CXCVII. THE EFFECT ON MALE RATS OF THE SIMULTANEOUS ADMINISTRATION OF MALE AND FEMALE SEXUAL HORMONES AND THE RELATION TO THE ASSAY OF THE HORMONES.

BY VLADIMIR KORENCHEVSKY AND MARJORIE DENNISON.

From the Lister Institute, London.

(Received July 1st, 1934.)

THE experiments on rats, of Moore and Price [1932] and of Freud [1933], relating to this question have already been referred to by us [1934, 2].

Schoeller and Gehrke [1933] also performed some interesting experiments on birds. In hens, injected with 10 c.u. of testicular hormone daily for 4 weeks, the comb-growth increased 23 %, while in those injected with 4000 m.u. of oestrin per week for 6 weeks the size of the comb decreased by 11 %. When capons were injected for 2 days, with testicular hormone (2 c.u.) and oestrin (5000 m.u.) simultaneously, the comb-growth on the third day was 50 % greater than that obtained with the same dose of testicular hormone alone. Thus, although in capons the degree of the reaction was less and the dose of oestrin used much larger, the simultaneous injection of oestrin with testicular hormone was found to have a similar stimulating effect on the comb-growth of capons as has been observed by Freud on the seminal vesicles of castrated rats. Freud performed his experiments on 3-5 weeks' old rats 3 days after castration, when atrophy of the seminal vesicles was not complete, used litter-mates only "as far as possible" and weighed the seminal vesicles fresh. This is perhaps the partial explanation of some of the "paradoxical results" mentioned on p. 1442 and seen in some of the other tables in Freud's very interesting paper.

In the present paper, besides a repetition of Freud's experiments on the seminal vesicles of castrated rats, we have studied the influence in castrated male rats of combined injections of testicular hormone and oestrin on the weight of the other secondary sexual organs, body weight, fat deposition and the endocrine and some of the other organs, which are influenced to a greater or less extent by testicular hormone as we have previously shown [Korenchevsky *et al.*, 1933, 1].

TECHNIQUE.

Experiments were performed on 73 castrated rats belonging to 16 litters. The number of rats in the litter, the final age, the age at castration and the number of days after castration that the animals were killed, the final weights and the preparation of testicular hormone injected are given in Table I.

A study of the effect of daily injections during 7 days was made in order to decide what changes in the effects of testicular hormone will be caused by the presence of oestrin in a preparation under assay.

A longer period of injection of 21 days was also used in order that more pronounced effects might be obtained for the study of the effects of the injection.

As in the previous experiments, crystalline oestrone¹ dissolved in olive oil was used for the injections. Two preparations of testicular hormone were used: one, designated in the tables as "Pr," was supplied by Messrs Schering, Ltd.; the other, designated as "H," was sent from Holland by Messrs Organon, Ltd.² The latter preparation is controlled in the pharmaco-therapeutic laboratory of Amsterdam University. Both preparations were used in doses of 1.5 c.u., though this does not imply that their actual strength in c.u. was the same, since the method of assay on capons is different in these two laboratories. In Tables II, IV, V, VI and VII, the average results obtained in the individual litters are given, while Table III gives only the general means averaged from the litters 6-13 and is constructed in the same way as the tables in the previous paper.

Table I. *Age, average weight and period of injections of castrated rats.*

No. of litter	No. of rats in litter	Period of injections Days	Age in days		No. of days after castration when killed	Final weights, g.					Testicular hormone preparation used
			At castration	When killed		Con-trols	Test. hor. alone	Test. hor. + oes. 20	Test. hor. + oes. 60	Test. hor. + oes. 180	
1	7	7	25	83	58	—	301	—	291	294	"Pr"
2	6	7	25	57	32	—	208	—	193	182	
3	4	7	22	54	32	—	188	—	176	—	
4	7	21	21	83	62	296	330	—	—	273	
5	5	21	21	83	62	241	305	—	—	255	
6	3	7	30	86	56	—	306	286	—	—	
7	5	7	23	76	53	—	263	272	—	—	"H"
8	4	7	23	78	55	—	304	—	275	—	
9	4	7	23	79	56	—	249	—	274	—	
10	4	7	25	73	48	—	282	—	265	—	
11	4	7	23	77	54	—	269	—	—	274	
12	3	7	24	77	53	—	259	—	—	241	
13	5	7	30	74	44	—	237	—	240	222	
14	5	21	24	95	71	345	357	—	335	—	
15	3	21	24	92	68	—	308	—	294	—	
16	4	21	21	89	68	—	327	—	—	305	

SEVEN-DAY EXPERIMENTS.

The controls in these experiments were injected with testicular hormone alone; the litter-mates received testicular hormone *plus* varying doses of oestrin.

Influence on gain in body weight and fat deposition. These data are not included in the table, but it is possible to say that on the average, as compared with the litter-mates receiving testicular hormone alone, the addition of the large dose of oestrin of 180 i.u. was followed by a decreased gain in body weight and slightly decreased fat deposition. For example, three control groups of rats injected with "H" alone in 7 days gained in weight 27, 19 and 22 g. respectively, while their litter-mates injected with "H" + 180 i.u. of oestrin gained 11, 5 and 3 g. respectively. In the same litters, the corresponding weights of retro-peritoneal fat were, in controls injected with "H" alone, 8.6, 9.2 and 9.0 g. and, in the litter-mates injected with "H" + 180 i.u. of oestrin, 6.1, 8.2 and 6.1 g.

