Penis (per unit of body weight)	± 3.9	4.7	

the organ increased proportionally to the weight itself was tested and found to be well supported by the data; the figures in col. II give this probable error as a percentage of the mean weight of the organ for a mean of 6 rats.

The most important results are the percentage increases due to the hormone, and these are affected by any variation in the 13 controls. The figures in col. III take this into account; as they stand they are not probable errors, owing to the variation increasing with the weight of the organ, but they must be increased by the "percentage increase" to which they apply.

For example, with a dose of 33.3 γ the actual weight of the prostate (see

Table I) showed a percentage increase of 80 %. Therefore the probable error in this case will be:

4.6	...	Table II, col. III.
3.7	...	80 % of 4.6.
8.3	...	Probable error required.

In the same way, for a dose of 167 γ which gives 154% increase in actual weight of prostate, we obtain a probable error of ± 11.7 . For the largest dose used 1410 γ , giving 326 % increase in actual weight of prostate, the probable error will be ± 19.6 .

II. PROLONGED PERIOD OF INJECTION (23 DAYS) OF TESTOSTERONE INTO CASTRATED MALES.

In long-duration experiments the relative fluctuations in the weights of the organs are smaller than in the 7-day experiments. Fewer animals therefore were used in these experiments. 29 rats belonging to 5 litters were distributed into 8 groups as shown in Table III. In 4 litters 1 or 2 rats of each litter were left

Table III. *Effect of prolonged injections of sexual hormones on male rats.*

The average actual weights of organs of castrated rats injected with sexual hormones compared with those of the organs of normal and castrated litter-mates injected with oil only.

	I	II	III	IV	V	VI	VII	VIII
			Castrated rats injected with (γ)					
	Normal control rats	Cas- trated control rats	Testosterone				Testost. 167 + androst. 450	Testost. 500 + androst. 450
Organ			33	167	500	1410		
Sem. vesicles (mg.)	999	10	29	81	180	276	98	199
Prostate (mg.)	1250	63	106	230	367	554	295	454
Penis (mg.)	328	79	159	208	243	277	226	256
Preputial gland (mg.)	144	65	65	120	129	185	158	161
Adrenals (mg.)	60	80	78	65	65	60	73	63
Thyroid (mg.)	25	22	23	27	26	30	24	24
Hypophysis (mg.)	10.8	12.6	12.4	14.1	13.3	15.5	14.2	14.2
Thymus (mg.)	315	560	474	500	409	382	548	420
Liver (g.)	13.6	10.3	10.8	12.9	12.6	13.8	13.0	13.6
Kidney (g.)	2.31	1.72	1.83	2.01	1.98	2.22	2.12	2.26
Heart (mg.)	960	795	989	976	919	1023	975	1092
Fat (g.)	14.5	17.2	16.5	15.3	17.1	17.2	17.1	18.9
Final body wt. (g.)	367	320	315	356	348	377	359	380
Gain in body wt. (g.)	80	64	69	85	85	92	78	100

Note. Bulb of penis cut off.

normal while the rest were castrated. These unoperated litter-mates form a control group of normal rats (col. I, Table III), with which to compare the castrated control rats (col. II) and the rats injected with the hormones (cols. III-VIII). The rats of both the normal and castrated control groups (I and II) were injected with 0.2 ml. daily of pure olive oil, since 0.2 ml. daily of a solution of the hormone in olive oil was injected into the rats of Groups III to VIII.

The average final age of the rats was 98 days, the injections being started 51 days after castration and carried on for 23 days. The number of rats in each group is also given in Table III. The technique used was the same as that given in our previous papers, except that the penis was weighed without the lower bulbous part, by which it is attached to the pelvic bones. This lower part was cut off after fixation in Bouin's fixative at the well defined point where the penis

begins to enlarge into the bulbous part. Our experiments show that this does not affect the percentage change in weight of the penis, but it does make the weighing of this organ more accurate.

Sexual organs. The experiments were carried out on rats a long time after they had been castrated and were therefore "recovery" experiments. In no case did the injection of testosterone restore the normal weight of the seminal vesicles, prostate or penis, although the largest daily dose used (1410 γ) contained about 176 rat units (as defined by us) or about 141 capon units. The weight of the preputial glands however not only returned to normal but, with the highest dose, exceeded the normal average.

