

STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION

PART XIV.—STUDIES IN THE DISTRIBUTION AND CONCENTRATION OF ALKALINE PHOSPHATASE IN THE VAGINAL SMEAR OF THE RAT DURING ESTRUS CYCLE: THE EFFECT OF OVARIECTOMY AND REPLACEMENT THERAPY

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THE PROBLEM

Thibault and Soulairac (1948) reported pronounced alkaline phosphatase activity in the vaginal mucosa of rats. Ovariectomy caused a total disappearance of the enzyme from the vagina which, however, was restored on replacement therapy with estrogen. Kamell and Atkinson (1948) made similar observations on mice. Recently, Ring (1950) noted a correlation between phosphatase activity in the vaginal epithelium of rats and the different stages of estrus cycle. He pointed out that the concentration of the enzyme was maximum in the proestrus and minimum in the metaestrus or diestrus stages. These findings were based solely on histological sections of the vagina and consequently do not provide a detailed picture of phosphatase distribution in the different cellular elements of the mucosa. The vaginal smear preparations, on the other hand, allow a much clearer elucidation of the enzyme pattern as the mucosal elements can be studied individually and with better precision.

In view of the above, an attempt has been made to investigate the distribution and concentration of alkaline phosphatase in the vaginal smear of the rat during different stages of estrus cycle. Attention has also been paid in this study to the responses evoked by ovariectomy and replacement therapy on the distribution of this enzyme in the vaginal smear elements.

EXPERIMENTAL PROCEDURE.

Albino rats, weighing 114.4 ± 10.6 gms., were used in this study. A total of 22 animals were used of which 12 were ovariectomized and the remaining 10 served as the normal controls. A group of 6 operated rats were injected intramuscularly with 1 mg. of estradiol dipropionate (in 0.2 c.c. of sterile sesame oil) daily for 6 days. The hormone therapy commenced 24 hours after the operation. An equal number of overiectomized animals were treated with sesame oil alone in a similar manner. All the animals were maintained under uniform husbandry conditions throughout the experimental period.

Daily examination of vaginal smears of the normal rats was made till each animal of the group completed all the four stages of the estrus cycle. In the overiectomized animals, however, the smears were taken from the 7th day after the operation in order to ensure a response of the genital accessories to the operative procedure and estrogen therapy.

The vaginal smears were fixed in chilled 80% ethyl alcohol and processed according to the technique of Gomori (1941) for the demonstration of alkaline

phosphatase. The sites of enzyme activity in the cellular elements are marked by the deposition of cobalt sulfide in fine black granules. In order to allow critical observation of these black granular deposits no counterstain was used. The smears were dehydrated and mounted in the usual manner. For comparison, parallel smears from each animal were stained with Ehrlich's hematoxylin followed by alcoholic eosin after ethyl alcohol fixation.

RESULTS

The cellular picture of vaginal smear of the rat during different stages of estrus cycle, is too well known to merit a repetition. No attempt is, therefore, necessary for a cytological characterisation of the vaginal smears. It would be quite appropriate to restrict our attention to the changes in the distribution of alkaline phosphatase in the cellular elements contained in the smears.

Diestrus stage.—The nucleus of the leucocytes stains positively for the phosphatase but the cytoplasm reacts in a somewhat diffuse manner. The epithelial cells, however, show considerable phosphatase activity both in the nucleus and in the cytoplasm. The overall picture at this stage is one of pronounced enzyme activity (Pl. III, fig. 1, table 1).

Proestrus stage.—As in the diestrus condition, the nucleus of the leucocytes stains positively for the phosphatase but the cytoplasm is virtually negative for the enzyme. A more or less similar pattern is noticeable in the epithelial cells but in some of these, the enzyme also occurs in the cytoplasm.

Different stages of cornification are seen in the epithelial cells. Some are slightly cornified and show a normal nucleus but in others the nucleus is degenerated and the entire cell is relatively more keratinized. A fully cornified cell, however, is scale-like and is conspicuous by the absence of a nucleus.

In the cornified cells with a normal nucleus an intense phosphatase activity is visible both in the nucleus and in the cytoplasm but those with a degenerated nucleus stain only faintly. The fully cornified cells, however, are devoid of any phosphatase activity (Pl. III, fig. 2).

