formation of binuclear cells from mononuclear at this time, and this period of reparative regeneration could be contrasted with that of ontogenetic development as regards the fate of the binuclear cells [6, 10-12].

The fact that maximal formation of binuclear cells from mononuclear observed in this investigation in the earliest stages of regeneration can evidently be explained on the grounds that mononuclear diploid cells are among the first to take part in proliferation [2], and the transition to a binuclear cell is most evident for such cells from the point of view of the ontogenetic principles of development of the liver cells [3].

The formation of binuclear cells from mononuclear in the course of reparative regeneration is an additional reserve in the mechanism of polyploidization of the liver cells, and it evidently explains why cells of degrees of ploidy that are not found during ontogeny of the organ can appear during repeated partial hepatectomy [3].

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CHANGES IN THE LEVEL OF EPIDERMAL G_2 CHALONE AND MITOTIC ACTIVITY IN THE VAGINAL EPITHELIUM OF RATS

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The content of epidermal inhibitor of mitosis (G_2 chalone) at different stages of the estrous cycle in rats was determined by the radial immunodiffusion method. The chalone level correlates with the mitotic index of the vaginal mucous membrane and is minimal in proestrus and maximal in estrus. In aging (14-16 months) rats with regular cycles the content of G_2 chalone in the vaginal mucous membrane at all phases of the estrous cycle was significantly lower than in young (3-4 months) rats with a regular cycle. In castrated rats the mitotic index begins to rise 18 h after a single injection of estradiol benzoate (1 μg/100 g body weight). This increase is preceded by a significant decrease in the concentration of G_2 chalone 12 h after injection of the estrogen.

KEY WORDS: chalones; mitotic index; estrous cycle; estrogens.

In recent years great importance in the mechanisms of maintenance of tissue homeostasis has been attached to endogenous tissue inhibitors of proliferative activity (chalones). It

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TABLE 1. Concentration of G_2 Chalone, Mitotic Index of Vaginal Epithelium, and Blood Estradiol Level in Rats at Various Phases of Estrous Cycle (M ± m)

Phase of estrous cycle	Concentration of G_2 chalone, μg/mg protein	Mitotic index, ‰	Blood estradiol concentration, pg/ml
Diestrus	4,3±1,3	4,6±0,4	35,1±3,3
Proestrus	3,0±0,6	3,7±1,1	58,6±4,0*
Estrus	8,6±0,6*	9,8±1,4*	44,7±4,5
Metestrus	4,6±0,2	5,9±0,9	—

*Difference from other phases of estrous cycle significant ($P < 0,05$).

is generally accepted that they act by a negative feedback mechanism, i.e., that chalones synthesized by differentiated cells inhibit the increase in cell population from cambial cells [5, 6]. Hence it follows that the quantity of chalones in a tissue is not constant, but depends on the level of its proliferative activity, which changes depending on the functional state of the individual. One such tissue, in particular, is the vaginal epithelium, fluctuations in the mitotic activity of which are determined largely by the level of the blood hormones [1, 4].

Until very recently there were no methods of quantitative determination of chalones in tissues; their concentration in tissues was estimated indirectly from the biological activity of extracts. As a result of the writers' previous immunochemical investigations which demonstrated the clear tissue specificity of rat epidermal G_2 chalone [2, 3], it became possible to use immunological methods characterized by high resolving power from both quantitative and qualitative respects for its measurement. By means of these methods, in the investigation described below, changes in the level of epidermal antimitotic G_2 chalones in the vaginal epithelium of rats were investigated in different stages of the estrous cycle and compared with the mitotic activity of the mucous membrane and the blood estradiol level; data are also given to show the effect of estrogens on the chalone content in the vaginal epithelium of castrated rats.

EXPERIMENTAL METHOD

In the experiments of series I 20 rats aged 3-4 months with regular estrous cycles were used. The blood estradiol concentration of each animal was determined by a radioimmunological method, using a kit (from CEA-Ire-Sorin, France); the concentration of G_2 chalone in an aqueous extract of the vaginal mucous membrane and its mitotic index also were determined. Quantitative determination of the chalone was carried out by Mancini's radial immunodiffusion method. Monospecific antiserum against G_2 chalone was used in a dilution of 1:20 [2]. A calibration curve was plotted from the results of immunodiffusion of 20 μg purified rat epidermal chalone in serial dilutions [3]. The reaction was carried out in 1% agarose, dissolved in 0.05 M phosphate buffer with the addition of 0.5% bovine serum albumin, and the results were read 48 h after incubation in a moist chamber at room temperature. The results were expressed in μg/mg total protein, determined by Lowry's method, in an aqueous homogenate of vaginal mucous membrane. The mitotic index was calculated after counting the number of mitoses in 1000-3000 basal cells of the vaginal mucous membrane in histological sections prepared in the usual way and expressed in promille. For this purpose, an area of mucous membrane from the proximal third of the vagina was taken in all cases.

