

It is generally accepted that the three fractions of estrogens usually studied — estradiol, estrone, and estriol — differ considerably in their biological activity. Some authors, for example, consider that estradiol is 20 times more active than estrone and that estrone is more active than estriol [6], while others claim that the same estrogenic effect may be obtained from 0.1  $\mu$ g estradiol, 0.8  $\mu$ g estrone, and 10  $\mu$ g estriol [10]. Because of these findings, estriol is often regarded as the inactive fraction of the estrogens.

This view of the properties of the estrogens was based on the comparative investigation of the ability of estradiol, estrone, and estriol to induce an estrual reaction and enlargement of the uterus in mice and rats, and the conclusion that estriol possesses weak biological activity was linked with the view that it is the end product of metabolism of the estrogens [3, 13].

Yet this conception of the weak activity of estriol is not in accordance with observations on its action on the mammary glands. Intensive development of the mammary glands takes place during pregnancy, when the level of the estrogens rises sharply (mainly on account of estriol). Furthermore, it is now known that a pathological reaction of the mammary glands — chronic mastitis — is regularly accompanied by an increase in the level of estriol in the urine, and after successful treatment of this disease this level falls [1].

As a whole these findings suggest that estriol, although possessing weak activity toward the uterus and vagina, may possess high biological activity in relation to the mammary glands. The object of the present investigation was to shed light on this problem.

