CXCVI. THE EFFECT OF OESTRONE ON NORMAL AND CASTRATED MALE RATS.

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It is now established that male and female hormones are produced in both sexes. In the urine of man and non-pregnant woman they are often excreted in approximately equal amounts [Dingemanse, 1931; Womack and Koch, 1932; Freud, 1933]. Zondek [1934] obtained the largest amount of oestrin from the urine of stallions, in which he found 170,000 M.U. per litre; while he obtained 200 M.U. from the non-pregnant and 100,000 M.U. from the pregnant mare; and from 30 to 200 M.U. in sexually mature woman and 10,000 M.U. in pregnant woman. Zondek and Euler [1934] suggest that in the organism oestrin is produced in the gonads, in the placenta and probably in some other tissues, since Loewe *et al.* [1932] found small amounts of oestrin even in the urine of castrated animals. These facts make necessary a careful examination of the influence of oestrin on males.

That oestrin had a physiological influence on young castrated rats was shown by Freud [1933]. Both oestrone and oestriol promoted the development of smooth muscle in the seminal vesicles of such rats and increased the influence of testicular hormone on the seminal vesicles.

That oestrin has a pathological influence has also been demonstrated. Thus Moore and Price [1932] injected 10–15 rat units of oestrin for 20 days into adult male rats and obtained a marked decrease in size of the testes, seminal vesicles and prostate, the atrophy of these last two organs sometimes being equal to that in castrates. Simultaneous injection of gonad-stimulating, or testicular hormone, however, prevented the injurious effect of oestrin (except the testicular damage which was not prevented when testicular hormone was used). In castrated male rats no effect was produced by injections for 20 days of oestrin alone (12–40 R.U.), or in doses of 12 R.U. combined with 6 C.U. of testicular hormone.

Lacassagne [1933] used young, adult and castrated mice injected for different periods with about 500 M.U. per week of oestrin. During the first 2 weeks atrophy of the seminal vesicles and prostate were noted, but later a pathological enlargement of the posterior lobe of the prostate developed, with stratification and keratinisation of the epithelium. These changes were usually observed after treatment for 3 months.

Burrows and Kennaway [1934] painted the skin of mice twice a week, for 6–22 weeks, with a solution of oestrin in a dose of about 125 R.U. The following changes were obtained in male mice; scrotal hernia, atrophy of the testes and seminal vesicles, enlargement of the posterior lobe of the prostate with histological changes similar to those observed by Lacassagne, and even in some cases resembling those in tumours. These pathological changes indicate the need for caution in following the suggestion of Freud [1933] that oestrin should be used in clinical practice in males.

In the present paper, only the changes in weight of the sexual, endocrine and some other organs are given. The histological results will be published elsewhere.

Table I. *Age, average weight, gain in body weight, and period of injections of normal rats.*

No. of litter	No. of rats in litter	Period of injections Days	Final age Days	Final weight, g.				Gain in body weight, g.			
				Controls	20 u.	60 u.	180 u.	Controls	20 u.	60 u.	180 u.
17	4	32	60	231	234	—	—	167	166	—	—
18	3	29	64	235	235	—	—	144	137	—	—
19	4	43	66	260	—	243	—	218	—	200	—
20	6	34	59	241	—	213	—	190	—	165	—
21	5	35	58	234	—	181	—	190	—	139	—
22	3	32	57	198	—	—	175	144	—	—	120
23	4	43	65	300	—	—	252	256	—	—	208
24	5	34	65	271	—	—	217	195	—	—	136
25	5	35	58	265	—	—	209	215	—	—	159

Table II. *Effect on sexual organs and thymus of prolonged injections of oestrone into normal rats.*

