THE BISEXUAL PROPERTIES AND CO-OPERATIVE ACTIVITY OF SEXUAL HORMONES AND THEIR EFFECTS ON FEMALES

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Both female and male sexual hormones have been found in the female organism (Koch, 1936; Dingemans, Borchardt, and Laqueur, 1937), and some have already been isolated in a pure form, while others, mostly those concerned in male activities, are as yet unidentified. In the experiments we performed on rats two lines of inquiry were followed systematically—namely, examination of the bisexual properties of the sexual hormones and investigation of their co-operative activity. It is now possible to draw general conclusions. Therefore in the present paper I propose to outline the results obtained at the Lister Institute in experiments some of which have already been published and some of which are unpublished, and also to discuss the possible clinical significance of these findings.

The effects on the male and female sexual organs of the following pure sexual hormones and their derivatives were investigated: androsterone (Korenchevsky, 1935; Korenchevsky and Dennison, 1935a, 1935c, and 1936; Korenchevsky, Dennison, and Levy Simpson, 1935a); androstanediol and its water-soluble esters (Korenchevsky and Dennison, 1935b and 1936; Korenchevsky, Dennison, and Levy Simpson, 1935 and 1935a); testosterone (Korenchevsky, 1936; Korenchevsky and Dennison, 1936a; Korenchevsky, Dennison, and Brovsin, 1936; Korenchevsky, Dennison, and Hall, 1937a); transdehydroandrosterone (Korenchevsky and Dennison, 1936b); Δ^4 -androstenedione and Δ^5 -androstenediol (Korenchevsky and Dennison, 1937; Korenchevsky, Dennison, and Eldridge, 1937 and 1937a); testosterone propionate (Korenchevsky and Dennison, 1937; Korenchevsky, Dennison, and Eldridge, 1937a; Korenchevsky, Dennison, and Hall, 1937 and 1937a); progesterone (Korenchevsky and Hall, 1937); and oestrone and oestradiol either alone or in combination with the hormones mentioned above (Korenchevsky and Dennison, 1934, 1934a, and 1935, and other references given above).

Of these hormones only androsterone, transdehydroandrosterone, testosterone, and oestrone (Häusler, 1934; Girard,* 1937) have been found as such in the male, while dehydroandrosterone (Callow, 1936), progesterone (corpus luteum or pregnancy hormone), oestradiol, and oestrone have been found in the female. Androstenedione and to a less extent androstenediol are regarded as possible intermediate links in the formation of the sexual hormones in the organism from cholesterol. Testosterone propionate is of special interest and importance, since its activity is much greater than that of pure testosterone. Some of the results obtained in our experiments were confirmed or independently obtained on rats or other animals by Collip and co-workers (McEuen, Selye, and Collip, 1936 and 1937; Selye, McEuen, and Collip, 1936; Selye, Browne, and Collip, 1936), Hain (1937), Deanesly and Parkes (1936 and 1937), Klein and Parkes (1936), Robson (1936 and 1936a), and Zuckerman (1937).

* Personal communication.

Technique of Investigation

The experiments were performed on 873 male and 437 female gonadectomized rats. The individual effect of any sexual hormone injected can be demonstrated only in those animals in which the sexual glands have been removed, and in which, therefore, there is no interference or complication due to the action of the other sexual hormones secreted by the gonads. The results obtained, however, were compared with those seen in 81 normal male and 109 normal female rats, though this comparison was made only in the case of some and not all of the hormones investigated. In order to draw correct conclusions with regard to the atrophy, restoration to normal, or hypertrophy of the sexual organs the histological changes must be considered together with the changes in the weight of the organs. For this reason the results obtained in our experiments were studied both by weighing the organs—that is, by the “method of weights”—and histologically. The details of our technique have been given in previous publications (Korenchevsky, 1932, and in those mentioned above).

