XLVII. THE MANIFOLD EFFECTS OF PROLONGED ADMINISTRATION OF SEX HORMONES TO FEMALE RATS

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IN his excellent book Simonnet [1937] has collected and analysed the extensive literature on the effects of oestrogenic hormones, while the papers on the effects on females of male hormones and their co-operative and antagonistic activities with oestrogens have recently been reviewed by Korenchevsky [1939].

Several workers have studied the effects of prolonged administration of oestrogenic hormones, but long duration experiments with male hormones on females are urgently needed.

In our previous papers [see references, Korenchevsky, 1937] we have studied the effects of male sex hormones on normal and spayed rats for the comparatively short period of 21 days. It seemed probable that certain weak and inconstant changes, produced especially in the non-sexual organs, might be more clearly defined if the period of administration of the hormones were prolonged. In the present experiments, therefore, the rats were injected for the comparatively long period of 3-3½ months, and, as will be shown in this paper, several aspects of the problem were made clear. Such an investigation is also of clinical importance, since the hormones are often applied for long periods and in large doses in order to obtain the desired results.

In the present experiments the following sex hormones have been investigated:

(1) The natural male hormones, i.e. those which are known to occur naturally in the female organism, namely androsterone [Callow & Callow, 1938] and *trans*dehydroandrosterone [Callow, 1936]. As the "female" activity of these hormones is weak [Korenchevsky *et al.* 1935; Korenchevsky & Dennison 1936, 1, 2], large doses of 7.5 mg. a week were given.

(2) Testosterone propionate was included as possessing the strongest female activity of all the known male hormones [Korenchevsky, 1937; Korenchevsky & Hall, 1937]. Medium (2.25 mg. a week) and large (7.5 mg. a week) doses were used.

(3) Of the oestrogens, oestradiol dipropionate was chosen because according to Miescher *et al.* [1938] it appears to be one of the most interesting esters of oestradiol, with a not too prolonged activity on oestrus and growth of the uterus, but of which otherwise little is known. Hormones with very prolonged activity (e.g. oestradiol benzoate-butyrate) have the disadvantage that, once they have been injected, their effects cannot be checked even if desired, and control over their physiological and pathological activities is lost for a very long period [Korenchevsky *et al.* 1939, 1].

Doses of 18, 90 and 200 μ g. a week of oestradiol dipropionate were used alone or simultaneously with the male hormones, in order to investigate their combined effects.

(4) In one group of our experiments, progesterone was given simultaneously with testosterone propionate and large doses of oestradiol dipropionate, the main object of this being to study the property of progesterone of preventing the pathological changes produced by oestrogens in the sexual [Korenchevsky & Hall, 1938, 1] and perhaps in some other organs.

Details of the results of histological investigation will be published elsewhere, a few statements only being included in this paper. Some preliminary results have already been published [Korenchevsky & Hall, 1938, 2].

Technique

The general arrangement of the experiments is clear from Tables I and II. A total of 88 rats was used: 9 normal and 13 spayed animals were kept as controls, and 4 normal and 62 spayed animals were injected with the hormones. The rats in Table I were injected for an average of 87 days, their average age when killed being 131 days. In Table II the average age of the spayed rats in groups XI–XVI was 150 days and that of the normal rats in groups X, XVII and XVIII was 201 days, all the rats having been injected for an average of 109 days. Ovariectomy was performed at the age of 21–24 days.

The male hormones and oestradiol dipropionate were supplied by Messrs Ciba Ltd., progesterone by Messrs Organon Ltd. The hormones were dissolved in sesame oil and were injected: androsterone, *transdehydroandrosterone* and testosterone propionate five times a week, oestradiol dipropionate three times a week (except the dose of 200 μ g. given once a week), progesterone six times a week. The weekly doses are given in the tables. Pure sesame oil was injected into the control rats.

For economy of space the data per unit of body weight have not been tabulated, but will where necessary be referred to in the text.

The other details of experimental technique were the same as in our previous papers.