Estrus stage.—Only the enucleated type of cornified cells are encountered at this stage. These cells stain negatively for the phosphatase (Pl. III, fig. 3).

TABLE I

The distribution of alkaline phosphatase in the vaginal smear elements of normal and experimental rats.

| | Diestrus. | Proestrus. | Estrus. | Metaestrus. | Ovariectomized. | Ovariectomized plus estrogen. |
|---------------------------------|------------------|------------------|---------|------------------|------------------|-------------------------------|
| <i>Leucocytes</i> .. | +++ ⁿ | +++ ⁿ | .. | +++ ⁿ | +++ ⁿ | .. |
| <i>Epithelial cells</i> .. | ++ | ++ | .. | .. | ++ | .. |
| <i>Cornified cells—</i> | | | | | | |
| (a) With normal nucleus .. | .. | ++ | .. | .. | .. | .. |
| (b) With degenerated nucleus .. | .. | + | .. | + | .. | .. |
| (c) Without nucleus .. | .. | — | — | — | — | — |

Legend:— ++ⁿ = Strong phosphatase activity only in the nucleus.
 ++ = Strong activity throughout the cell.
 + = Faint reactions.
 — = Negative reactions.

Metaestrus stage.—The nucleus of the leucocytes gives positive reactions for the phosphatase but the cytoplasm reacts only in a faint manner. The enzyme is completely absent in the enucleated cornified cells but a diffuse reaction is given by those with a degenerated nucleus (Pl. III, fig. 4).

Ovariectomized rats.—The vaginal smears of ovariectomized rats show a typical diestrus condition with an overall high concentration of phosphatase in the cellular elements. As in the normal rats, only the nucleus of the leucocytes stains positively for the enzyme but the cytoplasm shows a diffuse distribution. The epithelial cells give a positive reaction in the nucleus but the cytoplasm reacts intensely. The isolated cornified cells, however, show only negligible amounts of the enzyme.

Estrogen therapy.—Only enucleated cornified cells are visible in the vaginal smears of the ovariectomized rats treated with estrogen. These cells stain negatively for the phosphatase.

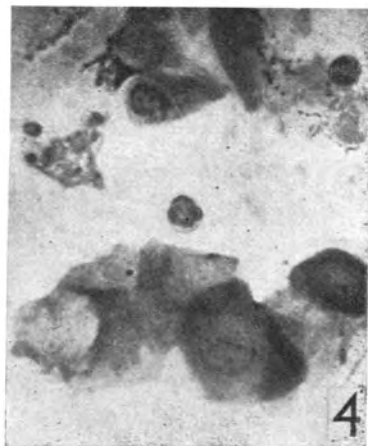
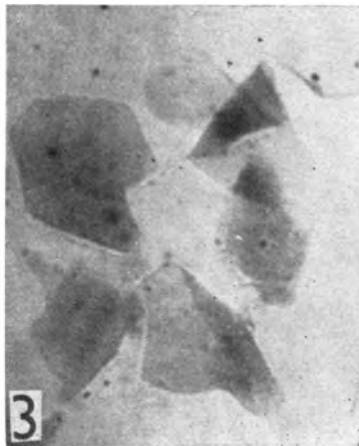
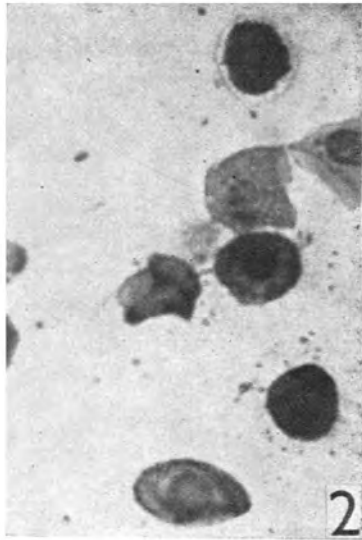
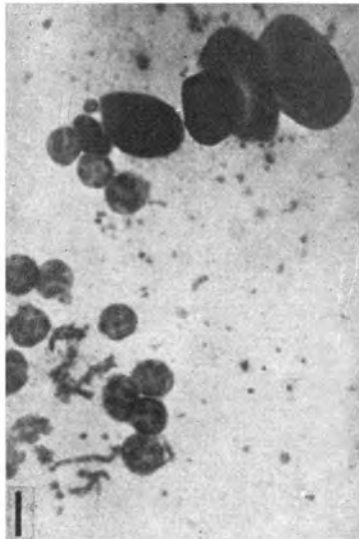
DISCUSSION

The present studies indicate clearly that there is pronounced phosphatase activity in the vaginal smear elements during the diestrus and the proestrus stages. However, the activity of the enzyme is considerably lowered during the estrus but reappears from the metaestrus stage. The ovariectomized rats exhibit a typical diestrus pattern and estrogen therapy is associated with a lowered activity of the enzyme as in the estrus condition.