		Weights					
		Actual			Per 200 g. body weight		
		20 u.	60 u.	180 u.	20 u.	60 u.	180 u.
Prostate with sem. ves., mg.	Controls	951	1443	1326	817	1185	1015
	Injected	1015	658	633	865	603	568
% change	Average	—	**-54**	-55	—	**-48**	**-45**
	Maximum	+17	-71	-72	+16	-65	-67
	variations	- 4	-39	-43	- 5	-31	-32
Prostate, mg.	Controls	513	809	895	441	660	552
	Injected	515	417	413	460	384	373
% change	Average	—	**-48**	**-45**	+5	**-41**	**-33**
	Maximum	+2	-67	-60	+9	-57	-54
	variations	-1	-35	-32	+1	-26	-19
Sem. ves., mg.	Controls	439	648	606	377	525	463
	Injected	471	241	221	400	218	197
% change	Average	—	**-62**	**-66**	—	**-57**	**-59**
	Maximum	+26	-79	-86	+26	-74	-83
	variations	-13	-46	-56	-14	-39	-48
Penis, mg.	Controls	336	310	309	289	253	246
	Injected	334	229	221	285	215	204
% change	Average	—	**-26**	**-28**	—	**-15**	—
	Maximum	-4	-36	-53	-6	-17	-46
	variations	+3	-20	-14	+3	-10	+ 2
Preputial glands, mg.	Controls	140	153	136	120	125	112
	Injected	146	117	107	125	111	101
% change	Average	—	**-23**	**-24**	—	**-11**	—
	Maximum	+29	-37	-40	+29	-18	-31
	variations	-12	-13	-14	-11	- 3	+ 8
Testes, mg.	Controls	2765	2351	2482	2381	1918	1966
	Injected	2601	1973	2140	2220	1855	2031
% change	Average	**-6**	**-16**	**-14**	**-7**	—	—
	Maximum	-8	-23	-34	-6	-10	+25
	variations	-4	-10	- 2	-7	+ 2	-24
Thymus, mg.	Controls	478	440	527	418	362	411
	Injected	516	518	431	441	493	403
% change	Average	**+8**	—	**-17**	**+6**	**+40**	—
	Maximum	+9	+54	-30	+7	+67	-14
	variations	+7	- 7	- 7	+5	+20	+ 8

Technique.

Experiments were performed on 39 normal rats belonging to 9 litters and on 73 castrated rats belonging to 16 litters. The number of rats in each litter, period of injection and daily dose of oestrin, the final age and weight of the rats and, in the case of castrated rats, the age at castration and the period after castration when killed, are given in Tables I and IV. In litters 14 and 15, control rats had very slight pneumonia and therefore were not excluded from the experiments, although there was some depressing effect on gain in body weight and fat deposition.

Table III. *Effect on adrenals, hypophysis, liver, kidneys, spleen, heart and food intake of prolonged injections of oestrone in normal rats.*

		Weights					
		Actual			Per 200 g. body weight		
		20 u.	60 u.	180 u.	20 u.	60 u.	180 u.
Adrenals, mg.	Controls	61·6	49·8	50·6	53·2	40·7	40·5
	Injected	75·0	67·1	72·7	64·1	62·1	68·1
% change	Average	+22	+34	+45	+20	+53	+73
	Maximum	+27	+57	+60	+24	+61	+94
	variations	+16	+18	+32	+17	+44	+42
Hypophysis, mg.	Controls	7·7	10·5	10·4	6·7	8·6	8·2
	Injected	9·2	11·5	13·0	7·9	11·1	12·2
% change	Average	+20	+12	—	+19	+29	+52
	Maximum	+29	+32	+46	+29	+41	+75
	variations	+12	+ 1	− 8	+ 9	+16	+ 5
Liver, g.	Controls	15·65	9·66	11·84	13·33	7·96	9·44
	Injected	16·46	9·06	10·11	14·04	8·56	9·62
% change	Average	+5	—	−15	+5	+ 8	—
	Maximum	+8	−16	−22	+8	+11	+8
	variations	+2	+ 0·2	−12	+3	+ 6	−1
Kidneys, g.	Controls	2·36	1·94	2·18	2·02	1·58	1·71
	Injected	2·33	1·76	1·89	1·99	1·66	1·79
% change	Average	—	−10	−13	—	—	—
	Maximum	−8	−16	−21	−9	+8	+8
	variations	+6	− 5	− 5	+6	−1	−1
Spleen, mg.	Controls	581	529	566	497	434	440
	Injected	713	595	531	608	564	500
% change	Average	+23	+12	—	+23	+30	—
	Maximum	+29	+16	−24	+29	+42	+27
	variations	+17	+ 9	+12	+17	+23	− 8
Heart, mg.	Controls	816	759	812	704	671	637
	Injected	823	728	760	702	692	714
% change	Average	—	—	− 7	—	—	+13
	Maximum	+2	−13	−11	−3	+12	+21
	variations	−0·4	+ 7	− 3	+2	− 9	+ 7
Food intake, g.	Controls	—	50·3	54·4	—	48·8	45·2
	Injected	—	44·9	48·4	—	50·4	48·2
% change	Average	—	−11	−11	—	+3	+7
	Maximum	—	−17	−13	—	+7	+9
	variations	—	− 6	− 8	—	+0·4	+5

