CLXXXIV. CONDITIONS OF HYPERTROPHY OF SEMINAL VESICLES IN RATS.

II. THE EFFECT OF DERIVATIVES OF OESTRONE (MENFORMON).

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HYPERTROPHY of smooth muscle and narrowing of the cavity in seminal vesicles of rats following treatment with oestrone (menformon) has been previously described [for references see Freud, 1933, 1, 2]. While stimulating the growth of non-epithelial structures, oestrone damages the columnar epithelium of seminal vesicles by reducing the surface of their cavity, hastening shrinkage of cells and desquamation in castrates. The detached epithelial lining often conglomerates in masses reminiscent of tumours or embryonic tissue. Eosinophil leucocytes invade the smooth muscle and submucosa.

Male hormone (hombreol¹, assayed on combs of capons) maintains or even increases the ramification of the cavity, prevents shrinkage and desquamation of columnar cells and stimulates them to secretion and mitosis. Hombreol does not interfere with the growth-stimulating effects of oestrone on the wall of the vesicles. The two substances mutually enhance their hypertrophic actions (pace-making). Contrary to the one-sided effect of oestrone, sufficiently large doses of hombreol (5 or more c.u. daily) stimulate general hypertrophy of seminal vesicles, even when thoroughly purified from contamination with oestrogenic substances.

Correspondence between the effects of oestrone in males and females.

Castrated female rabbits and rats, when treated with 100–1000 m.u. of oestrone daily during several weeks, have very thick uteri, with a narrow unramified cavity. Epithelium shrinks and becomes partially detached and conglomerated. The thickened wall consists for the most part of enormously hypertrophied submucous or interstitial fibrous tissue, reminiscent of fibroid tumours (see Fig. 1). An infiltration with eosinophil leucocytes is also noticeable. This favours Witherspoon's theory [1933] concerning the possible causes of uterine fibroids in human beings. Ramification of the uterine cavity and hypertrophy, mitosis and secretion of the epithelium are stimulated by the hormone of the corpus luteum (progesterin), which is thereby proved to be a female analogue of hombreol. The combined effects of oestrone and progesterin on the uterus are

¹ The commercial names of the substances when here referred to are used because they are short, and also imply a certain proved freedom from unwanted contaminations. Preparations less thoroughly purified are not certain to give results comparable with those here described.

The term oestrin is used as a generic name for oestrogenic substances such as may be obtained by simple extraction of urine; individual members of this class are oestrone (ketohydroxyoestrin, menformon) and oestriol (trihydroxyoestrin).

well known. According to our experience hembreol and progestin are not interchangeable with reference to seminal vesicles and uterus, and progestin does not stimulate comb-growth in capons [de Fremery *et al.*, 1932]. Its effect on the prostate is insufficiently known.

Fig. 1. Uterus of rabbit 58 after 6 weeks' treatment with 1000 m.u. of menformon daily. (Castration 8 weeks previously.)

1. Surface.
2. Longitudinal
3. Transverse
4. Submucous connective tissue enormously thickened.
5. Cavity without epithelial lining.

Oestrone stimulates thickening and cornification of vaginal stratified epithelium. De Jongh [1933, 1, 2; 1934], after treating male castrated mice with 2–50 m.u. of oestrone daily for 20 days, found stratified epithelium replacing columnar at the orifice of the ducts of the prostate and seminal vesicle, a level at which stratified epithelium never normally occurred. The prostatic ducts were cornified and the concentric desquamated epithelium which tended to obstruct the narrow ducts. When oestrone treatment was discontinued this abnormally localised (perhaps metaplastic) tissue soon disappeared¹. Similar observations were published by Lacassagne [1933]. De Jongh has further shown that hembreol effectively prevents metaplasia and cornification, just as a functioning corpus luteum, producing progestin, the female analogue of hembreol, is said by many workers [de Fremery *et al.*, 1932; Hisaw *et al.*, 1932] effectively to antagonise vaginal cornification. In male animals under forced treatment with oestrone the bladder is often engorged with urine so that its apex reaches the xiphisternum. Burrows and Kennaway [1934] have obtained similar results. No anatomical structure was found to obstruct the urethra, hence the retention may reasonably be assumed to be functional and similar to the "prostatisme sans prostate" of human pathology.

