Effect of Estrogen on Uterine ATP Levels.* (30428)

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The early phase of estrogen action in the uterus of an ovariectomized or immature rat is characterized by a rapid acceleration of RNA and protein synthesis(1,2,3). Although the mechanism of estrogen action is unknown, estrogen may stimulate the activity of transhydrogenase which reduces NADP(4), thus increasing the flow of electrons through the respiratory chain. Presumably this would be followed by an increase in production of ATP. The finding of Gautheron and Born(5) that administration of estradiol increased the ATP content of the uterus of immature rats lends support to this type of speculation, but that of Gorski(6), who reported no significant changes in ATP levels following administration of estradiol, leaves the situation confused. In view of the importance of ATP in biosynthetic processes, it was felt that this subject deserved more study.

We have found that the ATP level of the uterus decreases in the early stages of the estrogenic response and this decrease in ATP level is prevented by administration of actinomycin D, but not by puromycin. The possible significance of these findings in relation to RNA and protein synthesis will be discussed.

Materials and methods. Sprague-Dawley rats of the same age and weighing approximately 180 g were ovariectomized through the dorsal approach. They were maintained on Wayne Lab Blox rat food for one month before use. In other experiments we used immature female rats weighing approximately 60 g.

The ovariectomized rats were injected intraperitoneally with 10 μg of estradiol in 0.5 ml of saline at zero hour, whereas the immature rats received 5 μg of estradiol. Actinomycin D (500 μg) in 0.5 ml of 1,2-propanediol in saline (1:9) was injected intraperitoneally 30 minutes before zero time. Puromycin dihydrochloride (20 mg) in 2 ml of saline was injected hourly in four equal doses, starting from 15 minutes before zero time. Control animals were injected with saline solution only.

After sacrifice, the uteri were quickly excised and weighed, and ATP was extracted by dropping the uteri into about 50 ml of boiling water. After 10 minutes of boiling, the extract was quickly chilled in an ice bath and made to an appropriate volume with cold water. ATP levels were then determined by the luciferin-luciferase reaction using a Tricarb liquid scintillation counter(7).

Results and discussion. Fig. 1 shows that in both ovariectomized and immature rats, ATP levels in uteri decrease almost linearly after administration of the hormone and reach about 50% of the control value at 4 hours. The finding that uterine levels of ATP decrease following administration of estrogen contradicts the reports of others(5, 6), but it should be emphasized that the present method of ATP determination is very specific and rapid, enabling the performance of assays within 30 minutes after sacrifice of the animals.

Our findings of low levels of ATP in the uterus are comparable with the results of Clark and his associates(8). These workers found that the concentration of ATP in the liver of rats decreased to 30% of normal following ingestion of a high protein meal following a 10-hour fast. Both the uterus stimulated by estrogen and the liver stimulated by a sudden influx of amino acids may have increased energy requirements, attributable to increased synthetic activity and resulting in a drain on ATP. A reduction in ATP levels might occur in spite of increased rates of production if the demand is sufficient.

Fig. 2 shows that puromycin, an inhibitor of protein synthesis, does not block the decrease in ATP levels after estrogen administration. We may, therefore, conclude that

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the energy requirements for protein synthesis *per se* are not sufficiently demanding to cause a decrease in the cellular ATP levels. However, it is seen that actinomycin D, an inhibitor of DNA-dependent RNA synthesis, prevents the decrease in ATP levels following estrogen stimulation. This finding provides evidence that DNA-dependent RNA synthesis may be the critical energy-requiring system in uterine cells under these conditions. Furthermore, if we compare the data concerning estrogen-induced RNA synthesis obtained by Hamilton(1) with the data of Fig. 2, we observe an approximately inverse relationship between the rate of RNA synthesis in the uterus and the level of ATP. This provides support for the theory that RNA synthesis is an important drain on ATP in uterine cells.

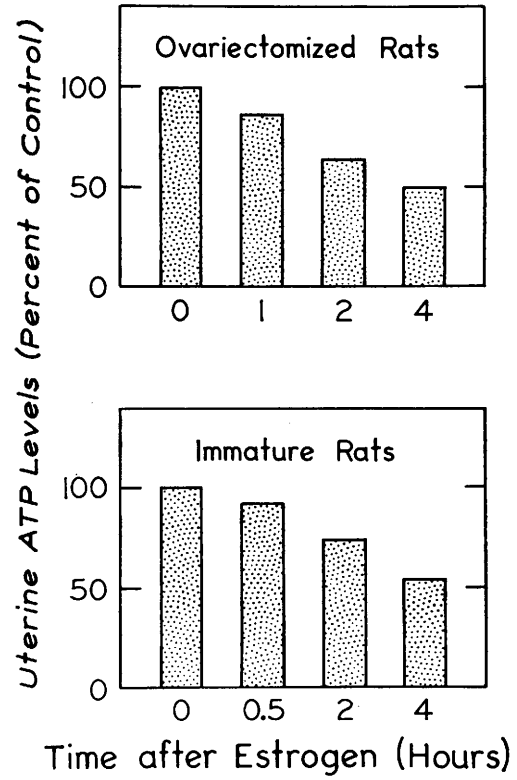

FIG. 1. Time sequence for estrogen-induced change of ATP levels in uteri of ovariectomized and immature rats. Each point represents the value obtained from extracts of pooled uteri from 4 rats. Actual values of control (zero-time) animals were 1.50 μmoles ATP per g wet weight of uterus for ovariectomized rats and 1.54 μmoles ATP per g wet weight for immature rats.

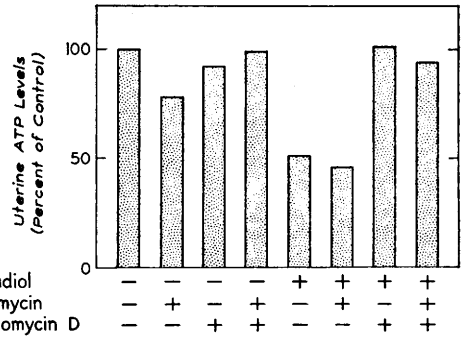

FIG. 2. Effects of puromycin and/or actinomycin D on ATP levels in control and estrogen-treated uteri of ovariectomized rats. Dose and time schedule of administration of estradiol and antibiotics are described in text. The animals were sacrificed at 4 hours after the hormone administration. Each point represents the value obtained from extract of pooled uteri from 2 rats. Actual value of control animals was 2.15 μmoles ATP per g wet weight of uterus.

Summary. The ATP levels were determined in the uteri of ovarectomized and immature rats following administration of estradiol. An almost linear decrease occurred with time so that the ATP level was reduced to 50% of control value at 4 hours. ATP levels seem to bear an inverse relation to the reported rates of RNA synthesis in a variety of experimental situations, thus RNA synthesis, not protein synthesis, is the most demanding utilizer of ATP in uterine tissue under the conditions of these experiments.

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