XLVI. THE ACTION OF THE DIPROPIONATE AND BENZOATE-BUTYRATE OF OESTRADIOL ON OVARIECTOMIZED RATS

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MIESCHER *et al.* [1938] have prepared several aliphatic di-esters of oestradiol which they have carefully tested, using the methods of vaginal smear and uterine growth. They gave subcutaneous injections of the di-esters to immature normal and ovariectomized rats, the total dose being usually 50 $\mu\text{g.}$, given in equal parts on two consecutive days, and found that, as compared with oestradiol, the di-esters have an extremely intense and prolonged effect. The di-esters are therefore important both for experimental and clinical purposes.

In the present paper are given the results of ten experiments (Tables I and II, groups III-VIII, XII-XV), in which the esters were injected for a period of 3 weeks, and of one experiment (Table I, group IX) for 19 weeks. The dipropionate and the 3-benzoate-17-*n*-butyrate were chosen as being typical representatives of the series of aliphatic and mixed aliphatic-aromatic di-esters.

Two esters, the effects of which last for very different lengths of time, were deliberately chosen: a long duration of effect would appear to be most advantageous for clinical use, but it is necessary to consider also the disadvantage of being unable to stop the effects of the injection if this should be required. In this respect there might even be danger, with a hormone which produces not only physiological but also pathological effects, as is the case with all oestrogens. According to Miescher *et al.* (p. 731, fig. 7) the maximal growth of the uterus with the dipropionate was obtained on the 12th day, after which period the effect started to decline, while with the benzoate-butyrate the maximal growth was still present and had not begun to decline on the 50th day. Robson *et al.* [1938], injecting a total dose of 50 $\mu\text{g.}$ of the benzoate-butyrate into mice, found that the effect of the hormone lasted more than 120 days, but that after 95 days the degree of its action was already halved.

Technique

The hormones were supplied by Messrs Ciba Ltd. Our technique of preparation was as usual: the hormones were first dissolved in alcohol, then mixed with warmed sesame oil and the alcohol distilled off *in vacuo* at a temperature rising slowly from 70 to 85°. The hormonal solution was then put into ampoules, sealed and sterilized for 3 days at 100°. The ampoules were kept in the cold store until needed. While oestradiol benzoate-butyrate was not affected by this method of solution, the dipropionate appeared to be weakened, since after keeping for a few months, even in the cold store, its effect was less strong. Dr Miescher has suggested (personal communication) that with our method of solution some of the ester might possibly be decomposed with liberation of pure oestradiol, and that shaking the dipropionate in oil warmed up to 70° without any additions

gives the most stable solution. However, since we discarded all experiments in which we noticed a weaker effect of the dipropionate and used fresh solutions, the duration of the effect in our experiments has been similar to that in the experiments of Miescher *et al.*

The rats used were ovariectomized on the 22–26th day of age. The hormones were injected as follows: the dipropionate was injected for three consecutive weeks in weekly doses of 18 and 90 $\mu\text{g.}$ (6 and 30 $\mu\text{g.}$ respectively three times a week, Table II, groups XII–XV); the smallest dose 18 $\mu\text{g.}$ of the benzoate-butyrate (Table I, groups III–V) was injected similarly, and the dose of 90 $\mu\text{g.}$ (groups VI–VIII) was given once a week for three consecutive weeks; in group IX, the weekly dose of 300 $\mu\text{g.}$ was given (100 $\mu\text{g.}$ three times a week) for 19 weeks.

In order to ascertain the duration of the effect after the last injection the rats in groups IV, V, VII, VIII, XIII and XV (“10–106 days rest”) were left without injection for the requisite number of days before killing. In order that in each experiment all the rats might be killed on the same day, the injections into the rats of groups “10–106 days rest” were started the respective number of days earlier than the injections into the rats which were killed on the next day after the last injection (groups III, VI, IX, XII and XIV).

In this way the final ages of comparable groups were the same. We have not noticed any definite effect through starting the injections at an earlier age in some groups than in others.