The addition of a small dose of androsterone, although improving the degree of recovery of the sexual organs, did not give even a summation result. After the injection of 0.45 g. androsterone for 22 days [Korenchevsky *et al.*, 1935, 3] the average weights of the sexual organs were: seminal vesicles 41 mg., prostate 210 mg., penis 209 mg. and preputial glands 115 mg. If these figures are compared with the corresponding figures in Table III, cols. IV and V (testosterone alone) and cols. VII and VIII (testosterone + androsterone), it is seen that the addition of androsterone brought about a very small increase in the "recovery" effect on the seminal vesicles, prostate and penis, causing a return to the normal weight only in the case of the preputial glands.

Adrenals. As always in castrated animals, the adrenals of control castrated rats were enlarged. Testosterone injected in doses of 167 γ or more caused a return towards the normal weight and with the highest dose the normal weight was obtained. This is shown both by the actual figures and the figures per unit of body weight (Table III).

Thyroids. The small changes in the weights of the thyroids may be significant, since they occurred with all doses, although the changes were less pronounced when calculated per unit of body weight. Histological investigation and repetition of the experiments will give a decisive answer. The addition of androsterone did not increase the "recovery" effect.

Hypophysis. These glands were fixed in formalin, since Bouin's solution is not suitable if the glands are to be used for histological investigation. The weights after formalin fixation are not so close to the original weights as after Bouin's fixation. In the castrated injected animals however as before (see our previous papers), hypertrophy of these glands was seen, which surprisingly was found to persist or even appeared to increase (shown more especially in the actual figures) after testosterone injections both alone and with androsterone.

Thymus. The usual effect of delay in the rate of involution of the thymus was seen in the castrated animals and a return towards but not to normal was obtained after injection of testosterone. The "recovery" effect of testosterone however does not appear to be so complete as with large doses of androsterone or diol [Korenchevsky *et al.*, 1935, 3; Table II, p. 2538].

Liver, kidneys and heart. The usual decrease in the weight of these organs was seen in the castrated animals (col. II) as compared with the normal animals (col. I). The injection of testosterone, alone or with androsterone restored the normal weight, even in the case of the heart which sometimes exceeded the normal value.

Deposition of fat and gain in body weight. The deposition of fat, as in our previous experiments, increased (but only to a slight extent) in the castrated control rats as compared with the normal litter-mates. The injections produced no definite effect (Table III). The injections however definitely increased the gain in body weight, which is decreased in castrated animals. Large doses of

testosterone, alone or with androsterone, even increased the gain in body weight above that of normal rats. Since there was no simultaneous increase in the deposition of fat (except in the actual figures for the injection of 500 γ testosterone and 450 γ androsterone, col. VIII), it may be concluded that this increased gain in body weight was due to growth of the other tissues and cannot be explained by an increased deposition of fat.

III. THE EFFECT ON MALE RATS OF THE ADDITION OF OESTRONE OR OESTRADIOL TO TESTOSTERONE INJECTIONS.

Experiments were performed on 20 rats belonging to 4 litters. The rats of each litter were evenly distributed into 4 groups each containing 5 rats. The control group in these experiments was injected with 33 γ testosterone only;

Table IV. *Effect of the simultaneous prolonged injection of testosterone and oestrone or oestradiol on the weight of the organs of male castrated rats.*

Organ	Groups injected with	Average actual weights	
		Weight	Percentage change compared with testosterone-injected rats
Seminal vesicles (mg.)	Testosterone	33	—
	Testosterone + oestrone	40	+ 21
	Testosterone + oestradiol	54	+ 64
	Oestradiol	25	- 24
Prostate (mg.)	Testosterone	107	—
	Testosterone + oestrone	129	+ 21
	Testosterone + oestradiol	138	+ 29
	Oestradiol	69	- 36
Penis (mg.)	Testosterone	148	—
	Testosterone + oestrone	149	+ 1
	Testosterone + oestradiol	144	- 3
	Oestradiol	74	- 50
Preputial glands (mg.)	Testosterone	67	—
	Testosterone + oestrone	75	+ 12
	Testosterone + oestradiol	87	+ 30
	Oestradiol	54	- 19
Hypophysis (mg.)	Testosterone	11.4	—
	Testosterone + oestrone	13.3	+ 17
	Testosterone + oestradiol	12.4	+ 9
	Oestradiol	13.4	+ 18
Thymus (mg.)	Testosterone	537	—
	Testosterone + oestrone	456	- 15
	Testosterone + oestradiol	467	- 13
	Oestradiol	447	- 17
Liver (g.)	Testosterone	10.09	—
	Testosterone + oestrone	9.41	- 7
	Testosterone + oestradiol	8.48	- 16
	Oestradiol	7.83	- 22
Heart (mg.)	Testosterone	870	—
	Testosterone + oestrone	773	- 11
	Testosterone + oestradiol	719	- 17
	Oestradiol	699	- 20
Gain in body weight (g.)	Testosterone	100	—
	Testosterone + oestrone	75	- 25
	Testosterone + oestradiol	53	- 47
	Oestradiol	53	- 47

