Early Stimulation by Estradiol of Amino Acid Penetration in Rabbit Uterus*

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The biochemical events by which estrogen causes rapid growth of the uterus have been investigated principally by studies of the intermediary metabolism or specific enzyme relations within the uterus (1, 2). The tissue-proliferating effect is apparent only after a period of sustained estrogen stimulation whereas the effect of estrogen on the water content and the electrolytes of the uterus is very prompt (3, 4). Electrolyte-water relationships and intermediary metabolism have been linked together by the observation that there is a reciprocal relation between the transport of amino acids into cells and the intracellular concentration of the inorganic cations (5). However, studies on the action of estrogen on uterine permeability and its relation to tissue proliferation have been difficult because of the metabolizable nature of most of the available substrates.

The demonstration by Christensen (6) that α-methylamino acids are transported across the plasma membrane in a manner similar to their natural counterparts has led to the development of α-aminoisobutyric acid-1-Cl4 as a tool with which penetration phenomena may be studied without the complication of further metabolism of the substrate (7). Brief communications have reported the utility of α-aminoisobutyric acid 1 Cl4 in the study of the estrogen-dependent transport process in the uterus (7, 8). The present study extends these observations to the very early effects of estradiol on amino acid transport in uterine tissue.

**EXPERIMENTAL PROCEDURE**

Radioactive Materials and Methods—Sucrose-U-Cl4 was purchased from Volk Radio-Chemical Company with an activity of 0.1 mc in 34.3 mg and estradiol-17β-16-C14 was obtained from Charles E. Froest and Company, Montreal, with an activity of 1.35 μc per mg. AIB 1 of specific activity 2.2 mc per mmole was prepared from potassium cyanide-Cl4 on a microscale by a procedure based on the report of Zelinsky and Stadnikoff (9). Samples of soft tissue were assayed for AIB or radioactive sucrose by previously described procedures (7, 8). Cartilage was rendered amenable to the method by placing the loosely stoppered grinding tubes containing the samples in a boiling water bath for 5 minutes before homogenization. Tissues obtained from experiments with estradiol-C14 were finely homogenized with water and aliquots of the total homogenate were plated. All counts were corrected to infinite thinness.

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1 The abbreviation used is: AIB, α-aminoisobutyric acid-1-C14.

Animals — Immature New Zealand white female rabbits weighing between 1800 and 2000 g were used in all experiments. All rabbits were ovariectomized at least 1 week before use except in some of the AIB distribution experiments in vivo.

### Extracellular Space Determinations

Extracellular spaces were determined by measuring the sucrose-U-Cl4 space and also the classical chloride space. Rabbits were nephrectomized under Nembutal-ether anesthesia and then were treated by intravenous injection of radioactive sucrose at the ratio of 2 μc per kg of body weight. Thirty minutes after the sucrose injection the rabbits were exsanguinated via the vena cava into a heparinized syringe before the tissue samples were excised (10). Duplicate samples were taken for analysis of water content. As a check on the method and the Cl4 determinations, the chloride space and sucrose space were simultaneously determined on several tissues. Chloride was determined on soft tissues by the nitric acid extraction procedure of Lowry and Hastings (11) and on plasma by the wet ashing method of Wilson and Ball (12). Space determinations were also made for the uterus after estrogen treatment. Rabbits were treated by subcutaneous injections daily for 4 days with 10 μg of estradiol-17β in 0.2 ml of sesame oil and then sucrose measurements were made on the 5th day. Spaces were calculated as grams of water per kilogram of wet tissue as previously described (11, 13).

### AIB Distribution in Vivo

All rabbits were nephrectomized while under Nembutal-ether anesthesia immediately before the intravenous injection of AIB at the ratio of 0.3 μc per kg of body weight. Control rabbits were ovariectomized at least 1 week before use. Estrogen-treated rabbits were injected for 4 days as described above and then used on the 5th day, 24 hours after the last estrogen injection. At various time intervals after the AIB injection the rabbits were reanesthetized and killed by exsanguination and tissue samples were excised and assayed for AIB. Distribution of AIB between intracellular and extracellular phases was calculated as previously described (7).

### AIB Distribution in Vitro

The studies were limited to the uteri from the ovariectomized rabbits. Rabbits were killed by air embolism and the uteri were quickly excised and floated on buffer solution. Each horn was freed of fat and fascia and then placed in an individual incubation flask. Each uterus served as its own control. The first horn was incubated without hormone whereas the contralateral horn was used to assess the effect of estradiol. The horns were individually incubated for various lengths of time at 38° in 20 ml of Krebs-Ringer phosphate buffer of pH 7.4 with a 100% O2 atmosphere and AIB at 0.85 ×
The uptake of AIB by the immature rabbit uterus was prepared for analysis by being split open longitudinally, quickly rinsed through two beakers containing nonradioactive buffer, lightly blotted, and then weighed. The AIB uptake in the experimental horns was calculated relative to the uptake in the contralateral controls. Estrogen effect in vitro was tested by adding a propylene glycol solution of estradiol to the experimental flasks to give a concentration of 0.5 μg per ml of estrogen and 0.26% of the glycol. The control flasks received a similar amount of estrogen-free glycol. Estrogen stimulation in vivo was determined by anesthetizing the rabbits, removing the control horn to an incubation flask, and immediately thereafter treating the rabbits by intravenous injection of 0.2 ml of propylene glycol containing 100 μg of estradiol. After allowing the estrogen stimulation in vivo to proceed for various times the contralateral horns were each removed and incubated in vitro in a similar manner. The incubation of each horn was terminated after 60 minutes.