Table I. *Effects of androsterone and transdehydroandrosterone injected alone or simultaneously with oestradiol dipropionate on actual weights of organs of spayed female rats*

	Spayed rats injected weekly with								
	Controls injected with oil		Oestradiol dipropionate		Androsterone, 7.5 mg.			Dehydroandro- sterone, 7.5 mg.	
					+ Oest- radiol dipropionate		Alone	+ Oest- radiol dipropionate	
	Normal rats	Spayed rats	18 μ g.	90 μ g.	Alone	18 μ g.	90 μ g.	Alone	90 μ g.
	I	II	III	IV	V	VI	VII	VIII	IX
Uterus (mg.)	448	34	266	283	59	327	357	63	366
Vagina with clitoris (mg.)	292	190	332	350	312	416	440	253	366
Preputial glands (mg.)	92	62	74	54	205	126	104	132	84
Adrenals (mg.)	78	70	85	62	57	73	84	57	63
Hypophysis (mg.)	15.4	12.3	37.3	98.0	10.8	30.3	48.0	11.8	55.5
Thymus (mg.)	261	380	253	178	274	223	125	344	172
Liver (g.)	8.66	8.93	8.17	7.61	11.39	10.01	8.96	9.81	8.90
Kidneys (g.)	1.71	1.61	1.78	1.56	2.25	2.11	2.01	1.64	1.70
Spleen (mg.)	515	618	465	446	739	481	630	824	376
Heart (mg.)	730	769	685	623	928	759	723	768	644
Abdominal fat (g.)	18	20	11	9	20	13	9	19	12
Final body wt. (g.)	230	308	218	205	339	257	228	308	225
Gain in body weight (g.)	126	169	82	73	199	103	112	165	76
No. of rats in group	3	8	3	5	4	4	3	4	4

Table II. *Effects of testosterone propionate injected alone or simultaneously with oestradiol dipropionate and progesterone on actual weights of organs of female rats*

Organs	Spayed rats injected weekly with													
	Controls injected with oil		Oest-radiol dipropionate 0.2 mg.	Testosterone propionate 2.25 mg.	Testosterone propionate 7.5 mg.	Testosterone propionate 2.25 mg. + oest-radiol dipropionate 0.2 mg.	Testosterone propionate 2.25 mg. + progesterone 4.5 mg. + oest-radiol dipropionate 0.2 mg.	Normal rats injected weekly with testosterone propionate						
	Normal rats	Spayed rats						0.75 mg.	7.5 mg.					
	X	XI						XII	XIII	XIV	XV	XVI	XVII	XVIII
Uterus (mg.)	526	33	326	262	395	511	337	383	3295					
Vagina with clitoris (mg.)	324	175	311	471	534	484	463	522	611					
Preputial glands (mg.)	85	49	50	198	196	203	121	141	245					
Adrenals (mg.)	66	63	53	50	62	64	60	47	66					
Hypophysis (mg.)	17.6	11.5	132.3	10.6	10.9	60.2	47.2	11.9	11.6					
Thymus (mg.)	155	327	128	123	26	75	55	129	59					
Liver (g.)	8.78	8.69	6.77	10.42	11.18	7.91	7.65	9.58	9.10					
Kidneys (g.)	1.74	1.54	1.52	2.08	2.54	1.86	1.88	1.84	2.51					
Spleen (mg.)	642	503	340	913	820	429	387	841	716					
Heart (mg.)	816	704	596	935	899	621	632	963	1065					
Abdominal fat (g.)	19	22	6	18	12	8	8	17	9					
Final body wt. (g.)	273	323	192	314	290	203	221	303	273					
Gain in body wt. (g.)	67	161	13	139	73	64	56	92	68					
No. of rats in group	6	5	9	7	4	6	9	2	2					

Ovariectomized rats

Effect of ovariectomy. In this paper the changes observed after ovariectomy will be only briefly summarized, the conclusions being drawn from the results of our previous and present experiments on rats. The only really striking changes, following ovariectomy performed before sexual maturity, occur in the sex organs, which become atrophic. Of the non-sexual organs, a definite change occurs in the thymus, the physiological involution of which becomes delayed, i.e. the gland is larger than in control rats of the same age. The gain in body weight increases after ovariectomy, this increase being only partly due to the slight increase in the amount of body fat. Unlike castration, ovariectomy did not produce any pronounced or constant changes in the actual weights of adrenals, hypophysis, liver, kidneys, spleen and heart. The weights of these organs when calculated per unit of body weight, however, were in most cases slightly decreased, which is to be explained by the greater body weight of the spayed rats as compared with that of the normal intact animals.