Note. The hormones were injected in the following doses: oestrone 6 γ and oestradiol 20 γ , three times per week; testosterone 33 γ daily. Bulb of penis cut off.

group II was injected with 33 γ of testosterone and in addition with 6 γ oestrone; group III with 33 γ of testosterone and 20 γ of oestradiol and group IV with 20 γ of oestradiol only. Testosterone was injected twice daily and oestrone and oestradiol were injected three times a week separately in another area of the subcutaneous tissue. The rats were castrated before reaching sexual maturity, the average final age being 74 days. On the average, the injections were started 28 days after castration and continued for a period of 23 days. The results are summarised in Table IV.

Sexual organs. The addition of oestrone or of oestradiol had only a small co-operative effect on the weight of atrophied seminal vesicles, prostate and preputial glands and no effect on the penis. The co-operative effect was more marked with oestradiol, which was not only injected in larger amount than oestrone, but has a greater effect on the seminal vesicles of castrated rats when injected in the same amount as oestrone, the effect of oestradiol being about twice that of oestrone [David *et al.*, 1934; David, De Yongh and Laqueur, 1935]. An increase in the dose of oestrone used would probably give a co-operative effect similar to that given by oestradiol. Oestradiol alone, as has been described by David, Freud and De Yongh, has a stimulating effect on the seminal vesicles and prostate (the authors obtained on the average 55% increase in the weight of the prostate and 129% increase in that of the seminal vesicles after injections of 0.2–6 γ daily for 5–6 days). The increase in the weight of the seminal vesicles was attributed to hypertrophy of the muscular layer. If the weights of the sexual organs of the rats in the present experiments are compared with those of uninjected castrated rats of similar age used in previous experiments, it is found that oestradiol injected during 23 days caused an increase of 108–150% in the weight of the seminal vesicles and of 19–28% in the weight of the prostate. The weight of the preputial glands increased in only two (by about 20%) out of 5 rats and there was no effect on the penis. The effect was much less than that brought about by 33 γ of testosterone (Table III).

Hypophysis. Oestradiol, alone or with testosterone, caused a slight increase in the hypertrophy of the already hypertrophied hypophysis.

Thymus. The rate of involution of the thymus, which is delayed in castrated rats, was slightly increased by oestradiol either alone or when added to testosterone injections.

Liver and heart. There was a quite definite decrease in the actual weights of these organs after the injection of oestradiol alone or with testosterone, which decrease disappeared or became insignificant when the results were calculated per unit of body weight. This shows that, after treatment with female hormone, the changes in body weight are related to the condition of these organs.

Deposition of fat and gain in body weight. The deposition of fat, as judged by changes in the amount of intra-abdominal fat, decreased by about 24% when oestradiol, alone or with testosterone, was injected. The effect of oestrone was less noticeable, the decrease with this hormone being only about 11%. These changes are small in view of the normally large variations. Taken however in conjunction with the quite definite decrease in the gain in body weight (Table IV) which is produced by oestradiol and oestrone these figures become significant. There is therefore an antagonistic action on the gain in body weight between testosterone and oestrone.

The adrenals, thyroid, kidneys and spleen did not show any definite changes in weight.

IV. THE EFFECT ON OVARIECTOMISED RATS OF TESTOSTERONE INJECTED ALONE OR SIMULTANEOUSLY WITH OESTRONE.

Experiments were performed on 42 rats, 7 normal and 35 ovariectomised before sexual maturity. These rats are represented in Table V in two groups, according to age, one group on the average 99 days old and the second group 56 days old. The rats belonged to 7 litters with the exception of 8 (out of 11) spayed control

Table V. *Effect 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.

