The effect of estradiol pretreatment on the serine aldolase activity of rat uteri*

Previous studies from this laboratory have demonstrated the high sensitivity of certain "one carbon" metabolic pathways to estrogen administration[1,2]. Six hours after a single injection of estradiol, the incorporation of glycine-2-^{14}C and formate-^{14}C into acid-soluble serine by surviving uterine segments was increased respectively to 2 and 3 times the control level[1]. The conversion of serine-3-^{14}C to formate was also stimulated[2]. These results prompted an investigation of the effect of estradiol pretreatment on the level of serine aldolase activity in the rat uterus.

At varying times after the intravenous injection of 10 μg of estradiol-3,17β to ovariectomized rats, the animals were sacrificed and 10% uterine homogenates prepared in the buffered sucrose medium of HOAGLAND *et al.*[3]. Aliquots of these homogenates were incubated in a N_2 atmosphere at 37° in the following medium: 3 μmoles glycine-2-^{14}C (1 μc/μmole), 3 μmole formaldehyde, 1.5 μmole tetrahydrofolic acid (THFA), and 0.05 ml 0.5 *M* K_2HPO_4–KH_2PO_4 buffer, pH 7.4, in a final volume of 1.5 ml. THFA was prepared according to the method of BROQUIST *et al.*[4]. The reaction was terminated by the addition of 0.125 ml of glacial acetic acid – 88% formic acid mixture (13:1). After dilution to 2 ml, the precipitated protein was removed by centrifugation and 1.5 mg each of nonradioactive glycine and serine added to the supernatant as carrier.

Duplicate 10 μl aliquots of this supernatant were applied as two separate bands, 2 cm long and 2 cm apart, on 15 × 92 cm strips of Whatman No. 1 paper which were buffered with 1 *M* acetic – 1 *M* formic acid mixture at pH 2.1. Good separations were achieved by high potential electrophoresis** after 45 min at 12,000 volts and 8 mA[5]. After drying in air, the duplicate pherograms were separated lengthwise and the amino acids localized by warming briefly the guide pherogram, which had been dipped through a solution of 0.2% ninhydrin in acetone. The corresponding section of the duplicate pherogram containing the serine band was eluted with water and plated on aluminum planchets for counting. The data are expressed as the total number of counts incorporated into serine/mg dry weight of protein residue.

Under the conditions used, the incorporation of radioactivity from glycine-2-^{14}C into serine was linear with time over a 30 min incubation period and proportional to tissue concentration with up to 1 ml of a 10% homogenate. The addition of adenosine triphosphate, diphosphopyridine nucleotide, triphosphopyridine nucleotide, hexose diphosphate, phosphocreatine, or pyridoxal phosphate did not facilitate the reaction. Attempts to convert formate-^{14}C into serine were unsuccessful, despite extensive efforts to fortify the system with various cofactors. The difficulty appeared to lie in the initial activation of the formate.

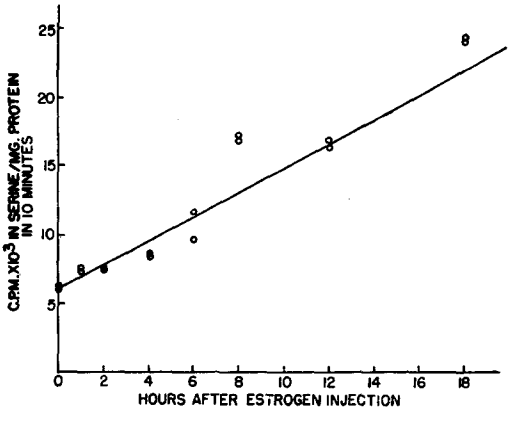

Fig. 1. Serine aldolase activity in 0.5 ml of 10% uterine homogenates at varying times after administration of estradiol. Incubated 10 min at 37°.