All the experiments were performed using the same technique as has been described in our previous papers. Oestrone, dissolved in olive oil after preliminary solution in a small quantity of the monoethyl ether of ethyleneglycol, was injected subcutaneously once a day. The pure crystalline preparation of oestrone was received from the Department of Biological Standards to which we wish to express our thanks. For litters 10, 12, 14 and 15 pure oestrone dissolved in arachis oil was kindly given to us by Dr A. S. Parkes, whom we also wish to thank.

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

No. of litter	No. of rats in litter	Age in days: At castration	Age in days: When killed	No. of days after castration when killed	Final weight, g.: Control	10 u.	20 u.	60 u.	180 u.	Period of injections Days
1	4	23	74	51	275	270	—	—	—	7
2	3	28	64	36	200	228	—	—	—	7
3	4	23	74	51	243	—	264	—	—	7
4*	2	23	74	51	—	—	266	—	—	7
5	4	21	72	51	267	—	—	254	—	7
6	2	27	63	36	208	—	—	176	—	7
7	6	25	65	40	245	—	—	244	233	7
8	3	21	72	51	268	—	—	—	244	7
9	4	25	61	36	205	—	—	—	192	7
10	4	26	106	80	285	—	303	—	—	29
11	3	23	78	55	340	—	265	—	—	43
12	4	25	104	79	284	—	—	277	—	28
13	3	23	78	55	289	—	—	252	—	43
14	3	28	93	65	261	—	—	—	294	28
15	3	23	88	65	281	—	—	—	291	28
16	3	22	77	55	380	—	—	—	270	43

* Control rat in litter 4 excluded because of severe pneumonia. In the following tables for litter 4 respective figures of control rats of the litter 3 are used, both litters being of the same age and castrated at the same date.

Table V. *Effect on sexual organs of 7 days of injections of oestrone into castrated rats.*

		Weights: Actual: 10 u.	20 u.	60 u.	180 u.	Per 200 g. body weight: 10 u.	20 u.	60 u.	180 u.
Prostate with sem. ves., mg.	Controls	72	72	70	70	62	60	59	58
	Injected	79	90	94	99	65	68	86	89
% change	Average	**+10**	**+24**	**+34**	**+43**	**+5**	**+13**	**+45**	**+55**
	Maximum	+18	+29	+42	+56	+5	+17	+57	+64
	variations	+ 3	+19	+28	+29	+5	+ 8	+35	+42
Prostate, mg.	Controls	61	58	58	57	52	48	49	48
	Injected	64	72	71	73	52	54	65	66
% change	Average	—	**+23**	**+23**	**+30**	—	**+13**	**+31**	**+38**
	Maximum	+14	+28	+42	+44	+2	+17	+43	+49
	variations	- 5	+19	+14	+16	-2	+ 8	+19	+26
Sem. ves., mg.	Controls	12	15	14	13	10	12	10	10
	Injected	16	18	23	26	13	14	22	23
% change	Average	**+37**	**+20**	**+ 89**	**+102**	**+37**	**+17**	**+110**	**+105**
	Maximum	+44	+27	+115	+122	+40	+25	+166	+140
	variations	+29	+13	+ 50	+ 75	+33	+ 8	+ 51	+ 82
Penis, mg.	Controls	91	100	96	94	76	82	84	79
	Injected	109	96	97	104	88	73	87	94
% change	Average	**+20**	—	—	**+10**	**+14**	**-12**	—	**+20**
	Maximum	+26	-10	-20	+18	+16	-17	+24	+30
	variations	+15	+ 2	+16	+ 5	+11	- 6	- 1	+12
Preputial glands, mg.	Controls	63	71	57	59	54	59	49	48
	Injected	70	68	65	80	55	51	63	75
% change	Average	—	**-5**	**+13**	**+39**	—	**-14**	**+27**	**+58**
	Maximum	+41	-6	+20	+49	+44	-14	+41	+73
	variations	-22	-4	+ 7	+30	-32	-14	+14	+42