Other details of our technique were the same as in our previous experiments. The total number of rats used was 99. The average age of the rats in Table I was 107–176 days (col. X), and in Table II 88 days.

Table I. *Effects of oestradiol benzoate-butyrate on actual weights of organs of ovariectomized rats*

Organs	Weekly doses of oestradiol benzoate-butyrate									Average final age in days X
	Control rats		Injected for 3 weeks						Injected for 19 weeks 300 $\mu\text{g.}$ IX	
	Normal I	Ovariec- tomized II	18 $\mu\text{g.}$ + 30 days rest III	18 $\mu\text{g.}$ + 52 days rest V	90 $\mu\text{g.}$ + 30 days rest VI	90 $\mu\text{g.}$ + 106 days rest VII	90 $\mu\text{g.}$ + 106 days rest VIII			
			18 $\mu\text{g.}$ IV	90 $\mu\text{g.}$ VI	90 $\mu\text{g.}$ VII	90 $\mu\text{g.}$ VIII				
Uterus (horns + cervix, mg.)	411 549	36 43	228 —	276 —	271 —	248 —	290 —	— 292	— 536	107 176
Vagina with clitoris (mg.)	232 326	175 189	314 —	311 —	353 —	258 —	328 —	— 365	— 409	107 176
Preputial glands (mg.)	97	58	73	67	67	78	81	76	58	107-76
Hypophysis (mg.)	14.7	12.5	18.8	23.2	20.2	30.7	42.2	32.0	76.0	107-76
Thymus (mg.)	283 213	440 221	397 —	385 —	313 —	257 —	277 —	— 197	— 110	107 176
Liver (g.)	8.34	9.08	8.69	11.02	10.67	9.36	9.41	8.22	8.45	107-76
Kidneys (g.)	1.53 1.87	1.61 1.79	1.82 —	2.02 —	1.83 —	1.99 —	1.86 —	— 1.92	— 1.82	107 176
Abdominal fat (g.)	21	20	10	15	17	12	12	16	7	107-76
Final body wt. (g.)	244 280	308 322	245 —	288 —	283 —	247 —	224 —	— 266	— 198	107 176
No. of rats in the group	10	9	4	6	3	7	7	4	4	107-76

Note. In the control groups I and II two average weights are given only for those organs of which the weight varies considerably according to the age (column X). In group I the vaginal weight of rats aged 107 days is given without clitoris.

Table II. *Effects of oestradiol dipropionate on actual weights of organs of ovariectomized rats*

Organs	Control spayed rats XI	Weekly dose of oestradiol dipropionate			
		18 μ g. XII	18 μ g. + rest 10 days XIII	90 μ g. rest XIV	90 μ g. + rest 10 days XV
Uterus (horns + cervix, mg.)	38	249	210	252	232
Vagina with clitoris (mg.)	170	285	313	268	281
Preputial glands (mg.)	49	74	63	67	65
Hypophysis (mg.)	10.9	22.1	21.3	35.5	32.4
Thymus (mg.)	467	299	313	218	223
Liver (g.)	8.56	9.00	11.35	9.38	9.32
Kidneys (g.)	1.57	1.84	1.97	2.10	1.82
Abdominal fat (g.)	17	9	13	7	11
Final body weight (g.)	276	223	273	223	211
Gain in body weight (g.)	76	21	39	28	27
No. of rats in the group	10	12	8	7	8

Effect on sex organs

The maximum effect, as studied by weight of the organs, was slightly greater with the benzoate-butyrate than with the dipropionate. We have not studied the duration of the effect of the dipropionate for a longer period than 10 days after the cessation of the injections. At the end of this period the weight of the uterus was only slightly decreased, while that of the vagina was slightly increased (groups XIII and XV) as compared with the weights of these organs in rats killed on the day after the last injection (groups XII and XIV). With the benzoate-butyrate, the maximum effect was still fully maintained on the 106th day after the last injection (group VIII).