Organ	I	II	III	IV	V	VI	VII	VIII	IX	X
	Spayed rats injected with (γ)									Average age of rats, days
	Normal control rats	Spayed control rats	Oestrone 6	Testosterone			Oestrone 6 + Testost. 167	Oestrone 6 + Testost. 500	Oestrone 6 + Testost. 700	
				167	500	700				
Uterus (mg.)	415	39	165	58	—	—	235	229	290	99
	—	32	—	—	75	124	—	—	—	56
Cervix of uterus (mg.)	117	17	59	25	—	—	94	98	104	99
	—	10	—	—	22	50	—	—	—	56
Vagina (mg.)	254	121	228	184	—	—	267	252	284	99
	—	130	—	—	214	225	—	—	—	56
Fem. preputial glands (mg.)	105	84	90	140	—	—	148	162	207	99
	—	109	—	—	124	119	—	—	—	56
Adrenals (mg.)	81	82	82	71	—	—	83	80	79	99
	—	94	—	—	82	61	—	—	—	56
Thyroids (mg.)	22	20	22	25	—	—	23	24	25	99
	—	18	—	—	20	21	—	—	—	56
Hypophysis (mg.)	13.8	12.1	12.6	11.2	—	—	12.2	14.3	12.5	99
	—	12.0	—	—	10.6	9.6	—	—	—	56
Thymus (mg.)	279	511	331	388	—	—	345	307	283	99
	—	672	—	—	458	339	—	—	—	56
Average final body weight (g.)	239	293	281	272	—	—	270	282	278	99
	—	204	—	—	202	203	—	—	—	56

Note added March 9th, 1936. The experiment with injection of testosterone into ovariectomised rats was repeated on 18 rats and similar results were obtained which will be published elsewhere.

rats in the second group, which were taken from our previous experiments. The number of normal control litter-mates was increased in the present experiment, in order to determine how far the injections restored the normal condition. The control rats were injected with pure olive oil. In order to ensure that there should be no regeneration of ovarian tissue from ovarian cells left after the operation, the ovaries were removed with the surrounding fat tissue and part of the horn. The length of the piece of horn removed varied and in any case shortened the horns of the spayed rats as compared with those of normal rats. Therefore in order to avoid a fallacious comparison with the uterus having horns of normal length, in this experiment we weighed not only the uterus with horns, but also the cervix of the uterus separately. These latter weights seem to give a more accurate representation of changes in the uterus of spayed rats compared with normal animals. Another difficulty in standardising the "normal" weight in the case of the female sexual organs, especially of the uterus, lies in the cyclic changes. The much greater "oestrus" weight of the uterus (obtained in one of the rats) was excluded from the average figure given in Table V (col. I). Theoretically, it would be expected however that oestrone would produce a uterus having an "oestrus" weight, which with the doses used we never

actually obtained. Testosterone was injected daily and oestrone three times a week. The period of injection of testosterone alone was 22 days and of oestrone alone or with testosterone 21 days.

Sexual organs. Testosterone injected alone (cols. IV–VI) caused hypertrophy of the atrophied sexual organs. The hypertrophy however was not sufficient (except in the case of the preputial glands) to represent complete recovery, nor in the case of the uterus was it equal to that produced by oestrone (col. III), though after injections of 700 γ daily of testosterone the vagina weighed about as much as after the injection of oestrone.

In the dose used, oestrone alone was unable to restore the normal condition of the sexual organs of ovariectomised rats. After simultaneous injections of oestrone and testosterone, recovery to the normal weight was complete in the case of the vagina but incomplete in the case of the uterus, although the weight of this organ was considerably greater than after injections of oestrone alone (especially if judged by the changes in the cervix of the uterus), when the largest dose of testosterone was added (col. IX). The weight of the preputial glands after the simultaneous injection of the hormones exceeded the normal weight.

Thus the co-operative activity of testosterone and oestrone in restoring the atrophied female sexual organs towards or in some cases, to the normal weight was clearly seen in all the experiments.

Other organs. The effect of ovariectomy on the weight of most of the organs, which are definitely affected in males by castration (adrenals, hypophysis, liver, kidney, heart, deposition of fat) could not be clearly seen, though the delay in the rate of involution of the thymus was quite definite (Table V). We do not however give definite conclusions with regard to the effect of ovariectomy on these organs, since for this a larger number of experiments would be necessary, especially considering the increase in weight of some of the organs of normal rats, which occurs during oestrus (*i.e.* adrenals, thyroids, hypophysis [Andersen and Kennedy, 1932; 1933, 1, 2]). According to Andersen and Kennedy, in rats spayed before sexual maturity and killed about 65 days after, there is no change in the weight of the adrenals as compared with that of rats in dioestrus; the thyroids are below normal weight, while the weight of the hypophysis lies between that of the normal controls in oestrus and those in dioestrus.