¹ Referred to as oestrin throughout the body of the paper.

² We wish to express our thanks to the Department of Biological Standards for oestrin and to Messrs Organon, Ltd., and Schering, Ltd., for testicular hormone preparations.

Table II. *Effect on sexual organs and hypophysis of 7 days of injections of testicular hormone preparation "Pr" alone or in combination with oestrone.*

No. of litter	Actual					Per 200 g. body weight				
	Weights, mg.			% change		Weights, mg.			% change	
	Pr 1-5	Pr 1-5 Oes. 60	Pr 1-5 Oes. 180	Pr 1-5 Oes. 60	Pr 1-5 Oes. 180	Pr 1-5	Pr 1-5 Oes. 60	Pr 1-5 Oes. 180	Pr 1-5 Oes. 60	Pr 1-5 Oes. 180
<i>Prostate with seminal vesicles.</i>										
1	147	149	167	+ 1	+14	98	102	113	+ 4	+15
2	131	148	154	+13	+18	126	151	169	+20	+34
3	144	165	—	+15	—	154	189	—	+23	—
Av.	141	154	161	+10	+16	126	147	141	+16	+25
<i>Prostate.</i>										
1	117	119	134	+ 2	+15	78	82	91	+ 5	+17
2	105	115	116	+10	+11	101	119	128	+18	+27
3	120	126	—	+ 5	—	128	144	—	+13	—
Av.	114	120	125	+ 6	+13	102	115	110	+12	+22
<i>Seminal vesicles.</i>										
1	30	30	32	± 0	+ 7	20	21	22	+ 5	+10
2	26	31	38	+19	+46	25	32	42	+28	+68
3	24	39	—	+63	—	26	45	—	+73	—
Av.	27	33	35	+27	+27	24	33	32	+35	+39
<i>Penis.</i>										
1	172	160	182	- 7	+ 6	115	110	124	- 4	+ 8
2	128	138	133	+ 8	+ 4	123	143	147	+16	+20
3	125	117	—	- 6	—	133	134	—	+ 1	—
Av.	142	138	158	—	+ 5	124	129	136	—	+14
<i>Preputial glands.</i>										
1	87	110	107	+26	+23	58	76	73	+31	+26
2	102	93	114	- 9	+12	98	96	125	- 2	+28
3	106	107	—	+ 1	—	112	122	—	+ 9	—
Av.	98	103	111	—	+18	89	98	66	—	+27
<i>Hypophysis</i>										
1	16.5	17.7	18.5	+ 7	+12	10.9	12.2	12.5	+12	+15
2	11.9	12.6	13.0	+ 6	+ 9	11.5	12.3	14.3	+ 7	+24
3	9.6	12.3	—	+28	—	10.7	14.0	—	+31	—
Av.	12.7	14.2	15.8	+10	+11	11.0	12.8	13.4	+17	+20

The influence on the secondary sexual organs. As can be seen from Tables II and III, the effect on the weight of the prostate of the addition of oestrin to testicular hormone "Pr" (Table II) or "H" (Table III), was small and similar in degree. The largest increase in weight of the prostate after the addition of oestrin to testicular hormone "Pr" was 27 % in litter 2 (Table II). With preparation "H," in several cases there was no effect at all on the prostate and the maximum increase was 20 % (Table III). The effect of increasing the dose of oestrin, when any, was small.

On the other hand, in the case of the seminal vesicles the addition of 60 or 180 i.u. of oestrin to the testicular hormone always greatly increased the effect of preparation "H," and in most cases and to a smaller degree, that of preparation "Pr." In the case of "H" the addition of larger doses of oestrin produced a larger effect on the seminal vesicles but not on the prostate. Thus, difference of action on seminal vesicles indicates a difference in the preparations "Pr" and "H." The effect of the addition of oestrin on changes in

Table III. *Effect on sexual organs and hypophysis of 7 days of injection of testicular hormone preparation "H" in combination with oestrone.*