Male hormone promotes the development of spongy glandular structures in the male genital region and reduces the experimental incidence of engorged

¹ In rats metaplasia occurs more readily in the seminal vesicles than in the prostatic ducts.

bladder. This observation may strengthen the theoretical basis for the treatment of prostatic hypertrophy by hombreol, suggested by Prof. Laqueur and successfully applied by van Cappellen [1933, 1, 2] and others.

Finally, the growth-stimulating effect of oestrone on accessory skin-organs, such as mammary and preputial glands, is similar in males and females. A special study of the preputial glands is in progress [see also Freud, 1933, 1, 2].

Pharmacology of oestrin.

The compiled data show that the conception of sex-specificity of oestrin, and perhaps of "sex-hormones" in general, is untenable. In terms of pharmacology, the known "sexual hormones" act selectively on certain tissues of specialised function. In secondary sex organs oestrone stimulates growth:

- (a) of smooth muscle tissue and interstitium;
- (b) of submucosa and stratified epithelium;
- (c) of submucosa and undifferentiated cylindrical epithelium;

the last, however, only in non-tubular organs, where smooth muscle is represented by interspersed fibres; in tubular organs a slight initial epithelial hypertrophy is soon superseded by that of the extra-epithelial structures of the wall, with results deleterious to the epithelium, unless hombreol (in males) or progesterin (in females) restores the equilibrium between these tissues. Hombreol and progesterin promote the spread, growth and differentiation of columnar and glandular epithelium. According to Pompen [1932] oestrone not only stimulates uterine growth but also sensitises the uterine muscle to functional stimuli, such as pitocin.

Oestrin derivatives.

While we were engaged in ascertaining these facts, new oestrogenic or chemically related substances were being discovered in various laboratories. To all these, according to present knowledge, a ring structure is ascribed similar to that of oestrone. The first of these to be tried in males was oestriol (Marrian); the experiments are described in the previous paper of this series. The oestrogenic activity of this substance and its growth-stimulating effect upon smooth muscle are very weak, but the synergism with hombreol almost equals that of oestrone.

In this paper will be described, comparatively with oestrone, the effects of the following substances on the seminal vesicles and other male accessories of immature castrated male rats:

I. *Oestrone* (crystalline menformon), extracted from the urine of pregnant mares. M.P. 255.5–256.5°; $[\alpha]_D^{20} + 163^\circ$ (in dioxan).

II. *Oestriol*, extracted from the urine of pregnant women, essentially according to the method described by Marrian [1932]. M.P. 273–276°; $[\alpha]_D + 58^\circ$ (in dioxan). Marrian has found M.P. 278–280°.

III. *Equilenin*. We are grateful to Dr Girard of Paris for supplying this substance. The constants determined in this laboratory were: M.P. 254–255°; $[\alpha]_D^{20} + 88^\circ$ (in dioxan). According to Girard [1932] himself, they were: M.P. 258–259°; $[\alpha]_D^{20} + 87^\circ$.

IV. *Equilin*, separated from crude oestrone crystals by repeated fractionation, white platelets crystallised from dilute alcoholic solution. M.P. 236–237.5°; $[\alpha]_D + 307^\circ$ (in dioxan). (Found: C, 80.64; H, 7.39 %. $C_{18}H_{20}O_2$ requires C, 80.55; H, 7.52 %.) According to Girard the constants are M.P. 238–240° (corrected); $[\alpha]_D + 308^\circ$.

V. *Reduced equilin*, prepared by reduction of equilin with sodium in alcohol. M.P. 174.5–175°; $[\alpha]_D + 220^\circ$ (in dioxan). (Found: C, 79.56; H, 8.37 %. $C_{18}H_{20}(OH)_2$ requires C, 80.0; H, 8.15 %.)

VI. *Oestradiol* (dimenformon), prepared by reduction of oestrone with sodium in alcohol. M.P. 176–177°; $[\alpha]_D + 80.5^\circ$ (in dioxan). (Found: C, 79.49; H, 8.98 %. $C_{18}H_{22}(OH)_2$ requires C, 79.36; H, 8.89 %.) From diluted alcohol the substance crystallises in shiny platelets, which variously contain either 0.5 mol. C_2H_5OH or 0.5 mol. H_2O .