In the experiments of series II, 41 rats ovariectomized 2 weeks before the experiments were used. An oily solution of estradiol benzoate in a dose of 1 μg/100 g body weight was injected once, subcutaneously, into 30 mice. The animals were killed by dislocation of the cervical spine 6, 12, 18, and 24 h after the injection. The 11 control mice received an injection of 0.1 ml vegetable oil. Vaginal smears were investigated from all animals and the concentration of G_2 chalone and the mitotic activity of the vaginal mucous membrane were determined quantitatively.

In the experiments of series III 39 rats aged 3-4 and 14-16 months with regular estrous cycles were studied. The animals were killed at different phases of the estrous cycle and the concentration of G_2 chalone was determined in the vaginal mucous membrane.

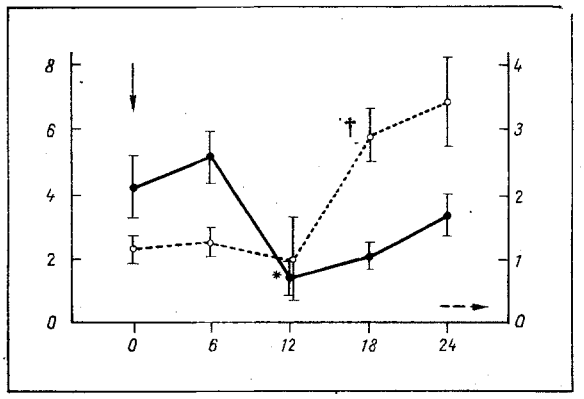

Fig. 1. Effect of estradiol benzoate on G_2 chalone concentration in vaginal epithelium of ovariectomized rats. Abscissa, time after injection of estradiol benzoate (continuous arrow above) in h; ordinate: on left — concentration of chalone (in μg/mg protein), on right — mitotic index (in ‰). Continuous line indicates concentration of chalone, broken line mitotic index; broken arrow below shows appearance of keratinizing scales in vaginal film. Values shown are M ± m. *) Difference from indices at 6 and 24 h significant ($P < 0.05$); †) difference from index at 12 h significant ($P < 0.05$).

EXPERIMENTAL RESULTS

The results in Table 1 show that the peak of mitotic activity in the vaginal epithelium was observed in the phase of estrus, in agreement with data in the literature [4]. In the same phase of the estrous cycle, the maximal concentration of G_2 chalone was observed, whereas its minimal concentration in the vaginal mucous membrane was found in proestrus when the blood estradiol level reached a peak. Meanwhile in rats in proestrus the largest number of cell layers was observed morphologically in the vagina [4] and in accordance with the principles enunciated above, this ought to be accompanied by the highest level of the inhibitor. In this connection it is important to note that 12 h after injection of the estrogen into castrated rats (series II) the chalone level in their mucous membrane was considerably reduced compared with the control animals (Fig. 1). This significant ($P < 0.05$) decrease was observed before the appearance of the first signs of proliferation of the vaginal epithelium. The peak of mitotic activity of the vaginal epithelium in rats with regular estrous cycles was somewhat delayed and took place in the phase of estrus, evidently on account of the time required for the estrogens to exert their specific mitogenic effect on the vaginal epithelium. Consequently, it can tentatively be suggested that estrogens specifically reduce the concentration of chalone — the natural inhibitor of cell division. In the phase of estrus, when the estrogens exert their biological effect and a peak of mitotic activity is found, accompanied by a simultaneous decrease in the estrogen level in the blood, the concentration of G_2 chalone in the vaginal mucous membrane rises considerably, and this is followed by a sharp decrease in the number of mitoses.

The results of these experiments thus showed that the increase in proliferative activity of the vaginal epithelium induced by estrogens is preceded by a decrease in the concentration of the natural inhibitor of cell division, G_2 chalone, also induced by them. The concentration of chalone is thus not stable and is not only under autonomous control, but is also dependent on other factors related to cell proliferation and, in particular, on the blood estrogen level.

The results of the experiments of series III are important. It was found that in the older animals the concentration of epidermal G_2 chalone in the vaginal mucous membrane in all phases of the estrous cycle was lower than in young females. For instance, the chalone

concentration (in μg/mg protein) was as follows in young and old rats respectively: in diestrus 5.3 ± 0.58 and 2.0 ± 0.35 ($P < 0.05$); in proestrus 2.6 ± 0.46 and 1.6 ± 0.42 ($P > 0.05$); in estrus it was 5.9 ± 0.69 and 3.5 ± 0.40 ($P < 0.001$). These results are the first direct evidence of a decrease in the G_2 chalone concentration in the tissues of the body with age. According to results obtained by biological testing of extracts of mouse skin, there is a decrease in the activity of antimitotic factors (G_1 and G_2 chalones) in this tissue also with increasing age [7]. The results now obtained indicate that with an increase in age there is no change in the activity, but a decrease in the concentration of G_2 chalone in the tissues.

The subsequent study of the mechanisms of interaction between chalones and estrogens will be of considerable interest. It can tentatively be suggested that a disturbance of these mechanisms plays an important role in the neoplastic transformation of the vaginal epithelium, for one group of substances are activators, the other blockers of cell division, The possibility cannot be ruled out that the decrease in the tissue chalone level with age, since chalones are factors of homeostatic regulation, can go some way toward explaining the age increase in the frequency of neoplasms.

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