		Weights					
		Actual			Per 200 g. body weight		
		20 u.	60 u.	180 u.	20 u.	60 u.	180 u.
<i>Prostate with sem. ves., mg.</i>	Controls	122	116	120	85	87	94
	Injected	122	134	140	88	104	115
	Average	—	+16	+17	—	+20	+22
	Maximum variations	{ +9 -8	{ +19 +13	{ +23 +7	{ +6 -1	{ +33 +9	{ +30 +17
<i>Prostate, mg.</i>	Controls	101	98	105	71	73	82
	Injected	95	105	106	69	80	87
	Average	-5	+8	—	-3	+9	—
	Maximum variations	{ -9 -1	{ +11 +3	{ +9 -2	{ -3 -3	{ +20 +1	{ +17 -2
<i>Sem. ves., mg.</i>	Controls	22	18	16	16	14	13
	Injected	27	29	34	19	23	28
	Average	—	+64	+110	+24	+73	+115
	Maximum variations	{ +50 -4	{ +113 +36	{ +157 +67	{ +47 ± 0	{ +140 +47	{ +136 +79
<i>Penis, mg.</i>	Controls	142	133	131	100	100	103
	Injected	128	145	134	92	112	110
	Average	-10	+9	—	-8	+12	+7
	Maximum variations	{ -11 -8	{ +18 +5	{ +8 -6	{ -10 -5	{ +18 +5	{ +12 +2
<i>Hypophysis, mg.</i>	Controls	12.3	12.0	11.6	8.8	9.0	9.1
	Injected	14.7	13.9	14.2	10.6	10.6	11.7
	Average	+20	+16	+23	+24	+18	+29
	Maximum variations	{ +30 +10	{ +39 +1	{ +30 +10	{ +40 +7	{ +26 +19	{ +39 +19

Note. In this table "controls" denotes rats receiving testicular hormone only; "injected" receiving the same dose of testicular hormone + various doses of oestrin, as shown in the heading of the table.

the penis and preputial glands was less constant but similar in degree to that on prostate alone.

Influence on hypophysis. One of the effects of the injection of oestrin into male normal and castrated rats was an increase in the weight of the hypophysis, as has been shown in the previous paper. This effect was again evident when oestrin was added to testicular hormone preparation, although the percentage increase in weight was sometimes small (Tables II and III).

Effect on other organs. Although the other organs were also weighed, no definite or constant changes in the weights were found to occur after the addition of oestrin to testicular hormone preparations, as compared with the litter-mates injected with testicular hormone alone.

TWENTY-ONE DAY EXPERIMENTS.

These experiments were performed on five litters, Nos. 4 and 5 containing 12 rats treated with testicular preparation "Pr" (Tables I, IV and VI) and Nos. 14, 15 and 16, also containing 12 rats, injected with testicular preparation "H" (Tables I, V and VII).

All the experiments were performed at the same time, using the same solution of oestrin, a point of importance when considering some of the differences between the results obtained with preparation "Pr" and preparation "H."

Table IV. *Effect on sexual organs and hypophysis of 21 days of injections of testicular hormone preparation "Pr" alone or in combination with oestrone.*

No. of litter	Actual					Per 200 g. body weight					
	Weights, mg.			% change on controls		Weights, mg.			% change on controls		% change on group
	Con- trol	Pr 1·5	Oes. 180	Pr 1·5	Pr 1·5 Oes. 180	Con- trol	Pr 1·5	Oes. 180	Pr. 1·5	Pr 1·5 Oes. 180	Pr 1·5 Oes. 180
<i>Prostate with seminal vesicles.</i>											
4	66	201	222	+205	+236	45	122	163	+171	+262	+34
5	61	191	263	+213	+331	51	126	206	+147	+304	+64
Av.	64	196	243	+209	+284	48	124	185	+159	+283	+49
<i>Prostate only.</i>											
4	55	163	168	+196	+206	37	100	123	+170	+232	+23
5	51	157	208	+208	+308	42	104	163	+148	+288	+57
Av.	53	160	188	+202	+257	40	102	143	+159	+260	+40
<i>Seminal vesicles only.</i>											
4	11	38	54	+246	+391	8	24	40	+200	+400	+67
5	10	34	55	+240	+450	8	22	43	+175	+434	+96
Av.	11	36	55	+243	+421	8	23	42	+188	+417	+82
<i>Penis.</i>											
4	85	143	145	+68	+71	57	92	115	+61	+88	+16
5	95	131	130	+38	+37	79	87	103	+10	+30	+18
Av.	90	137	138	+53	+54	68	90	109	+36	+59	+17
<i>Preputial glands.</i>											
4	52	125	127	+140	+144	35	76	93	+117	+166	+22
5	44	163	166	+271	+277	36	107	129	+197	+258	+21
Av.	48	144	147	+206	+211	36	92	111	+157	+212	+22
<i>Hypophysis.</i>											
4	11·6	11·2	13·0	-3	+12	8·0	6·8	9·5	-15	+19	+40
5	13·8	13·9	14·8	+1	+7	11·4	9·2	11·8	-19	+4	+28
Av.	12·7	12·6	13·9	—	+10	9·7	8·0	10·6	-17	+12	+34

Unfortunately the uninjected control rats of litters 15 and 16 had severe pneumonia and therefore, in accordance with our technique, these rats were excluded. Since there remained only the one control rat of litter 14, uninjected control rats of this experiment were not included in the tables. In Tables V and VII, therefore, the rats injected with testicular hormone alone (in the tables designated as "H 1·5") are used as the controls to those injected simultaneously with testicular hormone and oestrin. For the direct comparison of the results obtained with preparations "H" and "Pr," in the "Pr" experiments (Tables IV and VI) percentage changes per 200 g. of body weight were referred both to the uninjected control rats and to the rats injected with "Pr" alone. Therefore, the figures of percentage change per 200 g. of body weight in Tables V and VII can only be compared with those of the last column of Tables IV and VI.

