ANALYSIS OF THE EFFECT OF REPEATED ESTRONE INJECTIONS ON THE MITOTIC ACTIVITY OF UTERINE EPITHELIUM IN MICE

O. I. Epifanova

Laboratory of Histophysiology (Head – Candidate of Biological Sciences V. N. Dobrokhotov), Institute of Experimental Biology (Dir. – Prof. I. N. Maiskii), Akad. Med. Nauk SSSR N. N. Zhurkov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 3, pp. 102-105, March, 1961
Original article submitted July 5, 1960

The mitotic activity of the uterine epithelium in mice can be characterized throughout a full cycle as a diphasic curve [1, 2]. The first increase in the number of cell divisions, observable in the proestrus, coincides with an increase in the concentration of folliculin in the blood; the second increase occurs in the transition from metaestrus to diestrus, and, on the other hand, takes place during a fall in the blood concentration of estrogens.

The question of the type of effect that the estrogens have on the mitotic activity of uterine epithelium in mice has been investigated by us in experiments involving the administration of folliculin (estrone) to castrated females [3]. It was shown that estrone stimulates cell division in the iterine epithelium of mice only after the first injections. Continued administration of the hormone leads to a lowering of the mitotic activity and its maintenance at a low level. Cessation of the estrone injections causes a large elevation in the mitoric activity.

The results obtained suggested that in the process of estrogenic regulation of the mitotic effect the final effect depends not only on the presence of a stimulator in the organism, but also on the reactivity of the epithelial tissue itself, i.e., on its initial state at the moment of exposure.

The purpose of this work to test this hypothesis, and to investigate the question of possible reasons for the inhibitory effect of repeated estrogen injections on the mitotic activity of uterine epithelium.

EXPERIMENTAL METHOD

Sexually mature females were used in the experiment, 20 g in weight (about 200 mice). All the animals were castrated 20 days before the beginning of the experiment. Folliculin (estrone) was administered daily by means of subcutaneous injections in a dose of 2.5 μ per 0.1 ml of apricot oil.

The same number of injections were performed on the control mice, using the oil alone. The animals were sacrificed 24 hours after the last injection.

All the animals were sacrificed by decapitation, always at the same time of day – 11:00 to 12:00 A.M. The uterus was fixed in Zenker's fluid. The upper half of the right horn was made into a series of transverse sections, 7 μ in width, which were stained with hematoxylin according to Karachchi; in every 10 sections (with intervals of 3 sections) we counted the number of mitoses in the epithelium of the mucosal lining by the method described earlier [1, 2]. The mitotic coefficient (MC) was determined as the number of mitoses per unit area of epithelium. The degree of statistical significance of the differences in the mean values was established by the method of Fisher-Student.

EXPERIMENTAL RESULTS

As can be seen in Fig. 1, the number of mitoses in the uterine epithelium of the castrated animals 20 days after removal of the ovaries and at later stages was small (mitotic coefficient equal to 0.12, 0.11, 0.12 and 0.06 respectively; the difference between these values is not statistically significant). After 2 injections of estrone the mitotic coefficient reaches a considerable level (MC = 0.45; P = 0.04), but after 6 injections it decreases to the original level (MC = 0.11; P = 0.01) and remains low to the end of the experiment (10 injections).

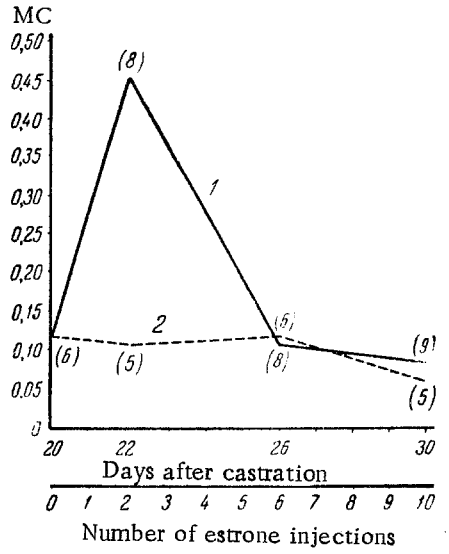

Fig. 1. Mitotic coefficient in the uterine epithelium of castrated mice and with estrone administration. 1) Experiment (administration of estrone); 2) control (castration). The number of animals is shown in parentheses.