The effect of pretreatment with a single 10 μg dose of estradiol on the level of serine aldolase activity is shown in Fig. 1. During a period of 18 h following the administration of the hormone, the serine aldolase activity increased linearly with time to 4 times the control level. Thus one of the early effects of estrogen on uterine metabolism has been demonstrated as a change in

* This work was supported by the Alexander and Margaret Stewart Trust Fund, Grant No. 1897 from the United States Public Health Service, and an institutional grant from the American Cancer Society.

** A high potential electrophoresis apparatus designed in collaboration with Gilson Medical Electronics, 714 Market Place, Madison, Wisconsin was used in these experiments.

enzyme activity. Experiments are now in progress to determine whether the increased serine aldolase activity results from activation of pre-existing enzyme or from the *de novo* synthesis of new enzyme, and to determine also the role of the hormone in this process.

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Received January 30th, 1957

Methylation of uracil deoxyriboside by soluble enzymes of thymus

Administration of serine-3-^{14}C or ^{14}C-labelled formate or formaldehyde to animals leads to extensive incorporation of ^{14}C into thymine of tissue deoxynucleic acids (DNA)[1-3], suggesting the synthesis of thymine from a uracil precursor. Of various (2-^{14}C)uracil derivatives administered to rats the best precursor of DNA thymidine was found to be uracil deoxyriboside[4], suggesting that in the intact animal the latter nucleoside is methylated to give thymidine. In confirmation of this view PRUSOFF, LAJTHA AND WELCH[5] observed that deoxyuridine stimulated the incorporation of ^{14}C-formate into DNA thymine by Ehrlich ascites tumour cells, and FRIEDKIN AND ROBERTS[6] reported the conversion of (2-^{14}C)uracil deoxyriboside to DNA thymidine by bone marrow cell suspensions and minced chicken embryo. Both groups of workers reported an inhibition of the conversion of uracil deoxyriboside to DNA thymidine by aminopterin.

A synthesis of thymidine from uracil deoxyriboside and serine-3-^{14}C has now been achieved in the presence of the clear supernatant from rabbit thymus homogenate. The reaction has been followed by isolating the synthesised thymidine with the aid of unlabelled carrier thymidine. After chromatography on paper using butanol-ammonia as solvent, the eluted thymidine was further purified by chromatography on Dowex-1-chloride. Untreated thymus supernatant catalysed the incorporation of ^{14}C from serine into thymidine in the presence of uracil deoxyriboside but, when boiled or treated with Dowex-1-chloride, incorporation did not occur. Thymus supernatant which had been treated with Dowex-1-chloride (100 mg/ml of supernatant) could be reactivated for optimum incorporation of ^{14}C from serine-3-^{14}C into thymidine by the addition to the solution of tetrahydropteroyl glutamate ($10^{-3}M$), adenosine triphosphate ($10^{-3}M$) and reduced diphosphopyridine nucleotide as well as uracil deoxyriboside. Omission of tetrahydropteroyl glutamate caused a 20 to 30-fold decrease in the reaction rate. Pyridoxal phosphate, triphosphopyridine nucleotide and cobalamine do not increase the rate of incorporation.

In a preliminary experiment, ^{14}C from formate or formaldehyde was incorporated into thymidine at a much lower rate than from serine-3-^{14}C. When uracil deoxyriboside was replaced by uridine, dihydrouridine or dihydrouracil deoxyriboside, the rate of incorporation of serine-3-^{14}C into thymidine was lowered considerably, so that uridine, dihydrouridine and dihydrouracil deoxyriboside are not intermediates in the conversion of uracil deoxyriboside to thymidine.

The incorporation of serine-3-^{14}C into thymidine with uracil deoxyriboside as precursor was much greater than incorporation into thymine using uracil as precursor, or into thymidine-5'-phosphate using uracil deoxyriboside-5'-phosphate as precursor. In a preliminary experiment, ^{14}C-thymidine formed by thymus extract was degraded to yield iodoform from the thymine methyl carbon[1]. The radioactivity of the isolated iodoform accounted for all the radioactivity of the thymine.

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Received January 21st, 1957