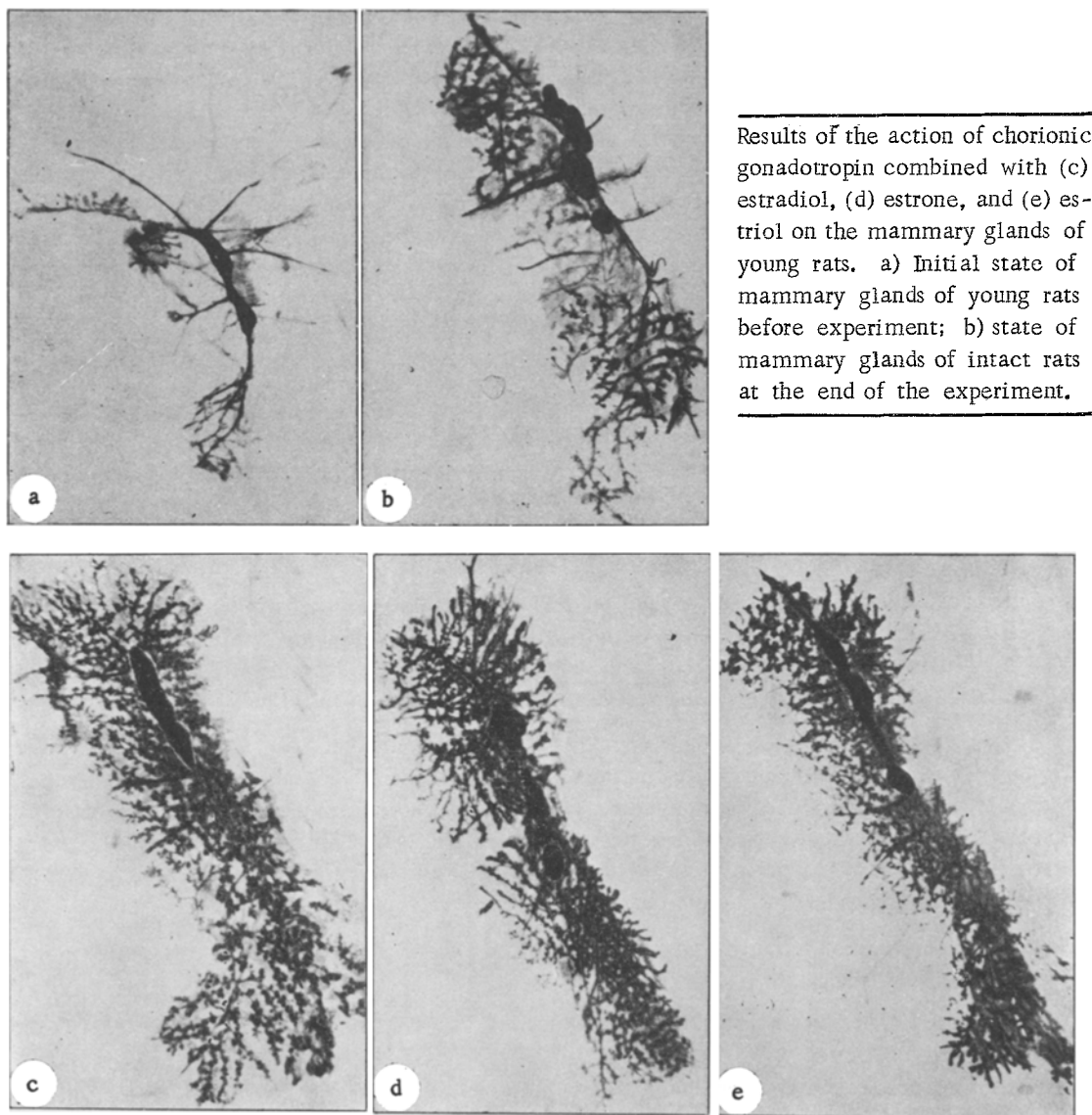
#### EXPERIMENTAL METHOD

Experiments were carried out on young noninbred rats 10 days old. Earlier experiments on these animals in the authors's laboratory showed that administration of synestrol (diethylstilbestrol dipropionate) or of a pituitary suspension does not cause development of their mammary glands, whereas the simultaneous administration of these two substances is regularly followed by hyperplasia of the mammary glands [8]. The same principle has also been established in the case of substitution of chorionic gonadotropin for the pituitary suspension [7]. Because of these findings it was expected that if a standard background of gonadotropin were established in such young rats, and against this background the different fractions of the estrogens — estradiol, estrone, and estriol — were administered in different experiments in the same dose, by comparing the degree of development of the mammary glands it would be possible to detect the assumed activity of estriol.

Experiments were carried out on 142 young rats. From the 10th day after birth the experimental animals received a daily injection of chorionic gonadotropin in a dose of 150 i.u., accompanied in the different groups of the experiment by estradiol, estrone, or estriol in a dose of 5  $\mu$ g of the aqueous suspension. The animals were sacrificed 5 and 15 days after the beginning of the experiment so that the development of the mammary glands could be followed at different stages. The development of the mammary glands was studied in total preparations obtained as follows. The inguinal and abdominal mammary glands with the surrounding connective tissue were dissected, fixed for 48 h in 20% formalin, washed in tap water for 24 h, and stained with Ehrlich's hematoxylin for 48 h. After a further washing in tap water (30 min), the preparations were differentiated at 70° in acid alcohol until a distinct pattern could be seen, after which they were washed for 10 min in distilled water and transferred to a 0.5% aqueous solution of ammonia, in which they were kept until the appearance of the required degree of staining. The preparations were then taken through 96° alcohol twice (24 h in each), kept in carboxylol for 24 h and in two changes of xylol (24 h in each), and then mounted in balsam. By this technique satisfactory total preparations of the mammary glands of rats for general study can be obtained.

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Results of the action of chorionic gonadotropin combined with (c) estradiol, (d) estrone, and (e) estriol on the mammary glands of young rats. a) Initial state of mammary glands of young rats before experiment; b) state of mammary glands of intact rats at the end of the experiment.

## EXPERIMENTAL RESULTS

The results obtained are illustrated in the figure. The mammary glands of the experimental rats gave a marked reaction to the combined administration of gonadotropin and all the estrogen fractions. As regards the degree of development of the mammary glands, practically no difference was seen between the rats of the three experimental groups. Admittedly the area of the mammary glands of the "estriol" rats was slightly smaller than in the "estradiol" rats, on account of the more compact arrangement of the ducts, but estriol caused the same degree of development of the mammary glands as estradiol and estrone.