In males, on the other hand, the gain in body weight decreases after castration. Therefore the effects of gonadectomy on the weights of the organs calculated per unit of body weight are more similar in both sexes with liver, kidneys, spleen and heart than they appear from the actual weights, but with hypophysis and adrenals the differences are more pronounced.

Effect of the hormones on sex organs

Oestradiol dipropionate (Table I, groups III and IV; Table II, group XII). With all doses the weight of the vagina reached normal or even supernormal level, but complete restoration of the uterus was not obtained. The largest

uterus was produced by the largest dose of 200 μ g. (group XII). No constant changes were found in the preputial glands.

In most cases the vagina showed oestrous cornification of the greatly enlarged epithelial layer, or in some cases swollen epithelial cells with dropsical vacuolation. The uterus was of abnormal fibrotic structure. With the smaller doses (18 and 90 μ g.) the epithelial cells were high columnar and in only one rat out of 8 contained a few very small patches of cells with squamous metaplasia (first stage of "precancerous" changes produced by oestrogens). With 200 μ g. the metaplasia of the uterine epithelium was present in about 44 % of rats, in some cases being very pronounced and involving the whole layer, with cornification of the upper row of cells.

Androsterone and transdehydroandrosterone (Table I, groups V and VIII). Both these hormones had only a very weak effect on the uterus, not significantly greater than that produced in our previous experiments of only 21–27 days' duration [Korenchevsky *et al.* 1935; Korenchevsky & Dennison, 1936, 1]. A much greater effect, especially with androsterone, was obtained on the vagina and preputial glands. Dehydroandrosterone, as in our previous experiments [Korenchevsky & Hall, 1937], caused mucification of the vaginal epithelium.

The addition of oestradiol dipropionate had a definite co-operative effect (as judged by weights of the organs) on the activity of androsterone, producing even superdevelopment of the vagina (Table I, groups VI and VII), while the co-operative effect with dehydroandrosterone was much less definite, especially on the vagina (group IX). After these simultaneous injections the effects of the oestradiol predominated on the structure of both vagina and uterus: the vagina in most cases showed oestrous changes, while fibrotic and other changes typical for oestradiol were seen in the uterus.

It is very remarkable that the *metaplastic changes in the uterine epithelium* occurred more frequently and were generally of more pronounced degree when androsterone, and apparently also dehydroandrosterone, were injected simultaneously with oestradiol dipropionate, than when the latter hormone was injected alone. With simultaneous injections the metaplastic changes were present respectively in 83 % (groups VI and VII) and 50 % (group IX), as compared with 13 % in groups III and IV (oestradiol alone).

However, before making a definite statement that androsterone and *transdehydroandrosterone* increase the "oestrogenic precancerous" changes, it will be necessary to repeat the experiment on a greater number of animals.

Testosterone propionate. When this hormone was injected alone (Table II, groups XIII and XIV), a large uterus and supernormal development of the vagina and preputial glands were obtained.

The structure of both the vagina and uterus was similar to that previously described in experiments of 21 days' duration [Korenchevsky & Hall, 1937], the chief features being: in the vagina—mucification of the epithelium; in the uterus—an increased amount of fibrous tissue in the stroma, hypertrophied muscle cells, and (with the large dose) progestation-like lace foldings of the mucosa, which were, however, much more pronounced and more closely reminiscent of those found during pregnancy than those obtained in our previous experiments of short duration.

The addition of oestradiol dipropionate (group XV) was followed by better growth of the uterus, but severe squamous metaplasia of the uterine epithelium with cornification was present in half of the rats. Testosterone propionate in the dose used (2.25 mg. a week) was unable to prevent these pathological effects of oestrogens. The effect of oestradiol also overruled that of the male hormone in

the vagina, in which the changes were of the oestrous type, though in some cases irregular.