Influence on food intake, body weight and fat deposition. It can be seen from Table VI that the actual food intake increased (up to 22 %) when "Pr" alone was injected and that this influence disappeared, or decreased, after the additional injection of oestrin. This latter effect is also shown in Table VII, although when calculated per unit of body weight the change in appetite is very small in both "Pr" and "H" experiments.

Table V. *Effects on sexual glands and adrenals of 21 days of injections of testicular hormone preparation "H" alone or in combination with oestrone.*

No. of litter	Actual					Per 200 g. body weight				
	Weights, mg.			% change		Weights, mg.			% change	
	H 1·5	H 1·5 Oes. 60	H 1·5 Oes. 180	H 1·5 Oes. 60	H 1·5 Oes. 180	H 1·5	H 1·5 Oes. 60	H 1·5 Oes. 180	H 1·5 Oes. 60	H 1·5 Oes. 180
<i>Prostate with seminal vesicles.</i>										
14	153	160	—	+ 5	—	86	96	—	+12	—
15	173	170	—	- 2	—	112	116	—	+ 4	—
16	182	—	210	—	+15	111	—	138	—	+24
Av.	169	165	210	—	+15	103	106	138	+ 8	+24
<i>Prostate only.</i>										
14	122	116	—	- 5	—	69	70	—	+ 1	—
15	142	133	—	- 6	—	92	91	—	- 1	—
16	156	—	170	—	+ 9	96	—	111	—	+16
Av.	140	125	170	- 6	+ 9	86	81	111	—	+16
<i>Seminal vesicles only.</i>										
14	31	44	—	+42	—	18	26	—	+44	—
15	31	37	—	+19	—	20	25	—	+25	—
16	26	—	40	—	+54	16	—	26	—	+63
Av.	29	41	40	+31	+54	18	26	26	+35	+63
<i>Penis.</i>										
14	142	125	—	-12	—	80	74	—	- 8	—
15	144	151	—	+ 5	—	94	103	—	+10	—
16	153	—	138	—	-10	94	—	91	—	- 3
Av.	146	138	138	—	-10	89	89	91	—	- 3
<i>Preputial glands.</i>										
14	72	80	—	+11	—	40	48	—	+20	—
15	93	111	—	+19	—	60	75	—	+25	—
16	100	—	108	—	+ 8	63	—	71	—	+13
Av.	88	96	108	+15	+ 8	54	62	71	+23	+13
<i>Adrenals.</i>										
14	81·1	88·0	—	+ 9	—	45·5	52·5	—	+15	—
15	90·0	100·5	—	+11	—	58·5	68·5	—	+17	—
16	64·6	—	82·2	—	+27	39·6	—	54·1	—	+37
Av.	78·6	94·3	82·2	+10	+27	47·9	60·5	54·1	+16	+37

During the injection of "Pr" testicular hormone alone, as compared with the uninjected controls the total gain in body weight increased, but decreased after the addition of oestrin to both "Pr" and "H" preparations, as is shown by the following figures:

		Litter Nos. ...	4	5	14	15	16
Gain in body weight, g.	{	Controls	75	56	—	—	—
		"Pr" or "H"	85	77	64	44	65
		"Pr" or "H" + oestrin	35	34	40	38	32

Corresponding changes were noted in the fat deposition. The weight of the retroperitoneal fat was increased with "Pr" alone (Table VI), but decreased with the additional injection of oestrin (Tables VI and VII).

Catabolic processes also seem to be favourably influenced by injections of testicular hormone alone and slightly depressed with additional injections of

Table VI. *Effect on thymus, liver, kidneys, heart, retroperitoneal fat and food intake of 21 days of injections of testicular hormone preparation "Pr" alone or in combination with oestrone.*

No. of litter	Actual					Per 200 g. body weight					
	Weights			% change on controls		Weights			% change on controls		% change on group
	Con- trol	Pr 1-5	Oes. 180	Pr 1-5	Pr 1-5 Oes. 180	Con- trol	Pr 1-5	Pr 1-5 Oes. 180	Pr 1-5	Pr 1-5 Oes. 180	Pr 1-5 Oes. 180
<i>Thymus, mg.</i>											
4	696	560	444	-20	-36	471	341	326	-28	-31	-4
5	445	634	346	+43	-22	369	416	270	+13	-27	-35
Av.	571	597	395	—	-29	420	379	298	—	-29	-20
<i>Liver, g.</i>											
4	10.28	13.69	11.33	+33	+10	6.94	8.31	8.31	+20	+20	± 0
5	8.13	12.14	10.33	+49	+27	6.74	7.96	8.08	+18	+20	+2
Av.	9.21	12.92	10.83	+41	+19	6.84	8.14	8.20	+19	+20	+1
<i>Kidneys, g.</i>											
4	1.677	2.052	2.028	+22	+21	1.132	1.245	1.484	+10	+31	+19
5	1.383	2.008	1.817	+45	+31	1.146	1.314	1.430	+15	+25	+9
Av.	1.530	2.030	1.923	+34	+26	1.139	1.280	1.457	+13	+28	+14
<i>Heart, mg.</i>											
4	813	934	803	+15	-1	550	567	588	+3	+7	+4
5	663	859	826	+30	+25	549	563	651	+3	+19	+16
Av.	740	897	815	+23	—	550	565	620	+3	+13	+10
<i>Retroperitoneal fat, g.</i>											
4	12.34	15.59	11.35	+26	-8	8.34	9.47	8.27	+14	-1	-13
5	8.63	13.50	10.06	+56	+17	7.15	8.88	7.71	+24	+8	-13
Av.	10.49	14.55	10.71	+41	—	7.75	9.18	7.99	+19	—	-13
<i>Food intake, g.</i>											
4	40.0	45.0	35.3	+13	-12	27.6	27.9	26.3	+1	-5	-6
5	34.1	44.7	34.9	+31	+2	28.6	30.3	27.8	+6	-3	-8
Av.	37.1	44.9	35.1	+22	—	28.1	29.1	27.1	+4	-4	-7

