Incorporation *in vitro* of Purine Ribosides into Deoxyribonucleic Acids of Ehrlich's Ascites Tumor Cells

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In *in vivo* experiments with rats Roll *et al.*[1] have found that the adenosine part of adenylic acid b may serve as a precursor of the deoxyribose of all four deoxyribonucleosides of deoxyribonucleic acid (DNA), and that some of the adenosine may have been converted to an adenine deoxyriboside derivative with the glycosidic linkage intact. There was no suggestion that the guanosine part of guanylic acid b was directly converted to a guanosine deoxyriboside derivative.

We have studied the incorporation *in vitro* of purine ribosides into deoxyribonucleic acids of ascites tumor cells. Suspensions of Ehrlich's ascites tumor cells in Tyrode's solution were incubated with glucose (10 mg/ml cell suspension), sodium succinate (10 mg/ml cell suspension), deoxycytidine (3 μmole/ml cell suspension), and uniformly ^{14}C-labelled adenosine or uniformly ^{14}C-labelled guanosine. After incubation at 37 °C with shaking for 3—4 h the suspensions were treated with ice-cold perchloric acid. The precipitate was washed with alcohol and with an alcohol-ether mixture (3:1), and digested with KOH overnight. After neutralization and dialysis of the digest the DNA present was degraded by incubation with DNA'ase and snake venom phosphodiesterase. The individual deoxyribonucleotides formed were separated and isolated by paper chromatography, and their specific radioactivity was determined.

It was found that when the cells had been incubated with uniformly ^{14}C-labelled adenosine most of the radioactivity (70—90 %) of the DNA was present in the deoxyadenylic acid, whereas the pyrimidine deoxyribotides contained insignificant amounts of radioactivity. The deoxyribose-5-phosphate of DNA-deoxyadenylic acid from such experiments was obtained by paper chromatography after acid hydrolysis. It was found that the average radioactivity per μmole carbon of this compound ranged from 70—100 % of to the average radioactivity per μmole carbon of the deoxyadenylic acid from which it had been derived. The experiments suggest, therefore, that under the conditions used adenosine serves as a precursor of DNA-deoxyadenylic acid, and that 50 % or more of labelled DNA-deoxyadenylic acid could have been formed from adenosine without cleavage of the glycosidic linkage.

In a similar way it was found that guanosine could serve as a precursor of DNA-deoxyguanylic acid. Furthermore, the average specific activity of the carbon atoms in DNA-deoxyguanylic acid obtained from such experiments ranged from 70—100 % of the average specific activity of the carbon atoms of its deoxyribose-5-phosphate part. This suggests that 50 % or more of the labelled DNA-deoxyguanylic acid could have been formed from guanosine without cleavage of the glycosidic linkage.

These results are only in partial agreement with the *in vivo* experiments of Roll *et al.* carried out with uniformly labelled purine ribotides.

1. Roll, P. M., Weinfeld, H., Carroll, E. and Brown, G. B. *J. Biol. Chem.* **220** (1956) 439.

Reversible Inactivation of Ribonuclease

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It has previously been shown that anhydrous formic acid induces an N,O-acyl migration in lysozyme followed with a loss of the enzymatic activity. However, titration of the inactivated enzyme with alkali at pH 7.5 reversed the peptidyl shift and restored the activity completely [1-3].

In the same way ribonuclease now has been studied to elucidate the mechanism of the enzymatic activity. In contrast to lysozyme the inactivation and reactivation of ribonuclease proceed slower. Furthermore the activity of ribonuclease is not completely restored at pH 7.5 but requires a further reaction at pH 8.5. However, the reactivation and alkali consumption strictly follow each other.

1. Josefsson, L. and Edman, P. *Acta Chem. Scand.* **10** (1956) 148.
2. Edman, P. and Josefsson, L. *Nature. In press.*
3. Josefsson, L. and Edman, P. *Biochim. et Biophys. Acta. In press.*