When, however, in another experiment (which will be described in detail elsewhere) a large dose of 7.5 mg. a week of testosterone propionate and a smaller dose (90 μ g. a week) of oestradiol benzoate-butyrate were injected, the development of the metaplastic changes was completely prevented, while in the uterus and vagina the effect of testosterone propionate (abortive progesterational changes) was present in most cases.

The addition of progesterone to the combined injections of testosterone propionate and oestradiol dipropionate (group XVI) had a definite preventive action on these pathological changes: metaplasia was present only in 1 rat out of 9, and was of a very weak degree. We have shown previously [Korenchevsky & Hall, 1938, 1] that progesterone was able to prevent these metaplastic changes in the uterine epithelium produced by pure oestradiol, and have given the literature relating to the subject.

The special pictures of the uterus with abortive epithelial foldings and of the vagina with vacuolated, swollen epithelium in rats of group XVI will be described elsewhere.

It is also noteworthy that the addition of progesterone was followed by the development of a smaller uterus (group XVI) as compared with that obtained in group XV.

Significance of varying amount of body fat in calculations per unit of body weight

The amount of body fat in the experimental animals varied considerably under the influence of the injections. Therefore there is a possibility that misleading conclusions might be drawn, if the weights of the non-sexual organs calculated per unit of body weight of fat rats (e.g. control groups, especially spayed rats) are compared with those of thin animals (e.g. those injected with oestradiol). The depots of inert adipose tissue require less activity from the organs regulating metabolism than do active tissues such as glandular, muscular etc. Consequently different requirements might influence differently the size and weight of the "metabolic" organs.

When studying the non-sexual organs, therefore, we have calculated the weights not only per unit of final body weight, but also per unit of "fat-free" body weight. This latter weight was obtained approximately by subtracting the total body fat from the final body weight (given in the tables) assuming that the abdominal fat (also given in the tables) represents about 30–32 % of the total fat in the body (for details see our previous paper [Korenchevsky *et al.* 1939, 2]. These calculations, however, gave results differing only in degree from the usual calculations per unit of body weight, without changing the essence of the conclusions.

Effect on gain in body weight and fat deposition

Growth, as judged by gain in body weight, was slightly stimulated by androsterone (group V), little or not at all affected by dehydroandrosterone and small doses of testosterone propionate (groups VIII and XIII) and depressed by oestradiol dipropionate (considerably, groups III, IV and, especially, XII) and large doses of testosterone propionate (slightly, group XIV).

The amount of body fat was considerably decreased by oestradiol dipropionate (the larger the dose the greater the decrease) and to a less degree by large doses of testosterone propionate, but unchanged by the other hormones or doses.

The remarkable neutralizing effect of the male hormones on oestrogens was again demonstrated by their power, when simultaneously injected, of preventing in most cases, and sometimes to a considerable extent, the depressing effect of the female hormone on body weight and fat deposition (groups VI, VII, IX, XV, XVI).

Effect of the hormones on non-sexual organs

Adrenals. The actual weights of these glands were slightly changed by oestradiol dipropionate, the smallest dose causing an increase (group III), the other doses a decrease (groups IV and XII). When calculated per unit of body weight, the weights showed with all doses a constant increase of 30–70%. This suggests that in spite of the evidence of actual weights, oestradiol dipropionate may have in reality some stimulating action on the adrenals, but fibrous degenerative changes found in the cortex did not support this suggestion.

The male hormones when injected alone (groups V, VIII and XIII, but not the large dose of testosterone propionate, group XIV) in most cases decreased both the actual weights of the adrenals and those per unit of body weight. Thus, in these prolonged experiments the effects of these hormones were similar in females and in males. In experiments of shorter duration on females [Korenchevsky *et al.* 1935; 1936; 1937, 1; Korenchevsky & Dennison, 1936, 1] the effects were similar but with some doses less definite and constant. Testosterone dipropionate also produced smaller adrenals [Korenchevsky *et al.* 1939, 3].

When male and female hormones were injected simultaneously (groups VII, IX, XV and XVI) the activity of the female hormone in most cases changed the effect of the male compounds, the adrenals becoming larger than those of rats injected with the respective male hormones alone. A similar effect was noted with some hormones in our previous experiments (mentioned above) of shorter duration.