Ring (1950) reported that phosphatase activity is maximum during the proestrus and minimum in the metaestrus or early diestrus stages. He added that the concentration of the enzyme is considerably less in the estrus condition. Our findings on the distribution of alkaline phosphatase in the vaginal smears are in general agreement with those of Ring. It may, however, be recalled that we observed a recurrence of enzyme activity in the metaestrus stage which is significantly enhanced during the diestrus. These apparently discrepant results could be ascribed to the fact that Ring focussed his entire attention on the epithelial cells but did not take into account the leucocytes which are found in large numbers during the diestrus and the metaestrus stages. As these cells exhibit fairly high enzyme activity, their contribution to the total phosphatase picture of the vaginal mucosa has to be taken into due consideration.

Thibault and Soulairac (1948) noted that ovariectomy causes a total disappearance of alkaline phosphatase from the vaginal mucosa which is adequately restored after replacement therapy with estrogen. Similar observations are reported by Kamell and Atkinson (1948) on mice. Ring (1950), on the other hand, described a marked concentration of the enzyme in the vaginal epithelium of the ovariectomized rats. Estrogen therapy produces a typical estrus picture of phosphatase distribution. The present studies on vaginal smear preparations lend support to Ring's findings and show that the activity of the enzyme in ovariectomized rats is similar to that of the normal diestrus condition and that estrogen therapy simulates the phosphatase pattern of the estrus stage. It may be recalled that the enzyme activity is considerably high in the diestrus stage but goes down markedly in the estrus.

A question may now be raised as to the physiological significance of the cyclic fluctuations in vaginal phosphatase activity during the estrus cycle. Ring (1950) suggested that the enzyme is concerned with the formation of keratin in the epithelial cells. The present findings also subscribe to such a concept. In this connection, reference may be made to our observation that there are three stages in the cornification of epithelial cells which are clearly discernible by the condition of the nucleus. In the initial stage when the formation of keratin has just commenced, the nucleus presents a normal appearance. With rapid progress in the process of keratinization, the nucleus becomes degenerated whereas in a completely keratinized cell it is absent. Concomitantly, the phosphatase activity is very high in the initial stage



of keratinization but is considerably reduced as keratinization progresses and is practically absent in a fully cornified cell. Apart from this, it is also probable that the enzyme is concerned with the proliferation of the epithelial cells, particularly during the proestrus stage when the vaginal mucosa thickens appreciably and numerous mitoses appear in the basal epithelial layers (Turner, 1948).

SUMMARY

1. Pronounced alkaline phosphatase activity is visible in the vaginal smears of the rat during the diestrus and the proestrus stages. The activity of the enzyme is considerably reduced during the estrus but there is a recurrence from the metaestrus stage.
2. Ovariectomy induces typical diestrus pattern of phosphatase distribution but replacement therapy with estrogen is associated with a low enzyme concentration as in the estrus.
3. The phosphatase is probably concerned with the keratinization and proliferation of the epithelial cells of the vaginal mucosa.

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EXPLANATION OF PLATE III

(All figures are photomicrographs and are magnified $\times 400$.)

- FIG. 1. Vaginal smear elements of a rat in diestrus condition. Note pronounced phosphatase activity in the large epithelial cells.
- FIG. 2. Vaginal smear picture of a rat in proestrus stage. Note the distribution of alkaline phosphatase in the epithelial cells which are in different stages of cornification. Leucocytes are not shown in the figure.
- FIG. 3. Vaginal smear elements of a rat in estrus. Phosphatase is absent from the scale-like cornified cells.
- FIG. 4. Vaginal smear picture of a rat in metaestrus condition. Note the distribution of the enzyme in the different elements.

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