The figures for these organs given in cols. I and II of Table V are in agreement with the conclusions of Andersen and Kennedy.

The effect of the injection of testosterone alone was slight, but occurred in some of the organs of all rats, the weights of the adrenals and hypophysis being decreased and of the thyroids being increased. The addition of oestrone to testosterone injections prevented the development of this effect on the adrenals and hypophysis.

Injections of oestrone and of testosterone separately or simultaneously restored the rate of involution of the thymus to or towards normal.

The body weight and the deposition of fat were not definitely affected by the injections, but the average body weight was increased by ovariectomy (col. II) compared with that of the normal control rats (col. I), in spite of the absence of an increase in the deposition of fat.

V. DISCUSSION.

It is only possible to estimate the activity of a hormone preparation and to compare it with that of other preparations, when all the results both of experiments of short and of long duration are taken into consideration. In the following

an attempt has been made to make such a comparison of testosterone with androsterone, androsterone-diol, gonadotropic hormone and the manifold and partly undefined factors which produce maturation of the sexual organs in normal animals.

Until the histological investigation has been completed, we cannot say how far the changes obtained in the weights of the organs after injections represent a true recovery. The results so far obtained indicate that the histological changes in the female sexual organs after the injection of testosterone are similar to those previously described [Korenchevsky and Dennison, 1936], which were obtained after the injection of androsterone-diol, *i.e.* they are similar to some of the changes produced by progesterone or observed during pregnancy.

Effect on castrated males.

On the method of assay. The statistical interpretation of the assay results by Mr Scott shows that the probable error for percentage changes of weight of organs increases with the weight of the organs. Therefore in the assayable part of the curve (*i.e.* from 8 to 334 γ , from which point the curve flattens sharply) the probable error for prostate gradually rises from ± 6.5 to ± 15.9 . Provided that not less than 6 rats are used for each group-dose, the results of assay with such errors should be considered as satisfactory, in spite of the simplification of the method introduced.

Using Mr Scott's coefficient the probable error for seminal vesicles is found to be greater than that for prostate. This supports our repeated statement that prostate is more suitable for assay experiments than seminal vesicles. However, the changes in seminal vesicles and penis are suitable for corroboration of the results obtained with prostate.

The rat unit. This unit, as defined in our previous papers (the minimum dose producing 40% increase in the weight of the prostate) only represents the smallest active dose of the hormone. As judged by changes in the actual weight of the prostate, one rat unit is contained in 8 γ of testosterone (present experiments), 21 γ of androsterone-diol [Korenchevsky and Dennison, 1935, 2] and 170 γ of androsterone [Korenchevsky and Dennison, 1935, 1]. A comparison of the rat units therefore indicates that testosterone has the greatest activity.

Comparison of the curves. The curves representing the percentage increase in weight of the seminal vesicles after the injection of androsterone, androsterone-diol and testosterone are given in Fig. 5, while those for prostate are given in Fig. 6. Because of the low solubility of fat-soluble (f.s.) androsterone-diol in oil the curve continues only to the point representing 350 γ of the hormone. Therefore androsterone-diol is also represented by the curve for water-soluble (w.s.) androsterone-diol (the Li salt of the monosuccinate of this hormone).

Since the curves are different in form, a comparison can only be made with reservations.

The curves for seminal vesicles (Fig. 5) show that fat-soluble and, less closely, water-soluble androsterone-diol are similar to testosterone in their activities and differ from androsterone by a greater effect on the seminal vesicles, the activity of testosterone being greatest.

The curves for prostate (Fig. 6) are nearly identical in the case of fat-soluble androsterone-diol and testosterone. The curve for water-soluble androsterone-diol differs from these, but not greatly. Androsterone curves show the lowest activity for all doses.

Average activity of the hormones. Another method of representing the activity has already been used by us [Korenchevsky and Dennison, 1935, 2, p. 2128;

Fig. 5. Comparison of the curves representing relation between percentage increase in the actual weight of seminal vesicles and dose for androsterone, fat-soluble (f.s.) and water-soluble (w.s.) androsterone-diol and testosterone.

