NUCLEOTIDE INTERCONVERSIONS V. INCORPORATION OF DEOXYADENYLATE INTO THE DEOXYGUANYLATE OF DEOXYRIBONUCLEIC ACID*

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In previous experiments (Maley and Maley, 1960), we have described the inhibition of DNA synthesis in chick embryo mince by deoxyadenosine. Similar findings have also been reported by Klenow (1959) and Prusoff (1959) with Ehrlich ascites cells.

Because of the important implications of the above results, a study of the mechanism by which deoxyadenosine effects the inhibition was undertaken. During the course of these investigations, it was found that dAMP-8-C^{14} was effectively incorporated into the deoxyguanosine of DNA (Table 1A). Since only the purine ring was labeled, it might be argued that dAMP or deoxyadenosine (the initial product entering the cell) is converted to adenine by a nucleoside phosphorylase and reutilized by the following pathway: Adenine $\longrightarrow$ AMP $\longrightarrow$ GMP $\longrightarrow$ dGMP. That this sequence of reactions is unlikely is seen in Table 1B, where the conversion of adenine to DNA guanine is demonstrated to be less effective than that of dAMP-8-C^{14} (1A). These data suggested a direct conversion of dAMP to dGMP (or derivatives of these compounds), similar to the pathway described by Lagerkvist (1958) for the conversion of AMP to GMP.

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Table I

Incorporation of Adenine-8-C^{14} and dAMP-8-C^{14} into DNA, Adenine, and Guanine

Substrate	DNA	Adenine	Guanine
	c.p.m./mg	c.p.m./μmole	
A. dAMP-8-C^{14}	2480	2700	1210
	2810	2680	820
	1820	2170	595
	1950	2740	610
B. Adenine-8-C^{14}	3340	5000	650
	2520	3400	385

The reaction mixture contained Krebs Ringer phosphate, radioactive substrate, and 1 gm of 4-day chick embryo mince in a final volume of 2 ml. The concentrations of adenine-8-C^{14} and dAMP-8-C^{14} were 1.16 μmoles, specific activity 1.55 x 10^6 c.p.m. per μmole, and 0.4 μmoles, specific activity 1.82 x 10^5 c.p.m. per μmole, respectively. Incubation with rapid shaking at 37° for 2 hours.

In order to test this hypothesis, uniformly labeled C^{14}-dAMP was prepared by incubating Escherichia coli Wc⁻ with uniformly labeled C^{14}-sucrose and isolating the nucleotides by described procedures (Maley and Maley, 1960). The isolated purine deoxynucleotides were labeled equally in the deoxyribose and purine portions of the molecule, whereas only the deoxyribose of the dTMP and dCMP was labeled.

The uniformly labeled C^{14}-dAMP was incubated with chick embryo mince and after degradation of the DNA with deoxyribonuclease and phosphodiesterase the 5'-deoxyribonucleotides were isolated by gradient elution chromatography (Hurlbert et al., 1954). The specific activities of dAMP and dGMP were determined, and the nucleotides were hydrolyzed at 100° in 0.01 N HCl for 15 minutes to deoxyribose 5-phosphate and the respective purine bases. The latter compounds were separated by passage of the hydrolysis mixtures through columns of Dowex 50-H+. The deoxyribose 5-phosphate in the water eluate

(30 ml) was concentrated *in vacuo* and analyzed by the method of Dische (1955). Aliquots were dried *in vacuo* over P_2O_5 for specific activity determinations. The purine bases were eluted from the columns with 20 ml of 6 N HCl subsequent to washing the columns with 20 ml of 2 N HCl in order to remove undesired absorbing materials. The accumulated data on the specific activities of the isolated components are presented in Table II, where it can be seen that dAMP is converted *in vivo* to dGMP without cleavage of the purine deoxyribose bond. To our knowledge, this is the first time such a conversion has been demonstrated. Whether the conversion occurs in a manner similar to that described for the AMP to GMP conversion (Lagerkvist, 1958), is still to be determined.

Table II

Incorporation of U.L. C^{14}-dAMP into DNA, Deoxyadenosine, and Deoxyguanosine

	Experiment I	Experiment II
	c.p.m./mg	c.p.m./mg
DNA	2560	4060
	c.p.m./μmole	c.p.m./μmole
dAMP	2140	4420
Deoxyribose	1110	2170
Adenine	1470	2070
dGMP	400	1160
Deoxyribose	175	658
Guanine	220	655

Reaction conditions were the same as those in Table I except that 3-day chick embryos were used in Experiment II and incubation was for three hours at 37° in both experiments. The concentration of U.L. dAMP-C^{14} was 0.5 μmoles, specific activity 1.16 x 10^5 c.p.m. per μmole (adenine/deoxyribose = 1.03).

Because deoxyadenosine is a precursor of deoxyguanosine in the chick embryo, it is doubtful that the inhibition of DNA synthesis by deoxyadenosine in this organism occurs solely by the GMP ⟶ dGMP[1] block proposed by Munch-Petersen (1960) from studies with Ehrlich ascites cells. Recent experiments in this laboratory have shown that pyrimidine nucleotide interconversions are also affected by deoxyadenosine and might provide an explanation for the almost complete inhibition of DNA synthesis that has been observed (Maley and Maley, 1960). If only the GMP ⟶ dGMP conversion is influenced by deoxyadenosine, complete inhibition would not be expected, for, as demonstrated above, deoxyadenosine can serve as a precursor of deoxyguanosine.

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[1]Reichard (1960) has presented tentative evidence indicating the reductive process to occur at the nucleoside diphosphate stage.