FAILURE OF ESCHERICHIA COLI TO FORM THYMINE FROM 3-METHYLASPARTIC ACID

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Recently we have stated that Escherichia coli strain B grown in the presence of tritiated 3-methylaspartic acid (3-MAA, 40 mg. per liter) formed tritiated thymine (Woolley and Koehelik, 1961). The cells were harvested, the desoxynucleic acids (DNA) were separated from them, hydrolyzed, and the bases separated by chromatography. The radioactive label was found only in the thymine. The identification of the thymine was made by comparison of it with an authentic sample by chromatography in 9 different solvent systems.

Further exploration has now shown that thymine is not formed from 3-MAA under these conditions. In the earlier experiments the amino acid (the DL-threo-form) was exposed to tritium gas, and the product was recrystallized from water. Careful examination has now revealed the presence of 2 radioactive impurities in this material which could be separated from 3-MAA by paper chromatography in isopropanol-HCl. One of these impurities was close to 3-MAA, but could be separated from it. In subsequent experiments the tritiated 3-MAA was recrystallized 4 times from water, at which time the radioactive impurities had been removed. When E. coli was grown in the presence of this highly purified material, the thymine

additions as indicated below in a total volume of 0.9 ml. At 0-time 0.1 ml of a 1 M sodium chloride solution containing ^{22}NaCl was added. After a suitable length of incubation at 30°C, usually 12 seconds, 10 ml of a chilled non-radioactive 1 M NaCl solution buffered with 20 mM tris-chloride, pH 7.5, was added rapidly. In the 0-time experiments the ^{22}NaCl was added together with this solution. The microsomes were centrifuged in the experimental tubes in the Spinco No. 40 rotor at 100000 g for 10 minutes, suspended in 11 ml of the washing solution and centrifuged again. The pellet was suspended in 2 ml of distilled water and the radioactivity was measured directly in the experimental tubes in a well type Scintillation counter.

Results. Experiments on the time course of sodium incorporation into the microsomes showed that in the absence of ATP the rate was slow and linear over the tested interval of one minute. In the presence of ATP, on the other hand, the incorporation rate was very rapid during the first few seconds after which time a saturation level was reached. It was therefore decided to study the reaction during a 12-second incubation, which permitted work in the rapid initial phase as well as reasonably good timing. Some of the more interesting results have been compiled in table 1. ATP produced a four to fivefold increase

Table 1. Incorporation of ^{22}Na into rat brain microsomes. The additions to and omissions from the standard incubation mixture given in the text are as indicated. Incubation time 12 seconds. The incorporation shown is that occurring in excess of the 0-time-value (1150 counts/10 min).

Additions (+) or omissions (-)	Counts/10 min
None	2863
- ATP	634
+ K^+, 5mM	2744
+ Ca^{++}, 1mM	2375
+ Ca^{++}, 1mM + K^+, 5mM	1725
+ Ca^{++}, 5mM	683
+ PAD, 10^{-4}M	3346
+ PAD, $2x10^{-4}$M	3787