Fig. 6. Comparison of the curves representing relation between percentage increase in the actual weight of prostate and dose for androsterone, f.s. and w.s. androsterone-diol and testosterone. Lower part of the curve for testosterone and the curve for f.s. androsterone-diol are very close, almost coinciding. They are distinguished in the figure by arrows.

Korenchevsky *et al.*, 1935, 1, Table VII, p. 2139]. Within the range of the curve, from the smallest active dose to the dose at which the curve begins to show pronounced flattening, comparison was made of the average percentage increase in the weights of the organs per 100 γ for each dose used. The figures of this comparison, given in Table VI, show that androsterone-diol appears to have the greatest average activity.

Table VI. *Comparison of the average effects of androsterone, androsterone-diol and testosterone (7 days' injections).*

Organs	Weights	Average percentage increase per 100 γ for daily doses		
		200-900 γ andro- sterone	25-200 γ andro- sterone- diol	8-334 γ testo- sterone
Seminal vesicles	Actual	23	223	205
	Per unit of body weight	23	216	206
Prostate	Actual	25	144	106
	Per unit of body weight	24	135	112
Prostate with seminal vesicles	Actual	24	156	122
	Per unit of body weight	23	149	121
Penis	Actual	11	100	66
	Per unit of body weight	10	93	65
Preputial glands	Actual	18	37	28
	Per unit of body weight	18	33	29

Comparison of the qualitative effect. Before forming a judgment about the activity of a hormone it is essential to analyse its specific activity from the point of view of its approximation to the natural biological process, which is partly or wholly controlled by the hormone. It is now clear that the natural development of the sexual organs is controlled by many factors, including various hormones. The activities of these manifold, but as yet not completely discovered, factors can be demonstrated if the weights of the different sexual organs of normal rats at different ages after sexual maturity are compared with the weights of these organs before sexual maturity. It is obvious however that the natural growth of the sexual organs, which develop during a period of some months, can only be compared with experiments in which (1) the effect of the injections develops during a period sufficiently long for the recovery to take place and (2) the doses injected are large enough to compensate the still relatively short period of injection (weeks instead of months) by increasing the dose of the hormone injected.

Our Table VII therefore is divided into two parts: in the first part experiments on the forced development of the sexual organs during a 7-day period of injection of artificial hormones are compared with a 5-day period of injection of gonadotropic hormone [Korenchevsky *et al.*, 1935, 2], which hormone is supposed to bring about normal stimulation of the normal secretion of the testicular hormones in their natural relationship. In the second part of the table the ratios obtained after a long period of injection of artificial hormones (the effect of large and smaller doses being given separately) are compared with the ratio given by naturally developed sexual organs. This "natural" ratio was obtained from the percentage increase of the actual weights of the sexual organs of normal rats 49-123 days old taken from our previous paper [Korenchevsky *et al.*, 1935, 3, Table I, p. 2535] compared with the weights of the organs of immature normal rats 26-27 days old [Korenchevsky *et al.*, 1935, 2, Table I, p. 2523]. The average

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

	Short-period injections		Long-period injections	
	Daily dose (γ)	Ratio prostate to sem. ves.	Daily dose (γ)	Ratio prostate to sem. ves.
Testosterone	8	68	33	36
	16	54	—	—
	33	50	—	—
	67	49	167	37
	125	51	—	—
	167	50	500	28
	334	52	—	—
	500	50	1410	29
	1000	45	—	—
	1410	42	—	—
Testosterone	Average	51	—	—
Gonadotropic hormone	Average	54	—	—
Androsterone-diol	Average	64	175-350	54
			700 or more	24
Androsterone	Average	106	450-2200	126
			3600 or more	55
Natural development in normal rats	—	—	—	25

for all ages of the ratio ($\times 100$) of percentage increase in the weight of the prostate to that of the seminal vesicles was 25.

The ratios given by testosterone are tabulated separately for each dose, whilst for the other hormones only the average ratios are given, since these have been given in detail elsewhere [Korenchevsky *et al.*, 1935, 1, p. 2141]. It is clear from the figures in Table VII that (1) the ratios of the short-period injections show that testosterone and, less closely, androsterone-diol produce development of the sexual organs similar to that produced by gonadotropic hormone and that therefore probably they bring about normal development, whilst androsterone does not do so; (2) the ratios of the long-period injections show a clear-cut difference (as was anticipated and explained above) between the results given by small and large doses, the small dose ratios being closer to those obtained for the short-period injections; (3) the ratios for long-period injections of large doses show a normal or nearly normal development of the sexual organs in the case of androsterone-diol and to a less extent in the case of testosterone, but not in that of androsterone.