oestrin, as is suggested by the following figures (g.) of gain in body weight per unit (10 g.) of food intake:

Litter Nos.	...	4	5	14	15	16
Controls		0.48	0.29	—	—	—
"Pr" or "H"		0.48	0.64	0.40	0.38	0.56
"Pr" or "H" + oestrin		0.29	0.28	0.30	0.30	0.43

Although in this experiment only litter 5 shows an increase on the injection of testicular hormone, litter 4 showing no change, in our earlier experiments an increase in gain in body weight per unit of food intake was found in all the litters injected with testicular hormone [Korenchevsky *et al.*, 1933, 1, p. 561, Table III]. It would seem therefore that, in general, testicular hormone had a slightly stimulating and oestrin a slightly depressing effect on the appetite, gain in body weight and fat deposition.

Influence on the secondary sexual organs. It can be seen from Table V that the addition of 180 I.U. of oestrin to the daily dose of "H" slightly increased the weight of the prostate, by 16 %, while when "Pr" was used the increases were 23 and 57 % in two litters respectively, on the average 40 % (Table IV). Thus, after prolonged injections, in contrast to the results of 7-day experiments,

Table VII. *Effects on hypophysis, thymus, kidneys, spleen, retroperitoneal fat and daily food intake of 21 days of injections of testicular hormone preparation "H" alone or in combination with oestrone.*

No. of litter	Actual					Per 200 g. body weight				
	Weights			% change		Weights			% change	
	H 1.5	H 1.5 Oes. 60	H 1.5 Oes. 180	H 1.5 Oes. 60	H 1.5 Oes. 180	H 1.5	H 1.5 Oes. 60	H 1.5 Oes. 180	H 1.5 Oes. 60	H 1.5 Oes. 180
<i>Hypophysis, mg.</i>										
14	14.6	15.6	—	+ 7	—	8.2	9.3	—	+13	—
15	12.6	17.2	—	+37	—	8.2	11.8	—	+44	—
16	12.3	—	17.6	—	+43	7.6	—	11.6	—	+53
Av.	13.2	16.4	17.6	+22	+43	8.0	10.6	11.6	+29	+53
<i>Thymus, mg.</i>										
14	586	562	—	- 4	—	329	343	—	+ 4	—
15	721	481	—	-33	—	468	329	—	-30	—
16	650	—	425	—	-35	396	—	279	—	-30
Av.	652	522	425	-19	-35	394	336	279	—	-30
<i>Kidney, g.</i>										
14	1.986	2.153	—	+ 8	—	1.112	1.285	—	+16	—
15	2.037	2.221	—	+ 9	—	1.324	1.512	—	+14	—
16	2.125	—	2.244	—	+ 6	1.297	—	1.473	—	+14
Av.	2.049	2.187	2.244	+ 9	+ 6	1.244	1.399	1.473	+15	+14
<i>Spleen, mg.</i>										
14	649	665	—	+ 3	—	364	396	—	+ 9	—
15	634	627	—	- 1	—	412	429	—	+ 4	—
16	500	—	524	—	+ 5	306	—	342	—	+12
Av.	594	646	524	—	+ 5	361	413	342	+ 7	+12
<i>Retroperitoneal fat, g.</i>										
14	19.51	17.68	—	- 9	—	10.94	10.54	—	- 4	—
15	13.21	9.63	—	-27	—	8.59	6.48	—	-25	—
16	14.54	—	11.80	—	-19	8.88	—	7.73	—	-13
Av.	15.75	13.66	11.80	-18	-19	9.47	8.51	7.73	-15	-13
<i>Daily food intake, g.</i>										
14	45.8	41.3	—	-10	—	26.2	25.0	—	- 5	—
15	36.6	36.8	—	+ 1	—	24.2	25.5	—	+ 5	—
16	43.9	—	42.0	—	- 4	27.7	—	28.2	—	+ 2
Av.	42.1	39.1	42.0	—	- 4	26.1	25.3	28.2	—	+ 2

the effect on the prostate of some testicular preparations may be considerably increased by the addition of large doses of oestrin. The addition of 60 i.u. of oestrin to the testicular preparation "H" did not give any change in the weight of the prostate and we made no experiments with this dose of oestrin in combination with "Pr."