The hormones were dissolved in sesame oil, and injected subcutaneously twice a day for twenty to twenty-one days. The daily doses, half of which was given at each injection, were as follows: androsterone, 450–3,600 γ ; water-soluble androsterone, 13,600–27,000 γ ; androstanediol, 180–700 γ ; water-soluble androstanediol, 1,460–5,310 γ ; testosterone, 8–1,500 γ ; testosterone propionate, 30–1,500 γ ; dehydroandrosterone, 1,000–4,000 γ ; androstenedione, 500–3,000 γ ; androstenediol, 200–600 γ ; and progesterone, 50–3,000 γ . In most of the experiments oestrone and oestradiol were administered in doses of 1–30 γ three times a week, but in some experiments doses of 30 γ daily were injected. In all the experiments in which progesterone and oestrone or oestradiol were combined, preliminary injections of 6 γ of oestrone were given on alternate days for ten to fourteen days before the combined hormones were injected.

Bisexual Properties of the Hormones

These experiments on gonadectomized rats have established the fact that nearly all the sexual hormones investigated tend to a greater or less degree to restore to normal both the male and the female sexual organs, which have atrophied as a result of gonadectomy; a striking example is the most powerful of the male hormones, testosterone propionate. This brings about a return to normal, or even to supernormal, of the atrophied sexual organs of castrated males. The same hormone also restores the structure of the atrophied uterus and vagina of an ovariectomized female to a condition not far from normal, while the weight of the vagina and the development of some of the tissues in this organ is even supernormal. In normal rats it stimulates the development of the uterus and vagina to much above the normal size and weight.

The sexual hormones investigated by us may be arranged in descending order of activity as stimulators of the development of the female sexual organs approximately as follows:

1.) Oestradiol and oestrone	6. Androstenediol
2.) Testosterone propionate	7. Androstenedione
3. Testosterone	8. Transdehydroandrosterone
4. Androsterone	9. Androsterone
5. Androstanediol	10. Progesterone

When these hormones are examined from the point of view of their co-operative activity this approximate order

no longer holds good. For example, progesterone, which when injected alone into ovariectomized animals has the least effect on the sexual organs, becomes one of the most active and important of the female hormones when oestrone or oestradiol is administered simultaneously with it.

Suggested New Classification

The bisexual properties of the sexual hormones make difficult the understanding of their mode of action, and render the old classification into male and female hormones inadequate. We suggest for critical consideration a new scheme of classification of the sexual hormones based on the results of our experiments, dividing them into three groups, of which a detailed definition has been given elsewhere (Korenchevsky, Dennison, and Hall, 1937). This classification according to bisexuality has as its basis the actual activity of the hormones on male and female sexual organs, irrespective of the mechanism of such activity. For example, in the female organism this mechanism could be in the form of activity of the male hormones (1) without chemical change, or (2) after their conversion into other male hormones, or (3) after their conversion into female hormones. Disregarding the mechanism involved we suggest a grouping, as follows:

I. PURELY MALE OR FEMALE HORMONES

So far as is shown by the results of our experiments, of the hormones investigated progesterone is the only member of this group. This hormone of the corpus luteum, having no definite effect on the male rat, may be defined as a pure female hormone.

II. PARTIALLY BISEXUAL HORMONES

1. *Hormones with Chiefly Male Properties.*

Although the hormones of this group do bring about some return towards the normal of the atrophied sexual organs of ovariectomized females, with the same doses the degree of recovery of these organs is less than that of the atrophied sexual organs of castrated male rats. To this group belong androsterone, testosterone propionate, androstenediol, and probably Δ^4 -androstenedione.

2. *Hormones with Chiefly Female Properties.*

Oestrone and oestradiol belong to this group. These two hormones have a marked effect on the female sexual organs, but do not stimulate to any considerable degree the normal development of the male sexual organs, in particular the prostate, penis, and preputial glands. They have a definite effect in males, however, on the seminal vesicles and on the coagulating glands (Korenchevsky and Dennison, 1935).

III. TRUE BISEXUAL HORMONES

To this group seem to belong transdehydroandrosterone, testosterone, and Δ^5 -androstenediol, since in prolonged experiments they bring about a return to or towards the normal of all the atrophied sexual organs to approximately the same degree in males and females.

It must be pointed out, however, that further investigation of the bisexual properties of these hormones might result in one or more of them being moved from one group into another.