The prostate to penis ratio is not included in Table VII as the penis of untreated castrated rats is larger than the penis of the young immature normal rats which were used for comparison with the older rats. This would therefore give percentage increases in the weight of the penis after injections of artificial hormone which would not be comparable with those given by injections of gonadotropic hormone or by natural development. The ratios of these organs in the castrated rats injected with artificial male hormone can however be compared with each other, in which case it is again seen that the development produced by testosterone is similar to that produced by androsterone-diol (ratios 168 and 158 respectively) and is different from that produced by androsterone (ratio 230).

Comparison of the "recovery" experiments on males. In the long-period experiments even the largest doses used (1410 γ =176 rat units) failed to restore

the normal weight of the seminal vesicles, prostate and penis of castrated rats injected for 23 days.

It is difficult to compare these experiments with our previous experiments [Korenchevsky *et al.*, 1935, 3, Table I, p. 2535], in which complete recovery of the prostate was obtained with androsterone and of all the sexual organs with androsterone-diol, since in these earlier experiments the period of injection and the period after castration were different from those of the present experiments. The experiments with water-soluble androsterone-diol (Nos. 19-21) might however be considered to be similar to the present experiments with testosterone. From these it is seen that about 80 rat units (5310 γ) of water-soluble diol brought about complete recovery, whilst 176 rat units of testosterone failed to do so. It would be necessary however to make comparative experiments on litter-mates before coming to definite conclusions on this matter.

If the restorative effects of testosterone on other organs are compared with those obtained with androsterone and androsterone-diol, they are found to be similar in the case of the adrenals, thymus, liver, kidney and heart. The gain in body weight was also found to be improved by all three hormones, though too large doses of water-soluble diol were toxic, as is shown by the decrease in the weight of the liver and in the gain in body weight.

The action of testosterone differed from that of the other two hormones in failing to decrease, even with the largest dose, the hypertrophy of the hypophysis, which with some doses was even increased.

Effect on ovariectomised rats.

A comparison of the figures of Table VI of this paper with Table V of our previous paper on androsterone and androsterone-diol [Korenchevsky *et al.*, 1935, 3, p. 2543] shows that testosterone restored the normal weight of the female sexual organs more completely than androsterone, the change being about the same as that obtained with androsterone-diol.

Whilst the effects on the thymus and hypophysis were similar with all three hormones, the decrease in weight of adrenals and the increase in those of the liver and kidney only occurred after androsterone or androsterone-diol injections. Each of these three male hormones when injected with oestrone showed about equal degrees of co-operative recovery effect on the atrophied female sexual organs (compare Table V of the present paper with the results given in Table VI of the previous paper and those given by the histological data [Korenchevsky and Dennison, 1936]).

Summarising this comparative analysis of these three artificial male hormones it may be concluded that the action of testosterone is similar to that of androsterone-diol, the most important difference between the two substances being the greater activity of the smallest doses of testosterone, *i.e.* in the amount of the substances per rat unit, while in the largest doses used the effect on the sexual organs seemed to be greater with androsterone-diol than with testosterone (long-period experiments).

On X-substance of testosterone.

Experiments on male rats have shown that in the doses used neither addition of androsterone nor of oestrone to testosterone injections was able to give such a large co-operative effect as was produced by the X-substance of Laqueur and his co-workers, only a small co-operative effect being obtained by the addition of the hormones mentioned above.

SUMMARY.

1. Experiments were performed on 5 normal males, 7 normal females, 124 castrated males and 35 ovariectomised rats belonging to 33 litters, in order to investigate the biological properties of artificial testosterone and the co-operative effect of this hormone with oestrone and with oestradiol.

Castrated males.

2. The hormone was assayed by a simplified form of the authors' method. One rat unit was found to be contained in 8γ (from actual weight of prostate increase) or 7γ (from weight per unit of body weight).

3. A relation between the dose of testosterone and the effect on the prostate, seminal vesicles and penis after 7 days of injection was investigated statistically and represented in the form of curves.

4. Prolonged periods of injection (23 days) in the doses used (up to $1410\gamma = 176$ rat units daily) failed to restore the normal weight of the atrophied sexual organs (except in the case of the preputial glands) of rats castrated about 51 days before the beginning of the injections ("recovery" test).