**Estradiol Distribution in Vitro**—Uterine horns were incubated together in 50 ml of Krebs-Ringer buffer at 38° with a 100% O₂ atmosphere. Radioactive estradiol was added in propylene glycol to give a concentration of 0.60 μg per ml. At 30 and 60 minutes of incubation time, tissue and medium were each sampled and assayed for total C¹⁴ content.

**Estradiol Distribution in Vivo**—Anesthetized rabbits were injected intravenously with 0.3 ml of a propylene glycol solution which contained 181 μg of radioactive estradiol. The observed specific activity was 2000 c.p.m. per μg. One horn of the uterus was removed at 30 minutes after injection and at 60 minutes the second horn was removed, the rabbit was exsanguinated, and other tissues were excised. Tissues and plasma were assayed for total C¹⁴ content.

**RESULTS AND DISCUSSION**

**AIB Distribution in Vivo**—The uptake of AIB in vivo, shown in Fig. 1 and Table II, has been calculated with the aid of the values given in Table I and represents the concentration of AIB in the intracellular phase relative to the extracellular. Fig. 1 shows the rate of uptake of AIB by the control uterus to be rather slow and characterized by a small initial slope of the curve, at least 10 hours being required to achieve a steady state distribution. In contrast to these results are those found in the estrogen-stimulated uterus in which the initial rate of uptake is markedly increased. The enhanced rate leads to a concentration gradient 0 times greater than that of the controls. The experiments were commenced 24 hours after the fourth injection of estradiol and represent the permeability of a uterus which has been well stimulated by the estrogen. The estradiol conditioning is seen to last for nearly 2 days after the last estrogen injection at which time the curve approaches that for untreated uterus. The early part of the curve represents the time interval 24 to 48 hours after the last injection of estradiol and is in the period during which the uterus was fully stimulated. Due to the time course of the estrogen treatment and the slow rate of entry of AIB into the cells, the highest figures presented are considered to represent minimal values for the estrogenic stimulation of AIB uptake by the uterus. The stimulated uterus concentrated AIB 27 times over the level of the extracellular fluid. Such high values appear to be characteristic of tissues which are metabolically very active. Thus, in Table II liver is seen to concentrate as much as 84 times and kidney has been found to achieve concentrations of 40 times (7).

The strong uptake of AIB by liver does not seem to be affected by estrogen. The 18-hour liver values are not significantly different (p > 0.1). Skeletal muscle, which also is not a primary estrogen target, similarly shows no effect. On the other hand, pubic symphysis is known to have its metabolism stimulated by estradiol as evidenced by the incorporation of glycine into

![Fig. 1. The uptake of AIB by the immature rabbit uterus in vivo.](image-url)
estrogen space. In the experiments of Table III the radioactivity of the tissues to accumulate estradiol by calculating what is termed an estrogen space. It has been found convenient to express the ability of the uterus to take up AIB after being thoroughly conditioned and those given in Fig. 1 suggested strongly that the mode of estradiol stimulation experiments in vivo and are shown in Table IV. The radioactivity of the uterus is seen to increase with time and at 60 minutes its activity is approximately the same as the value for plasma. In this type of experiment the plasma activity is initially high and rapidly decreases as the estrogen is metabolized. If the uterus accumulated the estrogen in vivo as easily as it does in vitro one would expect the 60-minute value to be considerably larger than the value for the plasma. The data suggest a more complex estrogen action in vivo than in vitro. Although estradiol per se may be extracted from the rat uterus after its intravenous injection (15) these data suggest the possibility that the active estradiol may be in an as yet unrecognized form. Estradiol in vivo strongly stimulates a number of metabolic activities of uterus and increases the ability of pubic symphysis to incorporate amino acids (14). Table IV in comparison with Table I shows that both of these tissues have an estrogen space greater than their extracellular space. Liver and muscle, which are not primary estrogen targets, show estrogen spaces which are in reasonable agreement with their extracellular spaces. Thus, the hormonal activity of estradiol correlates with its estrogen space.

Recently Halkerston et al. have reported a failure to observe early effects of estradiol on permeability in the rat uterus (16). The experimental plan we followed was found to be of consider-}

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time</th>
<th>Tissue or medium (c.p.m./g)</th>
<th>Estrogen space (g/kg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus</td>
<td>30 min</td>
<td>7,000 ± 50 (3)</td>
<td>7,700 ± 50 (3)</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>985</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>60 min</td>
<td>11,300 ± 300 (3)</td>
<td>12,200 ± 300 (3)</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>925</td>
<td></td>
</tr>
</tbody>
</table>

### Table IV

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue (c.p.m./g)</th>
<th>Estrogen space (g/kg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus</td>
<td>200 ± 20 (2)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>100 ± 9 (2)</td>
<td>300 ± 50 (2)</td>
</tr>
<tr>
<td>Biceps femoris</td>
<td>20 ± 3 (2)</td>
<td>72 ± 3 (2)</td>
</tr>
<tr>
<td>Pubic symphysis</td>
<td>250 ± 5 (2)</td>
<td>700 ± 50 (2)</td>
</tr>
<tr>
<td>Plasma</td>
<td>350 ± 30 (2)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. AIB uptake in vitro after estradiol treatment in vivo.
The ability of estradiol to influence the uptake of $\alpha$-amino-isobutyric acid by rabbit tissues has been studied. Uterus is strongly stimulated by 4 days pretreatment with estradiol whereas muscle, liver, and pubic symphysis are not affected. Stimulated uterus is characterized by an increased rate of entry of the amino acid and by a steady state distribution ratio at least 9 times greater than that of uterus from castrated controls.

The penetration of the amino acid into the uterus was increased 135% after only 30 minutes exposure to estradiol in vivo. In contrast, exposure to estradiol in vitro did not change the permeability in spite of the fact that the estrogen was strongly accumulated by the uterus.

REFERENCES

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