To compare in more detail the state of the mammary glands of the experimental groups of rats, drawings were made, initially of single ducts and later of single lobules. These showed clearly that there was no difference between the mammary glands as regards the degree of development of their duct system, and in fact the degree of development of the lobules was higher in the rats receiving estriol than in those receiving estradiol.

During the study of the state of the mammary glands of the rats sacrificed early (on the 5th day) and later (on the 20th and 25th days), no difference likewise could be found in the development of the mammary glands in the experimental rats of the different groups. On the 25th day marked hyperplasia of the mammary glands was found in all the animals, but the same in all the groups.

Hence it may be concluded from the results obtained that high biological activity of estriol has been demonstrated for the first time. In the light of these findings some paradoxical clinical observations may be analyzed.

For instance, one problem which has not been explained is the following. Many clinical observations have been made indicating a relationship between the development of chronic mastitis and carcinoma of the breast on hyperestrogenization of the organism, and the authors concluded from their own investigation [4] that a direct relationship exists between the development of chronic mastitis and carcinoma of the breast, on the one hand, and the presence of follicular cysts in the patients' ovaries, on the other. It has been shown experimentally that follicular cysts of the ovaries liberate an increased amount of estrogens, because in rats they cause a permanent estrus, lasting throughout life [2], although attempts to demonstrate an increased content of estrogens in patients with chronic mastitis and carcinoma of the breast have proved unsuccessful [9]. It is now known that in the overwhelming majority of patients with chronic mastitis an increased level of estriol is present in the urine [5], and this fact, in the light of the present discovery of the high biological activity of estriol, becomes of theoretical importance, because it indicates a special form of hyperestrogenization of patients with chronic mastitis, namely hyperestriolization.

Even in the treatment of patients with carcinoma of the breast methods of hormone therapy aimed at depressing the production of estrogens are widely used, although repeated attempts to demonstrate a correlation between the therapeutic effect and the lowering of the estrogen level have failed. It should be emphasized that all investigators have sought a correlation with the content of the active estrogen fractions — estradiol and estrone — and they have completely ignored the importance of estriol in this situation. Yet such a correlation with estriol has been reported more than once [11, 12], but it is only in the light of the present findings, demonstrating the high biological activity of estriol, that this correlation becomes understandable.

It is now clear from these findings why, during pregnancy, when the estrogen level rises excessively, the mammary glands react by intensive hyperplasia, whereas the vaginal smears do not demonstrate an increased content of estrogens. This may be associated with the fact that during pregnancy the estrogen content increases mainly on account of estriol, which is highly active in relation to the mammary glands but shows only weak activity against the genital apparatus.

Finally, the results now described explain why the simultaneous development of chronic mastitis and uterine polyps, caused by hyperestrogenization of the organism, is infrequent and the simultaneous development of estrogen-dependent tumors of the mammary glands and uterus is extremely rare. This may be explained by assuming that an increased estriol level is enough to cause the development of chronic mastitis and of carcinoma of the breast, but estriol has only a weak action on the uterus.

The high biological activity of estriol, demonstrated by the experiments described in this paper, may thus provide an explanation of certain clinical observations.

#### SUMMARY

The purpose of study was investigation of the biological action of estriol, which is considered slightly active, on the mammary gland. The experiments were made on suckling female rats, which were given injections of chorionic gonadotropin in a dose of 150 i.u. daily during a period of 15 days and, simultaneously estradiol, estron or estriol in a dose of 5  $\mu$ g. It has been found that under such experimental conditions estriol causes the same degree of development of the mammary glands as do estradiol and estron. It has been inferred from these findings that estriol has a high biologic activity with regard to the mammary glands. This fact throws light on a number of paradoxical clinical phenomena.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.

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