Table VI. *Effect on sexual organs and hypophysis of prolonged injections of oestrone into castrated rats.*

		Weights					
		Actual			Per 200 g. body weight		
		20 u.	60 u.	180 u.	20 u.	60 u.	180 u.
Prostate with sem. ves., mg.	Controls	80	74	80	52	52	52
	Injected	96	123	148	69	95	101
% change	Average	**+20**	**+68**	**+ 89**	**+33**	**+ 85**	**+100**
	Maximum variations	+20	+90	+110	+53	+102	+121
		+19	+46	+ 57	+13	+ 68	+ 84
Prostate, mg.	Controls	63	60	65	41	42	43
	Injected	68	85	92	48	66	64
% change	Average	**+ 8**	**+45**	**+44**	**+20**	**+59**	**+50**
	Maximum variations	+10	+66	+66	+41	+74	+52
		+ 6	+23	+ 9	± 0	+43	+46
Sem. ves., mg.	Controls	18	15	12	12	10	12
	Injected	29	38	49	21	29	35
% change	Average	**+66**	**+165**	**+322**	**+82**	**+198**	**+358**
	Maximum variations	+85	+200	+340	+86	+238	+443
		+48	+129	+292	+78	+158	+289
Penis, mg.	Controls	86	95	101	57	67	66
	Injected	100	98	107	71	75	75
% change	Average	**+17**	—	—	**+31**	**+12**	**+16**
	Maximum variations	+23	+12	+45	+57	+15	+41
		+11	− 5	−26	+ 4	+ 9	+ 3
Hypophysis, mg.	Controls	13·7	13·5	13·7	8·9	9·4	9·2
	Injected	15·3	17·9	21·3	10·9	13·8	14·9
% change	Average	**+12**	**+33**	**+61**	**+24**	**+48**	**+63**
	Maximum variations	+13	+45	+95	+44	+54	+72
		+11	+22	+19	+ 3	+41	+49

Table VII. *Effect on hypophysis, thymus, kidneys and heart of 7 days of injections of oestrone into castrated rats.*

		Weights							
		Actual				Per 200 g. body weight			
		10 u.	20 u.	60 u.	180 u.	10 u.	20 u.	60 u.	180 u.
Hypophysis, mg.	Controls	11·5	12·4	11·9	11·4	9·9	10·3	10·0	9·6
	Injected	15·5	14·6	14·3	14·7	12·3	11·1	12·9	13·3
% change	Average	**+35**	**+17**	**+22**	**+30**	**+26**	**+ 7**	**+30**	**+38**
	Maximum variations	+40	+20	+43	+37	+43	+10	+43	+44
		+30	+15	± 0	+25	+10	+ 5	+18	+34
Thymus, mg.	Controls	585	550	680	699	504	452	611	590
	Injected	547	697	583	515	449	525	540	475
% change	Average	—	**+27**	**−21**	**−25**	**−12**	**+16**	—	—
	Maximum variations	−24	+30	−20	−37	−23	+18	−26	−35
		+12	+23	− 8	− 5	− 2	+14	+ 8	+ 3
Kidneys, g.	Controls	1·94	1·79	1·90	1·98	1·66	1·47	1·60	1·66
	Injected	2·10	2·05	2·14	2·15	1·70	1·55	1·92	1·94
% change	Average	**+ 9**	**+15**	—	**+ 9**	**+3**	**+5**	**+20**	**+17**
	Maximum variations	+16	+17	+25	+17	+4	+7	+25	+23
		+ 1	+12	− 1	+ 1	+2	+4	+17	+11
Heart, mg.	Controls	725	682	705	694	617	562	588	582
	Injected	810	776	723	719	653	588	646	645
% change	Average	**+12**	**+14**	—	—	**+ 6**	**+5**	**+10**	**+11**
	Maximum variations	+16	+15	+18	+ 8	+11	+6	+18	+14
		+ 9	+12	− 3	− 1	+ 1	+3	+ 2	+ 5

Table VIII. *Effect on thymus, kidneys, spleen and heart of prolonged injections of oestrone into castrated rats.*