These data almost completely reproduce the curve of changes in the mitotic activity of uterine epithelium under the influence of repeated estrone injections which was described by us in a previous work [3]. As has already been stated, cessation of the hormone administration after 6 preliminary injections caused a marked elevation in the number of cell divisions after 5 days.

In the present work this question was subjected to more detailed study (Table 1). It was shown that an appreciable elevation in the number of epithelial mitoses takes place not only on the 5th day after cessation of the estrone injections (MC = 0.40; P = 0.002), but already on the 3rd day the MC = 0.39; P < 0.0001. It was then decided to investigate what influence cessation of the estrone injections during the period of its maximal activity, i.e., after 2 injections, would have on the mitotic activity of the uterine epithelium.

As would follow from Table 1, in this case a reduction was observed in the mitotic activity by a factor of over two on the third day following cessation of the injections (MC = 0.21; P = = 0.06), but on the 5th day after termination of the hormone injections the mitotic coefficient again rose twofold (MC = 0.40; P = 0.05).

Thus, termination of estrone administration is reflected by diverse changes in the epithelial mitotic activity: in a period of intensive cellular multiplication (after 2 injections of estrone) it leads to a reduction in the number of mitoses in the tissue, with a subsequent elevation; on the other hand, termination of the hormone injections in the setting of a low level of mitotic activity (6 injections of estrone) causes a marked elevation as early as the 3rd day, maintaining it up to the 5th day. The latter fact supports results obtained earlier, and is evidence that repeated injections of estrone lead to inhibition of the mitotic activity in uterine epithelium. In a previous work [3] it was shown that the simultaneous administration of estrone and progesterone to mice, the latter stimulating cell division in the absence of estrone, does not diminish this inhibitory effect. Further analysis of this question was undertaken in this work, with the purpose of clarifying whether or not the inhibition of cell division is related to disruption of nucleic acid synthesis, or to inadequacy in the energy sources.

TABLE 1

Mitotic Coefficient in the Uterine Epithelium of Mice after Termination of Estrone Administration

Number of injections	Mitotic coefficient		
	24 hours after the last injection	after termination of the injections	
		on the 3rd day	on the 5th day
2	0.45	0.21	0.40
6	0.11	0.39	0.40

Note: There were 8 mice in each group.

TABLE 2

The Effect of Folic Acid and ATP on the Mitotic Coefficient in the Uterine Epithelium of Mice after 6 Preliminary Injections of Estrone

Conditions of the experiment	Number of injections	Number of animals in the group	Mitotic coefficient
Injections of estrone and folic acid	4	8	0.08
Injections of estrone and ATP	4	10	0.05
Injections of folic acid	4	7	0.51
Injections of ATP	4	7	0.42

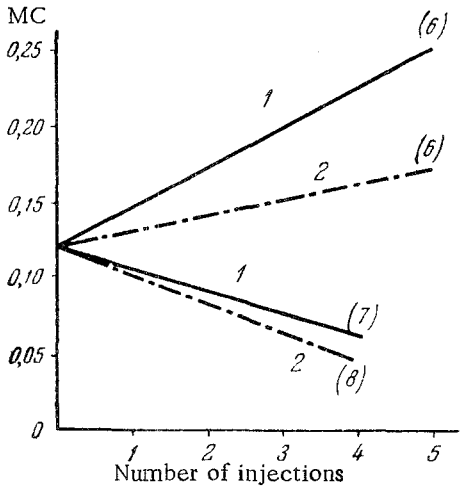

Fig. 2. Mitotic coefficient in the uterine epithelium of castrated mice and with administration of folic acid and ATP. 1) Administration of ATP; 2) administration of folic acid. The number of animals is shown in parentheses.

It is known [8] that the presence of folic acid is necessary for the development of mitoses in the uterine epithelium of rats under the influence of estrogens. In this case, the folic acid cannot stimulate the proliferative processes by itself [8-11], but it takes part in the synthesis of DNA [7, 8].

For the purpose of elevating the energy metabolism of the cells we chose adenosine triphosphoric acid (ATP), one of the most importanthigh energy compounds. According to existent data [6] ATP in a dose of 0.2 ml of a 1% solution causes a stimulation of cell division in the epithelium of the cornea in mice, 2 hours after a single injection, with a subsequent lowering to the normal level within 4-24 hours.

