Adaptation of *E. coli* to 2-Deoxy-D-ribose

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The degradation of the sugar component of deoxyribonucleosides has been demonstrated with whole cells and extracts of *E. coli* [1,2] but little is known concerning the bacterial utilization of the free pentose.

The present paper describes preliminary studies on the adaptation of *E. coli* to utilize 2-deoxy-D-ribose.

Standard techniques, *i. e.* incubation of the microorganisms on solid media in the presence of 0.25 % of specific substrate, was not practical because of a limited supply of 2-deoxy-D-ribose. A technique was therefore worked out in which a fairly large yield of adapted cells (1 g dry weight) was obtained in a fluid medium containing 20 mg of 2-deoxy-D-ribose. To obtain a similar yield of adapted cells on solid medium, 2.5 g of 2-deoxy-D-ribose was needed. The adaptation was made as follows: A loop inoculum from a slope was seeded into 5 ml of broth containing 1 % glucose. After 4 h growth at 37 °C, 0.2 ml of the culture was evenly distributed on each of 50 agar plates containing 1 % glucose. After 12 h at 37 °C the bacterial growth was scraped off the agar. The bacteria were suspended in ice-cold 0.05 M tris-HCl buffer (pH 7.4), filtered through gauze, centrifuged down at 0° and washed five times with cold buffer. The washed sediment was then suspended in 200 ml of fluid medium containing 2-deoxy-D-ribose (0.01 %), tryptic digested peptone (1 %), and sodium chloride (0.5 %). The mixture was stirred slowly in a 500 ml beaker at 37 °C. The degradation of 2-deoxy-D-ribose was followed by the disappearance of the Dische diphenylamine colour on aliquots withdrawn after different times of incubation. When about 90 % of the sugar was degraded, the bacteria were rapidly centrifuged down at 0° and washed repeatedly with cold 0.9 % saline. The degradation of 2-deoxy-D-ribose by cells adapted in this manner is shown in Table 1. A comparison is made with cells adapted on solid medium and non adapted cells.

Adapted cells could be lyophilized or stored at 4 °C without appreciable loss of activity. In the presence of glucose no adaptation occurred. By subcultivation of adapted cells in media containing no 2-deoxy-D-ribose, the adaptive ability was lost in one generation.

With cell free extracts, no significant degradation has been obtained in the presence of ATP and Mg++, neither could the formation of deoxyribose-5-phosphate be demonstrated. Under similar conditions the same strain of *E. coli* readily gave cell free extracts which metabolized D-ribose when adapted to this pentose. Under certain conditions [3] 44 % of the available D-ribose was isolated as D-ribose-5-phosphate. The formation of ribose-5-phosphate is the first step in the degradation of this sugar [3] by *E. coli*, and a similar pathway was anticipated for 2-deoxy-D-ribose. The

Table 1. Degradation of 2-deoxyribose by *E. coli.*

Incubation period (37°) min	2-Deoxy-D-ribose (DR) in mixture with					
	cells adapted on solid medium		cells adapted in fluid medium		not adapted cells	
	DR µg/ml	DR consumed µg/ml	DR µg/ml	DR consumed µg/ml	DR µg/ml	DR consumed µg/ml
0	370	0	370	0	330	0
10	345	25	355	15	325	5
35	270	100	265	105		
70	145	225	150	220	320	10
110	0	370	10	360	315	15
450					60	270

Experiments were made in tris-HCl-buffer (0.05 M pH 7.4). Each mixture contained the same quantity of cells.

failure to prepare a deoxyribokinase extract, compared to the ease with which a ribokinase could be prepared from *E. coli* make us believe that the degradation of 2-deoxy-D-ribose does not start with the formation of 2-deoxy-D-ribose-5-phosphate. It is also possible that the difference is due to extreme lability of the enzymes attaching 2-deoxy-D-ribose.

1. Manson, L. A. and Lampen, J. O. *J. Biol. Chem.* **193** (1951) 539.
2. Hoffmann, C. E. and Lampen, J. O. *J. Biol. Chem.* **198** (1952) 885.
3. Long, C. *Biochem. J.* **59** (1955) 322.

The Effect of Estradiol Pretreatment on Hexokinase Activity of Rat Uterine Muscle *

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Previous investigations in this laboratory have shown that administration of estradiol monobenzoate to rats increases glycogen content of uterine muscle and stimulates glucose uptake of the surviving uterine muscle. These findings indicated a study of whether hexokinase activity is increased after pretreatment with estradiol.

Hexokinase activity of extracts from uterine muscle of rats has been determined 48 h after subcutaneous injection of 50 μg estradiol monobenzoate in previously ovariectomized animals. 10 % homogenate of uterine muscle was prepared in 0.03 M tris(hydroxymethyl)-aminomethane buffer, pH 8.2, which contained 0.0012 M EDTA (versene). After centrifugation at 4 000 × *g* for 20 min the supernatant was used for determination of hexokinase activity. Aliquots of the extract (usually 20 mg tissue equivalent) were incubated with 1.5 μM glucose, 4 μM ATP, 5 μM $MgCl_2$, 20 μM KF at 37 °C for 30 min. Incubation was made in 0.033 M tris buffer in a total volume of 1.0 ml. Hexokinase activity was determined by the glucose disappearence method.

Hexokinase activity increased in linear correlation with time during incubation up to 30 min and was proportional with the amounts of extract added. After pretreatment with estradiol monobenzoate hexokinase activity was increased by 100 % above the control values in ovariectomized rats. This was a relatively specific effect as the "specific activity" (μmole glucose phosphorylated per mg protein in 30 min) was increased by 75 %.

Experiments were performed to decide if the increased hexokinase activity was due to activation of pre-existing enzyme or to stimulation of enzyme synthesis by pretreatment with estradiol. Therefore several properties of hexokinase activity of uterine muscle in controls and after administration of estradiol were studied. These experiments included the effect of pH, the affinity to ATP, protection by EDTA, inhibition by glucose-6-phosphate, inhibition by *p*-chloromercuribenzoate. No indication was obtained that these properties were changed after pretreatment with estradiol. The results indicate that administration of estradiol monobenzoate increases hexokinase activity of uterine muscle by stimulation of enzyme synthesis.

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Studies in the Mechanism of Bile Acid Formation

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In order to study the mechanism of the reactions involved in the transformation of cholesterol into bile acids we have prepared cholesterol stereospecifically labelled with tritium in the 7α- or 7β-position.

Some data on the metabolism of these compounds will be presented and discussed.