VII. *Monobenzoate of oestradiol*, prepared by benzylation of oestradiol in *N* NaOH solution. Small white needles (from diluted alcohol). M.P. 194–195°. Schwenk and Hildebrandt [1933] find M.P. 187.5–190°. (Found: C, 79.98; H, 7.37 %. $C_{25}H_{28}O_3$ requires C, 79.74; H, 7.50 %.)

VIII. *Dibenzoate of oestradiol* is obtained by benzylation of oestradiol with benzoyl chloride in pyridine solution. White, shiny platelets or needles (from alcohol). M.P. 169.5–170.5°. (Found: C, 79.96; H, 7.13 %. $C_{32}H_{32}O_4$ requires C, 79.96; H, 6.72 %.)

IX. *Diacetate of oestradiol*, prepared by boiling oestradiol with anhydrous acetic acid. Platelets shining like mother of pearl (from diluted alcohol). These crystals exhibit electrostatic phenomena immediately after filtration through a porcelain filter. M.P. 125–126°. (Found: C, 74.18; H, 7.86 %. $C_{18}H_{22}(OCOCH_3)_2$ requires C, 74.12; H, 7.92 %.)

X. *Benzoate of oestrone* (menformon B) prepared from oestrone as under VII. M.P. 218.5–221°.

XI. *Acetate of oestrone* (menformon A), prepared from oestrone as under IX. M.P. 125.5°.

XII. *Monomethyl ether of oestradiol*, prepared by methylating oestradiol in 2*N* NaOH solution with dimethyl sulphate [Marrian, 1933]. M.P. 94–96°.

In the foregoing description certain discrepancies will be noted between the constants of the substances as determined by the respective authors and by one of ourselves (K. D.). These differences, which will be discussed in detail elsewhere, are mainly due to:

(a) Incomplete separation of substances, or various crystal forms of the same substance [Kofler and Hausschild, 1934] having very similar solubilities or other physical properties used in isolation.

(b) Insufficient number of analyses to warrant consistent results.

(c) Lack of agreement between substances prepared at separate places (by exchange of materials and determination of their chemical and biological data at a laboratory other than that in which the preparative work was done). Our own determinations of M.P. were done with a short-stemmed Anschütz thermometer. No corrections were applied. The optical rotations were determined by the micro-method generally used for this purpose: 2–4 mg. substance being dissolved in 0.4 ml. dioxan.

Animals and method.

Immature castrated male rats were used. Transscrotal castration was done when the animals were 20–30 days old. Three days after the operation a course was begun of 10 (exceptionally 14) injections during 5 (7) days. The daily dose was given in 2×0.1 ml. arachis oil. The animals were killed the day after the last injection and the preputial glands (Pp.), seminal vesicles (Se.), prostate (ant. lobes) and periurethral tissue were dissected and weighed on a torsion balance. The technique of dissection was as follows.

Preputial glands. A triangular skin-flap is lifted, the apex being the hypochondrium, the base between the two inguines. The glands are found embedded in subcutaneous fatty tissue on the ventral surface of the abdominal wall. The ducts of the glands lead into the preputial sac and are often filled with a sticky brown secretion.

Seminal vesicles. The dissection of seminal vesicles is described in the previous paper of this series.

Prostate. The two anterior lobes are easy to isolate, when the pelvic bones are bilaterally resected laterally to the symphysis, the bladder drawn caudally until the urethra presents itself in the dorsal groove of the symphysis. The symphysis is now removed and the soft prostatic tissue cut off the urethra.

Periurethral tissue consists of glands not unlike the prostate, of prostatic ducts, vesicular ducts, ductus deferentes and urethra. All these structures are surrounded by a muscular funnel.

The prostatic tissue adherent to the inner and outer walls and ends of this funnel is macroscopically inseparable from it. The upper limit of the funnel is at the neck of the bladder, the lower at a transverse bluish line, corresponding to strong venous vascularisation.