Hypophysis. As with other oestrogens, oestradiol dipropionate produced hyperplasia of the hypophysis, the larger the dose the greater being the degree of hyperplasia (groups III, IV and XII). It is remarkable that while male hormones have no definite effect upon the weight and size of the gland (groups V, VIII, XIII and XIV), they can to some extent prevent the hyperplastic effect of oestrogens (groups VI, VII, IX, XV). If in addition progesterone is given (group XVI), the neutralizing effect appears to be even greater; the hypophyses of two rats in this group weighed only 19.8 and 25.6 mg. as compared with an average of 132 mg. with oestradiol alone (group XII). The weight of the hypophysis was thus restored nearly to normal, the average weight in normal controls being 17.6 mg.

Liver, kidneys, heart and spleen. With a few exceptions, the character of the changes in these organs was similar.

Oestradiol dipropionate (groups III, IV and XII) caused a decrease (considerable with large doses) in the actual weights of these organs, except the kidneys, while the weights of all of them per unit of body weight were increased. Therefore, as in the case of the adrenals, the ratio of the weights of these organs to body weight was greater than in control ovariectomized rats and often similar to that in control normal animals.

On this basis the influence of oestradiol on these organs cannot be considered as a true depressing effect, but may even suggest some stimulation, hidden by the effect of the stunted growth of the rats on the weight and size of these organs provided that histological investigation confirms this conclusion. This is also supported by experiments of shorter duration (21 days) with oestradiol dipropionate and oestradiol benzoate-butyrate [Korenchevsky *et al.* 1939, 1], when

the weights, both actual and per unit of body weight, of liver and kidneys were increased by those doses which did not cause much depression of body weight.

On the other hand, the effect of *male hormones* on liver, kidneys, heart and spleen appears to be definitely stimulating, both the actual weights and those per unit of body weight being increased (groups V, VIII, XIII, XIV). Even in experiments of shorter duration, similar results were obtained in some cases [e.g. Korenchevsky *et al.* 1939, 3].

This stimulating effect is further demonstrated by the property of androsterone and testosterone propionate of at least partly preventing the depressing effect of oestradiol on the actual weights of these organs (groups VI, VII, XV). The addition of progesterone (group XVI) does not increase this effect of testosterone propionate.

The weakest stimulant in this respect amongst the male hormones was dehydroandrosterone, which had a definite effect on liver and spleen, but not on heart and kidneys. It also possessed in only a very weak degree the property of preventing the changes produced by oestradiol.

Thyroids. The weights of these organs were unchanged.

Comparison of the effects of the sex hormones on the non-sexual organs in females with those in males

As shown above, gonadectomy does not produce such constant and definite effects on the majority of the non-sexual organs in females as in males. In spite of this, however, the effects of the male and female hormones on adrenals, hypophysis, liver, kidneys, spleen, heart, fat deposition and gain in body weight were similar in both sexes (compare the present results with those obtained in similarly arranged experiments on males [Korenchevsky *et al.* 1939, 2]. The remarkable property of male hormones of more or less neutralizing the effects of the female hormone was also present in both sexes. It should be noted, however, that while in males the weights of liver, kidneys, heart and spleen were restored to normal by male hormones, in the female these hormones produced super-normal development of these organs.

Effect of testosterone propionate on normal rats

Only six rats were used in this preliminary experiment, two in each of the injected groups and two control animals. The results, however, were constant, clear-cut and in many respects in good agreement with those of our previous experiments of shorter duration (21 days) [Korenchevsky *et al.* 1937, 2].

In normal females (groups XVII and XVIII) the effect of testosterone propionate on most of the organs investigated was similar in character to that in ovariectomized rats, but usually differed in degree. The following changes in weight (both actual and per unit of body weight) were found: a "gigantic" uterus with the large dose, and greatly enlarged vagina and preputial glands with both doses; decrease in weight of the adrenals with the small dose and no change with the large dose; decreased weight of hypophysis, and enlargement, varying in degree, of liver, kidneys, spleen and heart. The small dose caused increased gain in body weight but no definite change in fat deposition, while with the large dose fat deposition was decreased but the body weight remained unchanged. As in ovariectomized rats, therefore, the large dose affected fat deposition and body weight more than the small dose.