5. After the injections of testosterone the weights of the organs, which were changed by castration, were restored to the normal weights in the case of adrenals, thyroid, liver, kidneys and heart; towards the normal condition in the case of the thymus. There was no change, or an apparent increase in the "castration" hypertrophy of the hypophysis. The gain in body weight also increased to or even exceeded the normal standard value, without definite changes occurring in the deposition of fat; this indicates a favourable stimulation of anabolic processes in the metabolism of organs and tissues.

6. In the doses used, injections of oestradiol alone brought about changes similar to those obtained after injection of oestrone: *i.e.* a considerable increase in the weight of the seminal vesicles (up to 150 %) and small increases in the weight of the prostate (up to 28 %) and the preputial glands (up to 20 %), the penis remaining unchanged. Changes were also observed in the weights of other organs: in the hypophysis (slight increase), in the liver, heart and thymus (decreases). The gain in body weight and deposition of fat were both decreased.

7. Oestrone or oestradiol injected with testosterone showed only a slight co-operative effect on the weight of atrophied seminal vesicles and prostate.

Ovariectomised females.

8. Testosterone injected alone brought about hypertrophy of the atrophied sexual organs towards but not to the normal weight (except in the case of the preputial glands which reached normal weight) and decreased the weight of the thymus.

9. There was a marked co-operative effect between testosterone and oestrone in restoring the atrophied sexual organs to or towards the normal weight. The addition of oestrone to testosterone injections also increased the rate of involution of the thymus (co-operative effect).

10. Thus our previous results with androsterone and androsterone-diol, obtained by the method of weights and corroborated histologically, and also the present results with testosterone, show that the male hormones have some of the important properties of the female hormone; so that, if they are present in the female organism, it would be expected that they would act in co-operation with oestrone in controlling the condition and function of the sexual and of some other important organs in the female.

Comparison of the effects of the hormones.

11. Comparison was made of the biological properties of testosterone, androsterone, androsterone-diol and gonadotropic hormone. The effects of these hormones on the growth of the sexual organs were also compared with the natural growth of these organs in normal rats.

12. Androsterone-diol and testosterone, unlike androsterone, appear to bring about a qualitatively normal development of the male sexual organs, as judged by the ratio of the percentage increase in the weight of the prostate to those of the seminal vesicles and of the penis.

13. Both androsterone-diol and testosterone produce some changes which are similar to those observed after the injection of progesterone and, if injected simultaneously with oestrone, the changes produced are similar to some of those seen during pregnancy.

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REFERENCES.

- Andersen and Kennedy (1932). *J. Physiol.* **74**, 247.
— — (1933, 1). *Proc. Soc. Exp. Biol. Med.* **30**, 657.
— — (1933, 2). *J. Physiol.* **79**, 1.
Butenandt and Hanisch (1935). *Ber. deutsch. Chem. Ges.* **68**, 1859.
Callow and Deanesly (1935). *Biochem. J.* **29**, 1424.
David (1935). *Acta Brev. Neerland.* **5**, 85 and 108.
— De Yongh and Laqueur (1935). *Arch. Internat. Pharm. Ther.* **51**, 137.
— Dingemanse, Freud and Laqueur (1935). *Z. physiol. Chem.* **233**, 281.
— Freud and De Yongh (1934). *Biochem. J.* **28**, 1360.
Korenchevsky and Dennison (1935, 1). *Biochem. J.* **29**, 1720.
— — (1935, 2). *Biochem. J.* **29**, 2122.
— — (1936). *J. Path. Bact.* **42**, 91.
— — and Levy Simpson (1935, 1). *Biochem. J.* **29**, 2131.
— — (1935, 2). *Biochem. J.* **29**, 2522.
— — (1935, 3). *Biochem. J.* **29**, 2534.
Laqueur, David, Dingemanse and Freud (1935). *Acta Brev. Neerland.* **5**, 84.
Ruzicka, Goldberg and Rosenberg (1935). *Helv. Chim. Acta*, **18**, 1487.
— and Wettstein (1935). *Helv. Chim. Acta*, **18**, 1264.
— — and Kagi (1935). *Helv. Chim. Acta*, **18**, 1478.
Tschopp (1935). *Praxis*, **24**, 698.
Wettstein (1935). *Schweiz. med. Woch.* **65**, 912.