Co-operative Activity

The reason for investigating this aspect of the problem becomes clear when one remembers that (1) several sexual hormones have been isolated from both the male and female organisms, and (2) both male and female activities have been found in organisms of either sex. It is reasonable to suppose that the male activity may be explained by the presence of some of the sexual hormones found in the male (probably partly by dehydroandrosterone, Callow, 1936). We therefore investigated all the "chiefly

male" hormones, the "true bisexual hormones," and progesterone, the "pure female hormone," from the point of view of their co-operative activity with oestrone and oestradiol on the female sexual organs of ovariectomized rats. We summarized the results obtained by dividing the hormones (in the doses used) into five groups.

GROUPS I, II, AND III

Group I consisted of bisexual hormones appearing to have no co-operative activity in females with oestrone or oestradiol. To this group belong transdehydroandrosterone and Δ^5 -androstenediol. This group might even be termed antagonistic, since one of the "female" properties of these two hormones, mucification of the vaginal epithelium, is suppressed by oestrone. When either of these two hormones is injected simultaneously with oestrone the changes typically due to the action of oestrone are obtained, with or without cornification of the vaginal epithelium.

Group II, of bisexual hormones having co-operative activity with oestrone and oestradiol resulting in simple restoration to or towards the normal condition of the uterus and vagina without progestational changes, includes only androsterone.

Group III comprised bisexual hormones having co-operative activity with oestrone and oestradiol resulting in recovery to or towards the normal condition of the uterus and vagina with, in addition, the development of changes more or less reminiscent of those seen in pregnancy. To this group belong Δ^4 -androstenedione, androstenediol, testosterone, and testosterone propionate. In addition to an increase in size and weight, one of the chief progestational changes in the vagina of the rat is mucification of the epithelium, while in the uterus the mucosa develops lace-like foldings and the myometrium greatly hypertrophies. All the hormones in this group, even when injected alone, bring about mucification of the vaginal epithelium to a greater or less degree, but never to the extent seen in pregnancy. Only when oestrone is injected simultaneously does one obtain the complete mucification of the epithelial layer similar to that found in pregnancy. The structure of the vagina in other respects (larger size and better development of the various layers) is also reminiscent of the "pregnant" vagina. In contrast to this ease of production of the "pregnant" vagina, the progestational changes in the uterus (though even here poorly developed) were definitely seen only when either testosterone propionate alone, or more especially when testosterone propionate and oestrone together were injected.

GROUP IV

Group IV included only progesterone as a purely female hormone showing a co-operative activity with oestrone or oestradiol resulting in the production of changes closely approaching those of pregnancy. With the combined injections of progesterone with oestrone or oestradiol, histologically the nearest approach to the picture presented by the uterus and vagina in normal pregnancy was obtained by the use of 1 γ of oestrone injected three times a week along with daily injections of 1,500 γ of progesterone. Increasing the dose of oestrone, even to only 6 γ , or decreasing the dose of progesterone caused a decrease in, or with some doses even a disappearance of, the progestational changes, and especially those observed in the uterus. Even increasing both doses simultaneously to a combination of 30 γ of oestradiol three times a week with 3,000 γ of progesterone daily gave uterine progestational changes which were less than those obtained with the first combination of 1 γ of oestrone three times a

week and 1,500 γ of progesterone daily. Though the uterus and vagina showed typical histological progestational changes as a result of some of the combinations tried, both the size and the weight of these organs with all doses were considerably less than during pregnancy. In fact, the uterus was much smaller even than that of the normal rat in dioestrus. The bisexual hormones, especially testosterone and testosterone propionate, when combined with oestrone, produce progestation-like changes in the sexual organs of ovariectomized rats, while at the same time these organs hypertrophy considerably. Taking these facts into consideration, various doses and combinations of testosterone, testosterone propionate, and androstenedione (150 γ to 500 γ daily) were added to the combination of progesterone and oestrone. A greatly improved general development of the uterus and of the vagina resulted, as did progestational changes in the vagina approaching those seen in pregnant animals. But while in the uterus the myometrium considerably increased, the progestational changes in the mucosa definitely decreased. However, the degree of development of the uterus obtained in these experiments was still not identical with that of a natural "sterile pregnant" horn, and we are therefore continuing our experiments in an endeavour to reach this goal. The degree of development of the vagina, on the other hand, was exactly similar to that of the normal "pregnant" vagina.