The effect on the seminal vesicles of the addition of oestrin was similar to that in the 7-day experiments; in short a considerable increase in the weight of this organ follows the addition of oestrin to the injection of testicular hormone. A larger dose produced a greater reaction (Table V).

The changes in the prostate with seminal vesicles were closer to those in the prostate alone than to those in the seminal vesicles alone (owing mainly to the relative weights of these two organs).

The influence of the additional injection of oestrin on the weight of the penis and preputial glands was small, being practically absent in the penis of rats injected with "H" + oestrin.

Effect on hypophysis. After the addition of oestrin this endocrine organ increased in every litter (Table IV and VII), *i.e.* this typical effect of oestrin was again evident and the simultaneous administration of testicular hormone did not obscure it.

Effect on thymus. The addition of oestrin to "Pr" and "H" accelerated the involution of the thymus in most rats, a result which is in good agreement with the results obtained in the previous paper, on the injection of oestrin into castrated rats.

Effects on the kidney, spleen and heart. The effects on these organs of the addition of oestrin to testicular hormone were very small, but when present (Tables VI and VII) were similar to those described in the previous paper on the injection of oestrin into castrated rats.

The possible specificity of this effect of oestrin on the kidneys is supported by our earlier experiments, in which it was found that "Pr" injected for 21 days produced an increase in the weight of the kidneys varying, on the average, from 6 to 24 % [Korenchevsky *et al.*, 1933, 1, p. 562]. In the present experiments "Pr" alone (Table VI "% change on controls") produced a similar small, but constant increase, varying from 10 to 15 %, which was further slightly increased by the addition of injections of oestrin (last column, Table VI). The average figures for this additional effect of oestrin were practically identical in the "Pr" experiments (+14 %, Table VI), and in the "H" experiments (+15 % and +14 %, Table VII).

It must be remembered that after castration a small decrease in the weight of the kidneys was found in male rats [Korenchevsky and Dennison, 1934, 1]. Therefore, in spite of the small effects, the constancy of the results obtained with various arrangements of the experiments indicates that both male and female sexual hormones have a slight but specific stimulating effect on the weight of the kidneys of castrated rats.

Effects on the thyroid and adrenals. The addition of oestrin caused no changes in these endocrine organs.

Effect on the liver. Although the addition of oestrin to testicular hormone had no effect on the liver, we should like here to emphasise an important influence of "Pr" testicular hormone preparation injected alone (Table VI), namely the increased weight of the liver, shown both in the actual weight (on the average +41 %) and in the weight calculated per unit of body weight (on the average +19 %). These results are exactly the same as those published earlier [Korenchevsky *et al.*, 1933, 1; p. 562], in which the average increase in the weight of the liver (per unit of body weight) was shown to be from 17 to 25 % when castrated rats were injected with "Pr" for 21 days. On the other hand we obtained a decrease in the weight of the liver after the castration of male rats [Korenchevsky and Dennison, 1934, 1].

We have not sufficient data to come to any conclusion with regard to the preparation "H," since the control uninjected groups (not included in the tables) in two litters Nos. 15 and 16 had pneumonia (which reduces the weight of the liver), leaving only one healthy uninjected control in the third litter. In this last litter, however, there was no increase in the weight of the liver after the injection of "H" alone, or in combination with oestrin. It would therefore be interesting to repeat the experiments with "H" preparation, in order to decide whether the absence of influence of "H" was accidental, or indicated a lack in "H" of substances stimulating the liver of castrated rats.

It is possible from these experiments, however, to suggest that testicular

hormone, and not oestrin, has a stimulating effect on the liver. This is in agreement with the results published in the previous paper showing the absence of a stimulating effect on the liver of injections of oestrin alone.

DISCUSSION.

The assay of male sexual hormones.

The results of our experiments corroborated the result of Freud [1933], that oestrin, when injected simultaneously with testicular hormone, usually causes a considerable increase in the effect of the latter on the weight of the seminal vesicles.

Our results also showed that the effects of these combined injections on the weight of the prostate, penis and preputial glands were small, or absent, in 7-day experiments (the period of injection we proposed for the assay of testicular hormone). When the injections were made for 21 days, the effects were larger, only with the preparation "Pr."

We proposed, for the assay of testicular hormone, to use the percentage change in the weight of the prostate with seminal vesicles weighed together, 40 % increase being considered equivalent to 1 R.U. [Korenchevsky, 1932, 1, 2; Korenchevsky *et al.*, 1932].

It can be seen from Tables II and III that, although the differences were not very large in most litters with "Pr" and in all litters with "H," the addition of oestrin caused a smaller percentage increase in the weight of the prostate alone than in that of the prostate weighed together with the seminal vesicles. That the effect of the addition of oestrin is comparatively small, when these glands are weighed together, is chiefly due to the fact that the weight of the seminal vesicles is only from 1/4 to 1/3 that of the prostate and that to a considerable extent, therefore, the weight of the prostate masks the changes in the weight of the seminal vesicles. However, taking into consideration that oestrin in combination with testicular hormone may cause a considerable increase in the weight of the seminal vesicles and produces only a very small change in the weight of the prostate, it would be more cautious to use the change in weight of the prostate alone for the assay of testicular hormone.