		Weights					
		Actual			Per 200 g. body weight		
		20 u.	60 u.	180 u.	20 u.	60 u.	180 u.
Thymus, mg.	Controls	681	492	704	425	344	451
	Injected	467	439	479	336	333	338
% change	Average	−27	—	−27	−21	—	−24
	Maximum variations	−38	−17	−49	−21	−5	−34
		−15	+1	−7	−21	+0·4	−10
Kidneys, g.	Controls	2·06	1·98	2·38	1·32	1·38	1·55
	Injected	1·96	2·04	2·40	1·38	1·54	1·68
% change	Average	—	—	—	+4	+11	+8
	Maximum variations	−15	+13	−23	+9	+16	+9
		+7	−8	+22	+0·1	+7	+8
Spleen, mg.	Controls	633	530	620	408	369	395
	Injected	584	617	670	412	462	472
% change	Average	—	+17	—	—	+25	+23
	Maximum variations	−15	+29	+50	+9	+30	+46
		+0·3	+4	−21	−6	+20	+11
Heart, mg.	Controls	928	899	918	598	626	605
	Injected	862	868	930	602	656	653
% change	Average	—	—	—	—	+5	+8
	Maximum variations	−14	−8	+15	+10	+6	+19
		+0·2	+1	−15	−8	+4	+1

Litters 11, 13, 16, and 19–25 were killed by bleeding from the abdominal aorta, the remaining litters being killed by coal gas. The difference between the actual weights of some organs in these two groups of litters was considerable (*e.g.* liver). However, since, in every case, the control rats were litter-mates of the injected groups, the percentage changes were comparable. Therefore, general averages of each group were made, irrespective of the method of killing, the results being summarised in Tables II–VIII. The tables are constructed on the same principle as previously [*e.g.* Korenchevsky *et al.*, 1932, Table II, p. 2099]. The average weights and the average percentage changes in weight in each group of each litter were obtained for each organ. Then general means were obtained by summing the average weights or average percentage changes and dividing by the number of litters. These general means are given in the tables and therefore the percentage changes are slightly different from the results calculated from the general means of the weights given in the tables in the two first lines for each organ. In addition to the average percentage change, the greatest variations in the percentage change, above and below the mean value, which were observed in the litters, are given in the 4th and 5th lines for each organ. Where the percentage changes in the weight of the organ showed an increase (+) in some, and a decrease (−) in other litters, no average is made, a blank, signifying non-constant changes, being used in place of the average figure (3rd line for each organ). For example, in Table II, last column, the average weight of the testes as compared with the average for the control litter-mates was 25 % larger in one litter and 24 % smaller in another. No average figure of the percentage change, therefore, could justifiably be given. Thus these tables, although economising space, give an accurate representation of the degree, variations and constancy of the changes obtained. Our long experience in applying our technique to the study of changes in the weights of organs has shown us that, when this technique is strictly followed in all details, even small changes may be significant and specific if they are constant.

EFFECT ON NORMAL RATS.

Each litter was divided into a control group and a group injected with 20, 60 or 180 international units of oestrin per day for a period varying from 29 to 43 days (Table I). Although the injections were started before the age of sexual maturity, the rats should have been mature by the last injection, since they were killed at 57–66 days old, by which age the rats of our stock are sexually mature.

Influence on body weight, fat deposition and food intake. As can be seen from Table I, the rats injected with daily doses of 60 or 180 I.U. weighed less and had a smaller gain in weight than their control litter-mates.

A rough estimate of food intake did not show any noticeable change in the appetite of rats injected with 20 I.U. The food intake (Table III) was therefore only recorded in the litters receiving 60 and 180 units of oestrin. The actual food intake was decreased on the average by 11 % in all the injected rats. When calculated per unit of body weight, however, the food intake was unchanged or, in most cases, slightly increased. The decreased appetite therefore could only have been partly responsible for the retarded growth and decreased gain in weight. Probably excessive amounts of oestrin injected into normal male rats injure in some way the catabolic processes in the organism. This injurious effect is probably related to the size of the dose, for the gain in body weight per 10 g. of actual food intake was as follows: in two groups of controls, 1·1 and 1·1 g., in the group injected with 60 units, 1·0 g. and in the group injected with 180 units, 0·9 g., *i.e.* the definite decrease of about 19 % was only found in the group receiving the large dose, although the decrease in actual food intake was practically identical in the two groups (Table III).