We have improved our experimental method over that reported in our previous paper, by working with animals of known strain, litter and data of birth. If, say, six groups of animals are used at a time, the brothers of each litter are distributed over all six groups. Comparison of the average weight can be checked by comparing the brothers individually as they fall into parallel groups. Detailed protocols cannot be reported here. Hence the results are expressed in terms of the average weight of the animals in each group on the day of killing

Table I. *Experimental results.*

Substance injected	γ per day	Period days	No. of rats	Average weight g.	mg. per g. rat		
					Pp.	Pr.	Se.
None	—	—	30	44	0.34	0.22	0.14
None	—	—	85	58	0.34	0.17	0.13
I. Oestrone	2	7	5	37	0.30	0.44	0.25
	2	5	22	47	0.39	0.25	0.18
	6	5	5	45	0.35	0.42	0.29
	10	5	5	39	0.48	0.39	0.27
II. Oestriol	2	7	5	44	0.22	0.28	0.18
	2	5	5	38	0.50	0.42	0.19
	10	5	5	35	0.38	0.48	0.19
III. Equilenin	2	7	5	44	0.24	0.25	0.15
IV. Equilin	6	5	5	50	0.50	0.30	0.27
V. Reduced equilin	6	5	5	46	0.53	0.31	0.40
VI. Oestradiol	2	7	5	40	0.35	0.25	0.46
	0.2	5	5	38	0.21	0.31	0.19
	0.3	5	5	33	0.28	0.30	0.21
	0.5	5	10	40	0.31	0.39	0.31
	1	5	5	42	0.37	0.34	0.27
	2	5	27	48	0.40	0.30	0.37
	4	5	5	50	0.42	0.31	0.32
	6	5	5	38	0.45	0.74	0.45
VII. Monobenzoyloestradiol	0.5	5	5	41	0.49	0.54	0.29
	2	5	10	40	0.45	0.51	0.45
	6	5	10	43	0.45	0.58	0.46
	10	5	5	48	0.41	0.38	0.42
VIII. Dibenzoyloestradiol	2	5	5	46	0.20	0.31	0.28
	6	5	5	44	0.40	0.58	0.22
IX. Diacetyloestradiol	0.5	5	5	40	0.37	0.42	0.15
	2	5	5	43	0.44	0.40	0.40
X. Benzoyloestrone	2	5	8	55	0.38	0.13	0.16
XI. Acetyloestrone	2	5	8	54	0.34	0.12	0.23
XII. Monomethyloestradiol	2	5	5	39	0.30	0.29	0.17
Total			320				

and the weight of the various organs in mg. per g. rat. The average weight multiplied by the average mg. per g. organ weight gives the actual average organ weight in every group.

The increase in weight of the periurethral tissue as a result of treatment is mainly due to enlargement of the prostatic ducts and lateral lobes of the prostate. The periurethral tissue is not included in the table, the difference of its average weight in experimental and control groups being added to the weight of the prostate (Pr.).

DISCUSSION.

The *periurethral tissue* of 115 control animals averages 0.37 mg. per g. rat. The largest average actual prostatic weight of all groups was 0.32 mg. per g. rat. In those groups, where very large Pr. weights are noted in the table, this higher value is due to increase inside the periurethral muscle funnel which was added as a correction to the actual prostatic weight, as noted in the previous paragraph. Such is the case in groups I, II, VI, VII, VIII and IX, mainly where large doses were administered. De Jongh observed profound histological changes inside the funnel as a result of the prolonged administration of oestrin derivatives. These changes are in some respects analogous to human prostatic hypertrophy.

We chose to compare the effects of various substances according to the weight of the dose rather than to the oestrogenic potency expressed in units. Certain materials have practically no oestrogenic effect whatever; concerning others there is no agreement in the different laboratories; one might therefore assume that oestrogenic effects such as are described are in certain instances due to contamination.

Preputial glands respond to larger dosages in groups I, II?, IV, V, VI, VII and IX.