At the same time, a separate weighing of the seminal vesicles and a comparison of the changes obtained with those on the prostate alone may give a rough indication of the degree of purification of the testicular hormone from oestrin or oestrin-like substances, since the degree of increase of the seminal vesicles is usually much greater if oestrin is present in the testicular hormone preparation. While the addition of oestrin to the preparation "H" made the difference between the reaction of prostate and that of seminal vesicles quite obvious (Table III), this was not always so with the preparation "Pr" (Table II, litter 1). The occurrence of such large variations makes the explanation of the reaction of the seminal vesicles more difficult and probably will render these organs in castrated rats less satisfactory for the purpose of assay.

It would only be possible to work out an accurate relationship between the increase in weight of the prostate and that of the seminal vesicles on injecting testicular hormone alone or mixed with oestrin, by using pure testicular hormone, which is at present not available, and pure oestrin.

On the basis of the results obtained in the present paper, however, for the assay of the male hormone, we suggest the use of the percentage changes in the weight (per unit of body weight) of the prostate alone and not of the combined

weight of the prostate with seminal vesicles, which we suggested in our previous paper [Korenchevsky *et al.*, 1932].

This alteration raises the question as to whether, in the case of purified testicular hormone preparations (in the absence of oestrin), the number of R.U. as assayed on the prostate alone will differ from that as assayed on the prostate and seminal vesicles together. The following figures are an example of the average percentage changes, as compared with the uninjected control litter-mates, in the weight of the prostate alone and of the prostate and seminal vesicles of castrated rats, injected for 7 days with different doses of "H" or "Pr" in 11 litters used for the assay of these preparations. The two lower lines are obtained by dividing percentage change by 40 (see definition of R.U. [Korenchevsky *et al.*, 1932]).

	No. of litter	...	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
% increase in	Prostate + seminal vesicles		219	54	76	80	65	69	152	93	124	95	88
	Prostate alone		217	64	77	78	73	79	142	81	129	94	103
No. of R.U. given by	Prostate + seminal vesicles		5.5	1.4	1.9	2.0	1.6	1.7	3.8	2.3	3.1	2.4	2.2
	Prostate alone		5.5	1.6	1.9	2.0	1.8	2.0	3.6	2.0	3.2	2.4	2.6

A comparison of these figures shows that, in the case of sufficiently purified male hormone preparations, the number of rat units is the same or nearly the same when using the weight of the prostate alone or together with the seminal vesicles.

Again, a striking similarity in the results of the percentage increase in the prostate + seminal vesicles and in the prostate alone after 21 days of injection is seen in Table IV (see column "% change on controls, Pr 1.5").

Moreover, when assaying various batches of "Pr" and one batch of "H," we found that the strength of these batches in our proposed rat units was the same as the value given in capon units, *i.e.* 1 C.U. was about equal to 1 R.U. [Korenchevsky *et al.*, 1932; 1933, 2]. The percentage changes in the prostate are thus suitable for the assay of that male hormone which produces comb-growth in capons, for which purpose many investigators have found the comb-growth method to be satisfactory.

We have found in the previous papers, however [Korenchevsky *et al.*, 1933, 1, 2], that comparing the number of C.U. with the number of R.U. (as defined by us) the whole sexual activity in some preparations was about double that of the comb-growth activity. We should, therefore, have failed to recognise half the sexual activity of some preparations if we had not used our method of assay.

Thus the results obtained seem to justify the use of this method of assay, since it gives at least two data at the same time: (1) on prostate alone, an assay of the hormone promoting comb-growth, and (2) on prostate with seminal vesicles (weighed together), an assay of the whole male sexual activity of the preparation. In addition, the percentage changes in the seminal vesicles alone may give supplementary information as to the presence of oestrin, or oestrin-like substances, in the preparation.

It seems to us that the changes in weight of the seminal vesicles alone cannot be taken as a measure of the whole male sexual activity, since their exceptionally great reaction to oestrin and oestrin-like substances is not shown by the remaining male secondary sexual organs.

On the other hand, the change in the weight of the seminal vesicles + prostate gives a rough representation of the true result of the whole sexual activity, since the changes due chiefly to oestrin-like substances (seminal vesicles) and the changes due to the comb-growth-promoting substance (prostate) are given in their natural relationship. In addition, from the results of some of our present experiments, it would seem that the reaction of the seminal vesicles alone does not always give results which are regular enough for assay purposes in castrated rats.