Some unusual features of the changes in the uterus and vagina must be mentioned. With the small dose there was a decrease in size of the uterus (group XVII), but the structure was normal, with few and low foldings of the

mucosa. The small size of the uterus might be explained by the depressing effect of testosterone propionate on the secretion of gonadotropic hormone from the hypophysis, this dose of the hormone being unable to counterbalance the depressing effect by direct stimulation of the uterus. Analogous results were obtained previously [Korenchevsky & Hall, 1939] in normal male rats after injection of androsterone and *transdehydroandrosterone*, and the mechanism of this phenomenon was discussed in detail (p. 376). The increase in size of the vagina, however, does not accord with this theory, and therefore at present it is difficult to find a satisfactory explanation of the decrease in size of the uterus, especially on the basis of the results of an experiment on two rats only.

The "gigantic" uterus (group XVIII), produced by the large dose, was distended with secretion, the distension being reminiscent of that during oestrus (the columnar epithelium was normal and the myometrium hypertrophied). The epithelial layer of the vagina was narrow with narrow, mucified cells.

SUMMARY

1. Androsterone, *transdehydroandrosterone*, testosterone propionate and oestradiol dipropionate, alone, or each of the male hormones in combination with oestradiol dipropionate (in one group also with progesterone) were injected into ovariectomized and normal rats for a period of about 3½ months.

Effects on sex organs of ovariectomized rats

2. With oestradiol dipropionate alone, the vagina was restored to normal or supernormal weight, with normal (usually "oestrus") structure, but not even a weekly dose of 0.2 mg. was able to produce normal size and weight of the uterus, although this dose caused pronounced pathological changes in the structure of this organ.

3. The weak effects of androsterone and *transdehydroandrosterone* on the uterus were not significantly increased, as compared with experiments of shorter duration, but the vagina and especially the preputial glands were considerably hypertrophied.

4. There was a co-operative effect between oestradiol dipropionate and androsterone or (to a lesser degree) *transdehydroandrosterone* on the weight and size of the sex organs, but at the same time the number of rats affected with squamous metaplasia of the uterine epithelium (first step of "precancerous" oestrogenic effect) was increased and this condition became more severe.

5. Testosterone propionate produced pronounced progestational changes in the uterus and less strong ones in the vagina.

6. Testosterone propionate had a co-operative effect with oestradiol dipropionate on the weight and size of the uterus, but the occurrence and degree of epithelial squamous metaplasia in this organ were about the same as with the female hormone alone.

7. If, however, progesterone was added to the combination of male and female hormones, or a large dose of testosterone propionate was injected with oestradiol, these "precancerous" changes in the uterine epithelium were prevented to a remarkable degree.

Effects on non-sexual organs

8. Male hormones caused decrease in the weight and size of the adrenals of females similar to that previously described in male rats, while the effect of the female hormone was characterised by peculiar pathological changes. Enlarge-

ment of the adrenals in most cases followed simultaneous injections of male and female hormones.

9. The tumour-like hyperplasia of the hypophysis produced by the oestrogens was neutralized to a considerable extent (in two rats almost to normal) by male hormones.

10. Male hormones caused hypertrophy (in most cases exceeding normal level) of liver, kidneys, spleen and heart, but the female hormone produced either a slight decrease in their actual weights, or (kidneys) no change.

11. Oestradiol dipropionate produced stunted growth and decreased fat deposition, but with the male hormones this depressing effect was absent or (with the large dose of testosterone propionate) slight.

12. When injected simultaneously the male hormone more or less completely neutralized the effect of the oestrogen on liver, kidneys, heart, spleen, fat deposition and body weight.

Effect on normal rats

13. The effects produced by testosterone propionate on the weight and size of most organs of normal rats were in general similar to those in ovariectomized rats, in most cases differing in degree. A remarkable feature was the "gigantic" uterus distended with oestrus-like secretion.

14. Prolonged administration of male sex hormones produced in general similar results in both sexes.

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