Fat deposition was not tabulated since the changes, which were mostly small, were not constant. Out of seven litters, there was a decrease in five of from 4 to 26 %, and increases in two of 20 and 70 % respectively (calculated per unit of body weight). Such a degree of fluctuation in fat deposition may occur physiologically, as has been shown in our previous paper [Korenchevsky and Dennison, 1934, pp. 240, 241].

Influence on sexual organs. In agreement with the previous workers mentioned above, we have found injections of large doses of oestrin to have a depressing effect on the actual weights of all the sexual organs (Table II). The comparatively small dose of 20 I.U. did not produce a constant or large effect. The depressing effect of doses of 60 or 180 I.U. was greatest in the case of seminal vesicles and prostate and least in the testes, the influence on the penis and preputial glands being intermediate. When calculated per unit of body weight the influence was still evident in the prostate and seminal vesicles, but the changes in the testes, penis and preputial glands became inconstant or small. It must be taken into consideration that a decreased food intake can produce similar changes in the weight of the sexual organs [Sampson and Korenchevsky, 1932], but not to such an extent as was observed in the present experiment. Therefore, if there was any injurious effect on the sexual organs of reduced appetite, the chief cause of the changes in these organs was the specific injurious effect of oestrin and not of the decreased food intake.

Effects on adrenals and hypophysis. As can be seen from Table III, in male rats the adrenals and hypophysis belong to those endocrine organs the weight of which is influenced in every case (see figures calculated per unit of body weight) by both the small and large doses used. The maximum variations are not so large in the adrenals as in the hypophysis, but for both organs the

hypertrophy was considerable in most of the injected rats. The changes in the actual weight are less pronounced in most groups and, in the case of the hypophysis, less constant.

From the foregoing, the changes in these two organs are seen to be the results of the specific influence of oestrin.

Effects on thymus and spleen. The figures in Tables II and III show that the changes obtained were comparatively small and were more definite and constant in the spleen than in the thymus. A slight or moderate increase in the weight of the spleen was observed in most of the rats injected with 20 and 60 I.U. of oestrin and in some cases though to a smaller degree, in the rats injected with the very large dose of 180 I.U. In the thymus a constant and more significant delay in involution (per unit of body weight) was noticeable only in the group injected with 60 I.U. of oestrin, while with 20 units this effect was very small and with 180 units was both small and variable. The chief reason for giving the data relating to these organs is that the male sexual hormone increases the speed of involution of the thymus in both normal and castrated rats, castration delaying this involution [Korenchevsky *et al.*, 1933; Korenchevsky and Dennison, 1934], while a very slight decrease in the weight of the spleen was found in most castrated rats [Korenchevsky and Dennison, 1934].

More numerous experiments, however, using different doses of oestrin, are necessary before the delay in involution of the thymus obtained with some doses can be considered to be specific.

Effects on liver, kidneys and heart. The effects were similar in these three organs (Table III). The actual weights of these organs were slightly decreased, while when calculated per unit of body weight these changes became less pronounced or were replaced by very small increases in weight. Changes of this type in the liver and kidneys are typical of rats in which the appetite is decreased (our unpublished experiments). It is therefore probable that these changes were chiefly due not to the specific effect of oestrin but to the decreased food intake. The possible significance of small changes in the heart will be discussed later.

Effect on castrated rats.

In our previous papers we have studied the effect on castrated rats of testicular hormone injected for 7 days (chiefly for purposes of assay) and for the longer period of 3 weeks. Similarly, the injections of oestrin in the following experiments were made for a period of 7 days (Tables V and VII) and for longer periods of 28–43 days, after which period all the changes might be expected to be more pronounced (Tables VI and VIII). All the rats were castrated before sexual maturity and killed from 36 to 80 days after castration, at ages varying in different litters from 61 to 106 days (Table IV).

A study was made of the effects of four different doses 10, 20, 60 and 180 I.U. of oestrin.