With both hormones, the normal weight and size of the *uterus* was not obtained with any of the doses. Even with 300 μ g. (group IX) the large weight of the uterus was due to accumulation of secretion in the uterine lumen greatly distending the uterus, a similar condition to that observed during oestrus. Such a secretion greatly increases the weight of the uterus, while the control animals in group I were always killed during dioestrus, when this secretion is absent.

Furthermore, with all doses, metaplasia into squamous epithelium was observed in the uterine epithelium (the first stage of "oestrogenic precancerous" changes). This metaplasia, sometimes in a severe form, was still present in some rats even 106 days after the injections were ceased (group VIII). This means that not only physiological recovery but also the pathological effect was maintained and can be present as much as 15 weeks after the last injection.

One of the rats injected with 300 μ g. of the benzoate-butyrate developed pyometra in the greatly enlarged uterus (weight 4.3 g.), with adhesions to neighbouring organs. Such an effect has been found previously by other workers after injection of large doses of oestrogens.

In contrast to the uterus, the *vagina* returned to normal or in some groups (VIII and IX) was even larger and better developed than in normal control rats. In the rats killed on the next day or up to about two months after the last injection the vagina had a structure more or less typical of oestrus (cornified epithelial layer).

Progestational changes in the vagina. The most remarkable effect, however, occurred in the group of 4 rats killed 106 days (group VIII) after discontinuance of the injections: in two of these rats the appearance of the vagina was similar

to that found at metoestrus (with some cornification), but in the remaining two the epithelial layer was mucified—in one rat to a degree not far from that found during pregnancy.

From these results the following conclusions may be suggested, but confirmation on a larger number of ovariectomized animals is necessary:

(1) Oestrogenic compounds with a long period of action after discontinuing the injections (e.g. oestradiol benzoate-butyrate) may, towards the end of this period, produce a progestational instead of oestrous effect on the vagina.

(2) Since progesterone is naturally present in gonadectomized animals (e.g. in the adrenals [Beall & Reichstein, 1938; Beall, 1938]), the injected oestrogens, if present in the correct ratio with progesterone, might produce this progestational effect on the vagina.

(3) Probably, in the case of oestradiol benzoate-butyrate this ratio is reached toward the end of the after-period of "106 days rest".

(4) This explanation seems to us more probable than that oestrogens alone, without co-operation with progesterone, when injected in small amounts produce mucification, and when in larger amounts produce cornification of the vagina.

The latter theory, however, has been suggested by the workers who first noted the mucifying effect of very small amounts of oestrogenic concentrates or of purified oestrone [Robson, 1931; Robson & Wiesner, 1931–32; Meyer & Allen, 1932]. The latter authors also review the literature on the subject.

The *preputial glands* in most rats were slightly enlarged but did not return to normal.

Effect on body weight, fat deposition and non-sexual organs

In our previous experiments pure oestrone was injected in doses of 2–18 μ g. daily for 4 weeks [Korenchevsky *et al.* 1935] or 6 μ g. three times a week for 3 weeks [Korenchevsky & Dennison, 1936; Korenchevsky *et al.* 1936; 1937, 1 and 2] and the rats were killed on the day following the last injection. These doses of this compound did not produce constant changes in the non-sexual organs of ovariectomized females, with one exception—the thymus. The physiological involution of this organ, delayed in ovariectomized rats, was increased by oestrone. In some cases the weight of the liver was slightly decreased and that of the hypophysis slightly increased. A definite decrease in the gain in body weight and fat deposition was noted. Stunted growth after injections of oestrogens has been reported by several authors.

In the present experiments some of the effects observed were similar to those obtained previously, but other changes also occurred.

Body weight and fat deposition. As in our previous experiments, the di-esters produced stunted growth and decreased fat deposition in all groups—the larger the dose, the greater the effect. However, although during the injections the body weight remained stationary or increased less than in the controls, or even in some cases began to fall slightly, in the after-periods of "rest" the body weight and fat deposition began to return towards normal in spite of the persistence of the sex effect. With 18 μ g. these restorative changes were much more marked than with 90 μ g.