GROUP V

Group V comprised combinations of hormones producing normal oestrus. In our previous papers and in our present experiments we have found that oestrone and oestradiol do not produce typical changes in the uterus (distension of the uterus) in ovariectomized rats, and that even in the vagina the changes obtained are not absolutely identical with those seen during natural oestrus in normal rats. We concluded, therefore, that a combination of oestrone or oestradiol with one or more other hormones was responsible for the production of natural oestrus. Up to the present we have failed to find such a hormone among those which we have examined. Some approach to the "oestrus uterus" (considerable distension) was obtained when oestrone was combined with Δ^4 -androstenedione, but this change was only obtained in some of the injected animals and the vaginal changes were atypical. Kaufmann (1935) suggested that the co-operative activity of oestrone and progesterone was sufficient to produce normal menstruation in women, but this combination failed to produce typical oestrus in ovariectomized rats.

Physiological and Pathological Properties

Among the actions of the "chiefly male" and the "true bisexual" hormones the more or less pronounced hypertrophy of, and the restoration changes in the structure of, the uterus and vagina atrophied as a result of ovariectomy must be regarded as physiological effects. Another physiological feature of these hormones is their co-operative activity with oestrone and oestradiol. Their pathological properties are evidenced by the deficient quantitative effect of the hormones (incomplete return of the atrophied organ to the normal weight, size, and structure), and by their deficient qualitative effects, in that the changes they produce do not correspond to those seen at any phase of a physiological sexual cycle. In some instances the vagina would be typical of one period of the sexual cycle and the uterus typical of another. In general it may be stated that with most of the hormones investigated both quantitative and qualitative pathological defi-

ciencies were found more often and were more pronounced in respect of the changes produced in the uterus than of those in the vagina.

Supernormal development of the sexual organs is also a pathological feature, and it was particularly striking in the uterus ("gigantic uterus") and to a less degree in the vagina when normal rats received injections of testosterone propionate. The hypertrophy of the uterus greatly exceeded that seen in the sterile horn of a pregnant uterus. The same superdevelopment was also seen in the vaginas of castrated rats injected with testosterone propionate either alone or in combination with oestrone or oestrone and progesterone. The weights of these vaginas, however, were no greater than those of the vaginas of normal pregnant rats.

Other pathological changes seen, more usually after the injection of large doses, were: In the uterus, (1) irregular structure of the stroma of the mucosa; (2) vacuolation of the uterine epithelium; (3) mucous cystic enlargement of the glands; and (4) abnormally abundant development of the fibrous tissue of the myometrium. In the vagina corresponding pathological changes were: (1) deficient or more irregular mucification than that normally seen during pregnancy; (2) the persistence of a narrow epithelial layer in the otherwise normally developed progestational vagina; (3) the absence or only slight development of the cornification seen in normal oestrus and the presence of dropsical vacuolation with leucocytic invasion of the vacuoles and the spaces between the cells of the epithelial layer in some of the ovariectomized rats injected with oestrone or oestradiol.

Great development of the clitoris (Korenchevsky, Dennison, and Eldridge, 1937 and 1937a), and of the prostate (Korenchevsky, 1935a and 1937; Korenchevsky and Dennison, 1936), in normal and ovariectomized rats injected with male hormones are features indicative of masculinization—that is, of a further pathological effect. Hypertrophy of the female preputial glands (Korenchevsky and co-workers, 1935–7) is also pathological.

Clinical Significance

In the light of the facts given above an explanation may be advanced as to why treatment with oestrone or oestradiol alone often fails to give good therapeutic results when applied to diseases of the sexual organs and general endocrine sexual disturbances in women. The activity of the corpus luteum hormone or hormones is not limited to the period of pregnancy, since false corpora lutea appear and persist for some time after each ovulation. Apart from pregnancy, therefore, this hormone (or perhaps hormones), although secreted in much smaller amount than is the case with the fully developed corpora lutea, should produce some special effect if it is combined with the oestrogenic and other hormones. Kaufmann (1935) produced evidence of the successful treatment of some gynaecological cases by the combined administration of oestrone and progesterone.