Effects on body weight, fat deposition and food intake. The effect on the gain in body weight was noticeable in the long duration experiments in all the groups injected with 20, 60 and 180 units of oestrin. Thus, on the average, the gains in weight in the three groups mentioned above were 88, 83 and 86 g. respectively, while in the corresponding control litter-mates they were 127, 114 and 127 g. In the short duration experiments no definite effect was noticed in the groups injected with 10 or 20 units, but the check in the gain in body weight was significant in most of the rats injected with 60 and 180 units.

Changes in the food intake were recorded in only 3 litters, Nos. 11, 13 and 16, and since in these the food intake was decreased in 2 litters (injected with

20 units and 180 units of oestrin) and increased in the third (injected with 60 units of oestrin), more numerous experiments are required for definite conclusions.

Changes in the fat deposition were small. In the 7-day experiments the change in the amount of retroperitoneal fat did not exceed 36 %, 6 litters showing an increase and 3 a decrease. Of the 7 litters injected for a longer period the amount of retroperitoneal fat was slightly increased in 4 litters and decreased in 3 litters, the changes per unit of body weight in no litter exceeding the same figure of 36 %. Since such variations in the fat deposition are within the limits of physiological fluctuations, they are not given in the tables.

Effect on sexual organs. The effect on the sexual organs of castrated rats (Tables V and VI) was the reverse of that on normal rats, namely a constant increase in the weight of the prostate and seminal vesicles and a less constant increase in the weight of the penis and preputial glands. Between the doses of 10 and 60 units, small and large doses were followed by a corresponding difference in the effect. This difference in the response was small or not present in most organs between doses of 60–180 units. No direct proportional relation between the dose and the reaction could be seen however, except perhaps an approximate average percentage increase in the prostate or prostate with seminal vesicles in rats injected with 20 units as compared with rats injected with 60 units (Tables V and VI). The changes obtained were largest in the seminal vesicles and smallest in the penis.

The changes in the preputial glands were constant with large doses only in the 7-day experiments, becoming variable after prolonged injection, and are therefore not included in Table VI. For example, as compared with their control litter-mates the preputial glands (per unit of body weight) were increased by 13 and 30 % in 2 litters injected with 20 units of oestrin, increased by 56 % and decreased by 15 % in 2 litters injected with 60 units and decreased by 27 % and increased by 51 and 126 % in 3 litters injected with 180 units. Large variations, therefore, are obtained in preputial glands. This may partly be explained by the fact that these glands are difficult to dissect accurately, and the secretion is easily squeezed out during the dissection. The increase in the weight of the prostate was comparatively small, especially with small doses, being absent with 10 units and from 8 to 17 % with 20 units in 7-day experiments.

The effect on seminal vesicles of long duration injections was much greater than that of short duration injections, the former being, on the average, from two to five times the latter. The effect on the prostate of prolonged injection in most cases did not exceed, and was usually less than, double that obtained with 7 days of injection.

In the penis and preputial glands no definite or constant difference could be seen between the effects of short and long duration experiments.

Effect on hypophysis. As in normal rats, the weight of the hypophysis in castrated rats nearly always increased after the injection of oestrin both for 7 days and for the longer period, the increase however sometimes being small (Tables VI and VII). On the average the larger doses were followed by greater hypertrophy of the hypophysis only in the longer experiments, in which also the largest increases in this organ occurred (up to 72 %).

Effect on thymus. An increase in the involution of the thymus, although not constant or large, was present in most of the injected rats, especially after the injection of large doses for 7 days and after prolonged treatment. In castrated rats, therefore, the effect of oestrin on the thymus, though less constant, was similar to that of testicular hormone.

Effects on the liver, kidneys, spleen and heart. No definite changes were observed in the liver. In most rats, injections of oestrin slightly increased the weight of the kidneys and spleen, the changes in the spleen thus being similar to those obtained in normal rats.

If any significance can be ascribed to the very small increase in weight observed in the heart in most injected rats, this is also similar to that observed in normal rats.

Note on the effects of oestrone on the weights of organs in castrated female rats. In order to obtain some idea of the changes in the organs of castrated females as compared with those in males, a preliminary experiment was carried out with one litter of females, which was killed 50 days after castration at the age of 97 days. A total of 2340 I.U. of oestrin per rat was injected during a period of 23 days. In the injected rats, in addition to the usual large increase in the size of the uterus, an increase per unit of body weight as compared with the control litter-mates was obtained in the hypophysis (by 83 %) and in the kidneys (by 15 %). The changes were very small in all the other organs.