Non-sexual organs. The effect on the *thymus* (increased involution) was the same as in our previous experiments, but the enlargement of the *hypophysis* became more constant and much more pronounced. The larger the dose, the greater was the hypophyseal hyperplasia. With the benzoate-butyrate the hyperplasia increased during the first month after the end of the treatment

(groups IV and VII), but later restorative changes began to develop (groups V and VIII), while the sex effect was still fully maintained. The changes with the dipropionate (groups XIII and XV) were too small to suggest a definite decrease of hypophyseal hyperplasia 10 days after the end of the treatment.

A definite change in the actual weight of the *liver* was not always found during the period of injections, but in the after-periods of "rest" a considerable enlargement occurred (both in actual weight and that per unit of body weight) when small doses were given (groups IV, V and XIII). When the dose was increased up to 90 μ g. there was no significant change in the weight of the liver after the injections ceased (groups VII, XV). However, when the period of rest is longer, as the experiment with benzoate-butyrate shows (groups V and VIII), the enlargement of the liver decreases or disappears, in spite of the maintenance of the sex effect.

That the changes in the liver are significant is supported by the fact that, per unit of body weight, the weight of the liver was also increased in all the groups even during the periods of injections. It is clear, therefore, that during the period of the injections, or when large doses of the esters were injected the effect of oestrogens on the liver was veiled by the stunted growth of the animal, but the weights per unit of body weight and the enlargement of the organ after the end of treatment suggest a stimulating action of oestrogens on this organ, provided that histological investigation will confirm this conclusion.

The *kidneys* were enlarged in varying degree in all groups both in actual weight (except group IX) and those per unit of body weight.

In *adrenals and heart* no constant or pronounced changes were observed in the actual weights, but per unit of body weight there was an increase in most of the injected rats.

Thyroids and spleen showed no definite changes in weight.

Comparison of the effects of female hormones on females with those of male hormones on males

If the general results of previous experiments (of about 3 weeks' duration) performed by Korenchevsky and co-workers on castrated male rats injected with male hormones are compared with the present observations, some similarities and differences in the action of male and female hormones on males and females respectively can be noted. Thus, as judged by weights, the di-esters of oestradiol and the male hormones produced similar effects on sex organs, thymus, liver (with the reservations for oestrogens already mentioned) and kidneys. The two groups of hormones, however, in most cases influenced differently the body-weight, fat deposition, hypophysis and adrenals.

Thus the oestrogens do not act on females in the same way as the male hormones on males, although some similar properties are present in both groups.

SUMMARY

1. The effects of the dipropionate and benzoate-butyrate of oestradiol were studied on 99 ovariectomized rats.
2. With the doses used, the effects on the sex organs were maintained in the case of the dipropionate during the investigated period of 10 days without injections, while the action of the benzoate-butyrate had not begun to decline even on the 106th day after the last injection.

3. Complete restoration to normal weight and structure of the vagina but not of the uterus was obtained. Moreover, an important pathological change, squamous metaplasia, occurred in the uterine epithelium and was still present up to 106 days after cessation of the injections.

4. While the restorative effect on the sex organs in the case of oestradiol benzoate-butyrate was maintained at its maximum for more than 106 days, the effect on the structure of the vagina in this later stage of the period without injections may be changed from oestrous to progestational form (mucification of the vaginal epithelium).

5. Most probably this progestational effect on the vagina was due to co-operative action at this period of a small amount of oestrogenic hormone (liberated from the injected depot) with progesterone which has probably originated from the adrenals.

6. Body growth and fat deposition were depressed by the di-esters but a partial recovery took place in the period after the injections.

7. Involution of the thymus was hastened; hyperplasia of the hypophysis was present during and after the period of the injections; pronounced enlargement of the liver during some after-periods, and of the kidneys during and after the periods of injections was noted.

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