We give as an example the following experiment taken at random and performed on a litter of castrated rats in the assay of one batch of preparation "H" (changes per unit of body weight):

		1.5 "H" alone	1.5 "H" + 60 oestrin
% change in weight of	Prostate + seminal vesicles	76	133
	Prostate alone	77	92
	Seminal vesicles alone	67	300

Assuming the effect of oestrin on the prostate to be comparatively negligible for assay purposes, the strength in the preparation of the hormone promoting comb-growth was between 1.9 and 2.3 R.U. (77/40 or 92/40); the whole sexual activity of the preparation "H" injected alone was 1.9 R.U. (76/40); and that of "H" injected with oestrin 3.3 R.U. (133/40). The presence of the "oestrin-like substance" is suggested by the very large difference in the percentage increase of the seminal vesicles alone and of the prostate alone; and by a considerable increase in the number of R.U. of the "whole sexual" activity as compared with "comb-growth" activity. It should be noted that for our method of assay an average of the results of at least 3 litters is used.

On the manifold function of the sexual hormones in males.

Korenchevsky [1930], from the results of his experiments on castrated and cryptorchid rats, suggested that the male gonads produce at least two hormones, both of which influence the secondary sexual organs. The results of the present experiments indicate a similar conclusion, since both testicular hormone and oestrin have a stimulating effect on the secondary sexual organs.

In the paper already mentioned, as well as in a later paper [Korenchevsky and Dennison, 1934, 1], it was shown that in male rats castration produces changes in many non-sexual organs and functions: a decreased gain in body weight, increased fat deposition, slight atrophy of the thyroid, liver and kidneys, delay in involution of the thymus and hypertrophy of the adrenals and hypophysis. On the other hand, the results in our previous and present experiments show that testicular hormone preparations cause a return towards normal of the changes in the thyroids, adrenals, thymus, liver and kidneys and improved the gain in body weight but do not influence the hypophysis or decrease the deposition of fat. This of course only refers to the purified preparations which we were using and not to a pure testicular hormone.

In castrated male rats, oestrin produced in addition to the changes in the secondary sexual organs which have been mentioned above, a decrease in body weight, hypertrophy of the hypophysis, acceleration in the rate of involution of the thymus and possibly had a slightly (but not constant) stimulating effect on the kidneys and spleen.

Thus, on the basis of our experiments on the effects of castration and of injections of testicular hormone and oestrin, alone or combined, these two hormones cannot be regarded as sexual hormones only, but as hormones having

manifold functions, of which one is a sexual function. Even before the completion of the histological investigation such a conclusion seems to us to be justified.

Before drawing any definite conclusions as to the influence on the catabolic processes, which from our results also seems to be present, it is necessary to carry out more numerous and direct experiments, including investigations of the gaseous, nitrogenous and mineral metabolism.

SUMMARY.

1. Experiments were performed on 73 castrated rats belonging to 16 litters and injected for 7 or 21 days with preparations of testicular hormone, alone or in combination with oestrin.

2. The comb-growth method does not assay the whole sexual activity of male hormone preparations, while this is given by the assay on the prostate with seminal vesicles of castrated rats.

3. The addition of oestrin to testicular hormone injections greatly increased the weight of the seminal vesicles (especially with one of the preparations) and (more particularly in the 7-day experiments) caused only a slight, if any, increase in the weight of the prostate, penis and preputial glands.

4. An alteration in the method of assay previously described and in the definition of the rat unit is therefore suggested; namely, that the percentage change in weight of the prostate alone shall be used for the assay of the hormone "promoting comb-growth," and that of the prostate and seminal vesicles weighed together for the assay of "the whole male sexual activity" of the sexual hormone preparations. For this latter assay the changes in the weight of the seminal vesicles in relation to the changes in the weight of the prostate are also important.

5. This alteration in the method does not cause any significant change in the number of R.U. in purified testicular hormone preparations, but eliminates those produced by additional sexual activity (*e.g.* oestrin) in less purified preparations.

6. The rat unit suggested is the minimum daily dose, which when injected twice a day in half doses, during 7 consecutive days, into at least three litters of rats castrated before the 30th day after birth, will produce, on the average, an increase of 40 % in the weight of prostate alone (for the assay of "comb-growth hormone") or in the weight of the prostate with seminal vesicles (for the assay of the "whole male sexual activity") as compared with the average weight of the respective organs in the uninjected litter-mates. The rats for assay should not be used earlier than 30 days after castration.

7. The reasons for not using the changes in weight of the seminal vesicles alone for the assay of the "whole male sexual activity" of the preparations were discussed.

8. The male and female hormones in the male organism are not sexual hormones only, but hormones with manifold effects on other important functions and organs.

9. For ascertaining the other properties of testicular hormone preparations, in addition to the assay of sexual activity, it is most suitable to use rats which have been castrated for not less than 70 days and injected with suitable doses for not less than 21 days.

10. The effect of testicular hormone of increasing the weight of the liver and kidneys (which has been previously described) was corroborated in these experiments.

11. Besides the effect on the sexual organs, the addition of oestrin to testicular hormone injections was followed by a decreased gain in body weight and fat deposition, an acceleration in the speed of involution of the thymus, an increased hypertrophy of the hypophysis and a slight increase in the weight of the kidneys.

12. Some differences were noted between the effects of two preparations of testicular hormone when used in combination with oestrin injections.

A grant from the Medical Research Council and the hospitality of the Lister Institute have enabled us to carry out this research and to them our thanks are due.

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