It is most improbable that the presence of male compounds in the female organism is without significance or without effect on the sexual and other organs and functions; there can be no doubt that they also play an important part in the general endocrinological co-operative and antagonistic activities in the female organism. It follows that the endocrine treatment of diseases of women cannot be perfected until (1) the male sexual hormones present in females have been isolated and identified; (2) a thorough experimental investigation of the physiological and pathological properties of these hormones, alone and

in combination with oestrogenic hormones and progesterone, has been carried out; (3) the normal ratio of oestrogenic hormones to progesterone and to male hormones in normal women has been ascertained; (4) the pathological disturbances of this ratio during endocrine and gynaecological disturbances in women have been discovered.

Danger of Masculinization

Even if all the above criteria should be fulfilled yet another problem remains to be solved before the therapeutic application of sexual hormones possessing definite male properties can safely be started—namely, the danger of masculinization. The cause of the natural occurrence of masculinity as a pathological condition in women may be understood better if the results of the experiments described above are taken into consideration. Our experiments have also shown that by the injection of some of the “chiefly male” hormones the rudimentary periurethral glands of female rats can be developed into a comparatively large and typical prostate, and in the same way the clitoris-like structure can be greatly enlarged. The supernormal development of these organs, and the possible growth of a beard or moustache with the appearance of other signs of masculinity, must be avoided in treating women. There is also the danger of a possible disturbance of the normal sexuality leading to the production of pathological perversions of sexual feeling.

These difficulties, however, can probably be overcome by working out the correct ratio of the male and female hormones. Experimental tests that might be suitable for this purpose are concerned with the development of the female preputial glands, the clitoris-like structure, and, though technically more difficult, the “female prostate” in rats. But only after the completion of the whole scheme of investigation outlined above can the male hormones—and of them only those which are found to be normally present in women—be applied therapeutically with the object of restoring to normal the pathological hormonal ratios which will be found in certain diseases of women.

Summary

1. Bisexual properties have been found in nearly all the sexual hormones, and the co-operative activity of most of them with oestrone or oestradiol has been demonstrated by experiments on female rats.

2. Suggestions are advanced as to the significance of these facts in regard to sexual physiology and pathology, and in relation to the therapy of general sexual disturbances in females.

3. In the present state of knowledge, however, the results obtained in our experiments and by other workers do not in any circumstances justify the use of the bisexual hormones with pronounced male activity in the treatment of diseases of women.

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TREATMENT OF THE FEMALE CLIMACTERIC BY FOLLICULAR HORMONE

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Although some years have now elapsed since preparations of oestrin and its derivatives became commercially available, considerable differences of opinion still exist on their clinical value. Since in my own case ignorance of certain principles underlying the administration of oestrin led at first to disappointment, and since further experience has convinced me of its unique value, I feel that the following observations on its employment during the last three years in the treatment of disorders of the menopause may prove of general interest.

The climacteric is associated with the decline in the secretory activity of the ovary and a secondary rise in the gonadotropic secretion of the pituitary. Clinically these changes find expression in the diminution and ultimate cessation of the menstrual flow. Amenorrhoea by itself, however, must not be accepted as a reliable index of the menopause. On the one hand the endocrine changes which characterize the climacteric, together with their secondary clinical symptoms, may originate several years before the occurrence of menstrual irregularity. On the other hand amenorrhoea may not infrequently be due to causes other than ovarian, and in such cases the signs characteristic of the menopause, such as hot flushes, gain in weight, and nervous disturbances, may appear many years after the actual cessation of the menstrual cycle.

It will be well to emphasize once again the frequent occurrence of menopausal symptoms. In a group of 1,000 normal women investigated by the Medical Women's Federation (*Lancet*, 1933, **1**, 106) only 15 per cent. passed through the climacteric without symptoms. Of the remainder 62 per cent. suffered from hot flushes, 44 per