Because of this, we initially investigated the effect of daily repeated injections of 0.2 ml of a 0.03% solution of folic acid and 0.2 ml of a 1% solution of ATP on the mitotic activity of the uterine epithelium in castrated mice. The animals in this group of experiments were sacrificed at 2 and 24 hours after the last injection. As can be seen in Fig. 2, with the administration of either of the preparations two hours before sacrifice of the animals an elevation is observed in the mitotic activity, and with administration 24 hours before, a depression, as compared with the original level. However, these differences are not statistically significant; the differences in the values of the mitotic coefficients as dependent upon the times of the last injections is also not statistically significant. Thus, in subsequent experiments with repeated injections of folic acid and ATP we normally sacrificed the animals 24 hours after the last administration. The results of these experiments are presented in Table 2.

As follows from Table 2, neither the administration of folic acid nor of ATP stimulates cell division during the continued injection of estrone (mitotic coefficients equal to 0.08 and 0.05 respectively). If the preparation is injected after 6 preliminary injections of estrone without further application of the latter, then a considerable rise is observed in the mitotic activity (mitotic coefficients equal to 0.51 and 0.42); however, the number of cell divisions is not greater than that which is seen following termination of hormone administration (see Table 1). Hence, it would follow that this rise is not related to the administration of folic acid or ATP, but rather to termination of the estrone injections as in the preceeding experiment.

Thus, under the conditions of our experiments, not one of the substances studied (folic acid, ATP, and according to the data of a previous work, progesterone) was capable of elevating the level of cell division during continued injections of estrone, while termination of its administration caused a marked rise in the mitotic activity. It is our impression that we are here dealing with the phenomenon of activity on the part of one of the numerous systems with reciprocal relationships that guarantee the process of autoregulation in the living

organism. M. M. Zavadovskii [4] concluded that not only does the developing animal organism as a whole act as a self-regulatory system, but each of its separate sections as well. One of the basic characteristics of autoregulation involves the fact that the conditions created under the influence of certain factors in turn inhibit the further action of these factors, and simultaneously cause and stimulate antagonistic sources, which finally leads to stabilization of the process [5]. It seems to us that this is the biological significance of the phenomena we have described. Concerning the mechanisms lying at the foundation of cell division regulation by ovarian hormones, further study is needed.

SUMMARY

Estrone administration stimulates mitosis of the uterine epithelium in castrated mice, inhibiting it, however, in repated injections. Discontinuance of estrone administration at the time of intensive cell multiplication leads to reduction of mitotic activity of the tissue with its subsequent rise; as distinct from this, suspension of hormone administration against the background of low mitotic activity provokes its sharp rise already on the 3rd day.

Folic acid and adenosintriphosphate exert no stimulating effect on cellular division during oestron therapy. Evidently, the inhibitory effect of repeated oestron injections is not connected with the disturbed DNA synthesis or with the exhaustion of the high energy phosphorus compounds stored in the cell.

LITERATURE CITED

1. O. I. Epifanova, Byull. Éksp. Biol. i Med., 46, 11, 113 (1958).
2. O. I. Epifanova, in the book: Regenerative Processes in Vertebrates (Moscow, 1959) p. 199.
3. O. I. Epifanova, Byull. Éksp. Biol. i Med., 48, 12, 96 (1959).
4. M. M. Zavadovskii, The Conflicting Interaction Between Organs in the Body of the Developing Animal (Moscow, 1941).
5. A. A. Malinovskii, in the book: Problems in Cybernetics 4, 151 (1960).
6. I. A. Utkin, Experimental Study of Certain Principles of Cell Division in the Organism (Avtoref. Diss. Dokt., Moscow, 1958).
7. W. W. Gravens and E. E. Snell, Proc. Soc. exp. Biol. (N. Y., 1950) 75, 43.
8. S. Gelfant, R. K. Meyer, and H. Ris, J. Exp. Zool. 128, 219 (1955).
9. M. Härk Önen and A. Kiviranta, Ann. med. exp. Fenn. 36, 213 (1958).
10. J. C. Penhos, Acta physiol. lat.-amer. 5, 22 (1955).
11. A. Cardeza and J. C. Penhos, Rev. Soc. argent. Biol. 33, 121 (1957).

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 this issue.