Discussion.

It is clear, from the results obtained, that oestrin in the doses used had a quite definite effect on the gain in body weight, the weight of the sexual organs, of the adrenals and of the hypophysis of male rats. The hypertrophy of the hypophysis in both normal and castrated rats and of the adrenals in normal rats was an unexpected result of the injection of oestrin. We have previously shown that castration is always followed by such changes in these endocrine organs. The effect of oestrin on the hypophysis of normal or castrated rats may, therefore, be compared with that of castration, irrespective of the presence of the testes. The effect of oestrin on the adrenals of normal rats is also similar to that of castration. Without histological examination of the glands (which is now in progress) it is not possible to give a correct interpretation of these results.

In normal rats oestrin checks the development of the sexual organs. Moore and Price [1932] explain this by the depressing influence of oestrin on the secretion of the hormone of the anterior lobe of the hypophysis, since the simultaneous administration of the gonad-stimulating hormone, or making transplants of the hypophysis, is followed by the normal development of the sexual organs.

It is rather difficult to relate a depression in the activity of hypophysis with the hypertrophy of this gland which we obtained as the result of the injection of oestrin. However, the possibility of the depression of the special cells producing the gonad-stimulating hormone together with the hypertrophy of the other cells of the hypophysis cannot be excluded.

Of the sexual organs oestrin has the greatest effect on the seminal vesicles, which respond both to increase in the dose and in the period of injection. The response of the prostate to oestrin is much less, and the increase in reaction on increasing the dose or period of injection is also less than in the case of the seminal vesicles.

This sensitivity to oestrin, which is great in the case of seminal vesicles and only slight in the prostate, together with the occurrence of both male and female hormones in both sexes, suggests that, of these two glands, the prostate should be used for the assay of testicular hormone and the seminal vesicles as evidence of the possible presence of oestrin in the preparation (though both these organs are sensitive to testicular hormone).

Using the method of assay, previously proposed for male sexual hormone, but using the percentage increase in the weight of the prostate alone (Table V), doses of 60 and 180 I.U. of oestrin would contain, on the average, about 0·75 and 1 R.U. of male sexual hormone activity, while as judged by the percentage increase of the seminal vesicles they would contain about 2·75 R.U. We shall return to this problem in our next paper on the effect of the combined injection of oestrin and testicular hormones.

The stimulation of the penis and preputial glands by oestrin is not so regular as that of the seminal vesicles and prostate.

With regard to the changes in weight of the other organs no definite conclusion as to their specificity can be drawn until the histological examination is completed. Among the more constant changes were the slight increase in weight of the spleen and kidneys. The presence of a definite decrease in the weight of the heart of animals killed a long time after castration (oxen and geldings) necessitates an accurate investigation of this organ in all experiments dealing with the study of the sexual inner secretions. This question has been discussed in detail elsewhere [Korenchevsky and Dennison, 1934, p. 245]. In the present experiments, the increase in the weight of the heart in rats injected with oestrin was very small and not always constant.

Summary.

1. The effect of subcutaneous injections of pure crystalline oestrone was studied in 39 normal and 73 castrated rats.

2. In *normal rats* large daily doses (60 and 180 I.U.) of oestrone decreased the appetite and gain in body weight without any definite change in the fat deposition, depressed development of the sexual organs and produced hypertrophy of the adrenals and hypophysis. Small and less definite changes were found in the thymus and spleen (an increase with some doses), liver and kidney (decrease in actual weight). The changes in these last two organs were explained by a decreased food intake.

3. In *castrated rats*, in both 7-day and long period experiments, oestrone decreased the gain in body weight of most animals (except with 10 and 20 I.U. in 7-day experiments), but did not produce definite changes in the fat deposition, greatly increased the weight of the seminal vesicles, slightly increased that of the prostate and, less constantly, that of the penis and preputial glands and produced hypertrophy of the hypophysis. With larger doses, or after a prolonged period of injection, the rate of involution of the thymus was increased in most rats. Less definite changes (slight increase with some doses) were observed in the kidneys and spleen. The changes in the remaining organs investigated were very small.

4. It may therefore be concluded that the presence of oestrin in the male organism has a definite effect on some of the organs which are also influenced by testicular hormone.

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