Early effect of estradiol on amino acid penetration in rabbit uterus

The stimulation of protein synthesis by estrogen in uterine tissue has been observed to follow the early initial water and electrolyte changes[1]. Estrogenic stimulation of the incorporation of radioactive substrates into uterine tissue components has required several hours pretreatment *in vivo* or long incubation in tissue culture[2]. These apparent differences have lead to separate experimental consideration of electrolyte and protein processes. However, RIGGS[3] has shown in the Ehrlich ascites tumor cell that the penetration of amino acids into the cell, the earliest step in cellular protein synthesis, is intimately related to potassium metabolism. With the demonstration of a correlation between electrolyte metabolism and cellular penetration of amino acids it becomes pertinent to consider the earliest effect of estrogen on amino acid penetration into uterine tissue. Amino acid transport has been examined with the non-utilizable amino acid [1-^{14}C]AIB and the association of uterine tissue with estrogen has been observed with [16-^{14}C]estradiol-17β.

Immature, ovariectomized New Zealand white rabbits were used in three types of experiments: estradiol stimulation *in vivo* followed by AIB penetration *in vitro*, estradiol AIB incubation together *in vitro*, and radioactive estradiol penetration *in vivo* and *in vitro*. Rabbits were anesthetized and control horns removed to incubation flasks containing buffer and AIB. After the intravenous injection of estradiol the contralateral experimental horns were removed at various times and incubated with AIB in a manner similar to the controls. In the experiments *in vitro* the rabbits were sacrificed and the control horns placed in incubation flasks with buffer and AIB while the flasks with the contralateral experimental horns had estradiol in addition. After the incubations the tissues and media were assayed for AIB as previously described[4,5]. Estrogen-penetration studies *in vivo* were carried out by injecting rabbits intravenously with radioactive estradiol, removing uterine tissue at various

TABLE I

EFFECT OF TREATMENT WITH ESTRADIOL ON ABILITY OF UTERUS TO CONCENTRATE AIB

Each uterine horn was incubated at 38° with continous oxygenation in 20 ml of Krebs-Ringer phosphate buffer, pH 7.4. AIB of specific activity $1.7 \cdot 10^9$ counts/min/mmole was at a concentration of $8.78 \cdot 10^{-6}$ *M*. Estradiol was at level of 0.5 μg/ml. AIB was determined as counts/min/ml of medium or total tissue water, from which the concentrating ability of the tissue relative to the medium was calculated. The figures reported show the per cent increase in the ability of the estrogen-treated uteri to concentrate AIB as compared to the controls. The figures in parenthesis are the number of experiments.

	Incubation time	
	30 min	*60 min*
Estradiol *in vivo*		
30 min		131 ± 10 (3)
60 min		159 ± 1 (2)
120 min		195 ± 14 (2)
Estradiol *in vitro*	0.46 ± 6.7 (19)	7.3 ± 9.6 (11)

Abbreviation: AIB, α-aminoisobutyric acid.

times and obtaining a terminal blood sample. Estrogen penetration *in vitro* was observed by incubating the uterine horns in buffer containing radioactive estradiol. The tissues, blood plasma and media were analyzed by determining the ^{14}C content. All radioactivity determinations were corrected to infinite thinness.

TABLE II

[16-^{14}C]ESTRADIOL UPTAKE BY UTERUS

In vivo experiments. Rabbits were injected with 181 μg [16-^{14}C]estradiol-17β, 1963 counts/min/μg. Uterine horns were removed after being exposed to the circulating estrogen for 30 and 60 min. *In vitro* experiments. Uterine horns were pooled and incubated for 30 and 60 min in 50 ml of Krebs-Ringer phosphate buffer containing 0.605 μg/ml of radioactive estradiol. Plasma was prepared for ^{14}C determination by plating an aliquot; incubation media were diluted with 10% egg albumen prior to plating. Tissues were ground with 10 vol. water and aliquots of the total homogenates were plated for counting.

Reaction time (min)	*In vivo*		*In vitro*	
	counts/min/g uterus	*counts/min/ml plasma*	*counts/min/g uterus*	*counts/min/ml medium*
30	204 ± 20 (2)		7,590 ± 50 (3)	985
60	340 ± 4 (2)	346 ± 32	11,276 ± 310 (3)	925

The results show that estradiol when injected *in vivo* has an immediate effect on amino acid penetration, which is concomitant with the earliest time when electrolyte shifts may be observed. The early effect *in vivo* is accompanied by an association in uterine tissue of radioactivity which was derived from estradiol. The stimulation *in vivo* of penetration of amino acids by estradiol is not found in analogous experiments *in vitro*. The failure *in vitro* cannot be attributed to inability of estradiol to penetrate uterine tissue since the experiments *in vitro* show that uterine tissue can concentrate the estradiol from the medium.

These experiments demonstrate that the very early effects of estradiol on the uterus include a stimulation of the first step in protein synthesis, the cellular penetration of amino acids. The dependence of the stimulation on the presence of the organism suggests that estradiol is converted *in vivo* to some other active form.

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