Prostates respond strongly in the groups already noted. Considering however that the largest average weight of the free prostate is 0.32 mg. per g., it is apparent that the growth of this organ and of the preputial glands is stimulated at most only moderately by the substances here used. Anticipating some unpublished experimental data concerning the effect of male (comb-growth) hormone on these two organs, we may state that with the same type of treatment (or one day less) the growth of prostate and preputial glands may be stimulated by male hormone considerably more than by the largest doses of any of the substances here mentioned. Seminal vesicles stand in a sharp contrast to the other organs and structures in this respect, in that:

(a) they remain unaffected by: equilenin (III), benzoyloestrone (X), and monomethyloestradiol (XII);

(b) they are moderately influenced by: oestrone (I), oestriol (II), equilin (IV), less than 0.5 γ daily of oestradiol (VI), less than 0.5 γ daily of monobenzoyloestradiol (VII) and dibenzoyloestradiol (VIII), less than 1–2 γ daily of diacetyloestradiol (IX), acetyloestrone (XI);

(c) they are strongly stimulated to growth by: reduced equilin (V), oestradiol (VI), its monobenzoate (VII) and diacetate (IX), in doses of or above 2 γ daily for 5 days.

The weight of the seminal vesicles after the usual treatment is increased to nearly three times that of the controls.

Within certain limits increasing doses have increasing effects. So does prolonged treatment, even if prolonged by only 2 days.

Histological comparison reveals no qualitative difference from the effects of oestrone.

Oestradiol and its active esters cause muscular hypertrophy and ultimately destroy cylindrical epithelium; their relation to the effect of male (comb-growth) hormone is the same as that which was noted in the first section of this paper.

The increased oestrogenic potency of these substances is associated with an increased effect on the seminal vesicles. Oestradiol is about twice as oestrogenic as oestrone [David, 1933], but its monobenzoate and diacetate are (*qua* oestrus) almost the same as oestrone. Substances which have no effect on the seminal vesicles are not oestrogenic. The dibenzoate of oestradiol has no oestrogenic effect administered in the usual doses (more than $5\gamma=1$ mouse unit); its moderate influence upon seminal vesicles may be due to some contamination with the mother substance or to a very slow hydrolysis of the ester in the body; the latter is a possible explanation of the marked activity of the diacetate of oestradiol and other esters.

The purpose of this study of oestrin derivatives was to investigate certain effects of these substances apart from their oestrogenic capacity. It was thought possible that a modification of chemical structure might permit of a qualitative or quantitative differentiation of myotropic and epitheliotropic effects. Although we were able to show clearly that the muscular effect may increase relatively more than the epitheliotropic, as in the case of oestradiol, its monobenzoate and its diacetate (about 4-7 times more active upon muscle tissue, while only twice as active upon oestrus), yet on the basis of these experiments, neither of the two distinct functions could be consistently associated with the phenolic or aliphatic hydroxyl or ketone groups in the molecular formula of oestrin.

The myo- and epithelio-tropic effects may be regarded as two functions, regularly associated with the oestrogenic substances and their known derivatives. The former may be seen in the muscular walls of the uterus or of the seminal vesicles, the latter in the vaginal epithelium or in the metaplastic changes which occur in the excretory ducts of the prostate and seminal vesicles. The presence or absence of such effects, as well as of the other effects of oestrin (*e.g.* on the mammae, preputial glands, uterine vessels, feathering of birds and the antimasculine action) should be investigated for every new derivative of oestrin, to ascertain its complete pharmacological and physiological significance.

SUMMARY.

1. Sex hormones are not sex-specific, nor do they specifically affect whole organs but only certain tissues. In sex organs oestrone (menformon) and certain of its derivatives are growth-promoters to the smooth muscle, stratified epithelium and some glandular epithelia of ectodermal origin, the latter when not located in tubular organs with strong muscular walls, such as mammae and, to a moderate degree, the preputial glands.

2. Glandular epithelium in its development to the secretory stage is subject to either male or corpus luteum hormone; these probably also counteract noxious effects of oestrin derivatives upon the structures concerned.

3. An unsuccessful attempt was made to associate the epitheliotropic and myotropic effects of oestrogenic substances and derivatives with certain known alterations of their structure.

4. However, it was ascertained that oestradiol, its monobenzoate, its diacetate and also reduced equilin, all have myotropic effects which are higher than that of oestrone and some other substances derived from or related to oestrone.

5. Between oestrogenic and myotropic effects a rough parallelism was found, though within this parallelism a limited number of experiments suggest a comparatively enhanced myotropic effect for substances named in paragraph 4.

6. Without an extensive investigation of their actions on the various components of the primary and secondary sex glands of the male and female, our knowledge of the substances belonging to the chemical group of oestrin will necessarily remain incomplete in its most important features.

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