EFFECT OF CORDYCEPIN ON THE INCORPORATION OF P^{32}-ORTHOPHOSPHATE INTO THE NUCLEIC ACIDS OF ASCITES TUMOR CELLS *IN VITRO*

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Cordycepin has been isolated from the liquid culture of the mold *Cordyceps militaris* (Linn.) (Cunningham *et al.* 1951) and has been identified as an adenine nucleoside containing a 3-deoxy-pentose with a branched chain (Bentley *et al.* 1951). The compound inhibits the growth of several bacteria (Cunningham *et al.* 1951) and has recently been shown to prolong the survival time of mice bearing the Ehrlich ascites tumor cells (Jagger *et al.* 1961).

In the present communication the effect of cordycepin on incorporation of P^{32}-orthophosphate into the nucleic acids of Ehrlich ascites tumor cells *in vitro* is reported. Since the compound can be regarded as a structural analog of deoxyadenosine the name iso-deoxyadenosine seems to be both more descriptive and more convenient and will here be preferred to cordycepin.

The effect of iso-deoxyadenosine on the incorporation of P^{32} into DNA and RNA of Ehrlich cells has been studied under different experimental conditions with the methods previously used (Klenow 1959). The compound was found to inhibit P^{32} incorporation into DNA almost completely at 2 µmoles per ml cell suspension, and about 60 % inhibition was obtained with 0.5 µmoles per ml. For RNA the

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inhibition was less pronounced and appeared to have a lag period of about 1 hour before maximal inhibition was obtained. The effect of iso-deoxyadenosine on P^{32} incorporation into the two nucleic acids could not be abolished by the simultaneous addition of deoxyadenosine or deoxyguanosine, whether added separately or together (see Figure 1). Deoxycytidine, whether added alone or together with deoxyguanosine, was also without effect. On the other hand, adenosine (2 μmoles per ml) was found to prevent the inhibiting effect of both iso-deoxyadenosine and deoxyadenosine. Preincubation of iso-deoxyadenosine with highly purified adenosine deaminase did also abolish the inhibiting effect of this compound for both DNA and RNA. Since it has been shown (Coddington 1961) that adenosine deaminase converts iso-deoxyadenosine to iso-deoxyinosine it is concluded that the latter compound has no effect on P^{32} incorporation into the nucleic acids.

The ascites tumor cells appeared to accumulate three hitherto unknown nucleotides on incubation with iso-deoxyadenosine. After incubation for 3 hours with iso-deoxyadenosine (4 μmoles per ml) the cells were treated with 2.5 % $HClO_4$ and the extract was neutralized with KOH. Ribonucleoside 5-phosphates were degraded by treatment with $NaIO_4$ and alkali (Lehman et al. 1958), and the solution was then passed through a column of Dowex-1 formate. After elution of free bases and nucleosides three different uv-absorbing peaks were obtained. They all had absorption spectra characteristic for adenine compounds and were present in almost equimolar amounts, i.e. about 2 μmoles per g cells (wet weight). On paper chromatograms developed in ethanol - ammonium acetate - borate solvent (Plesner 1955) the three compounds had Rf-values almost identical with those of deoxyAMP, deoxyADP, and deoxyATP. On analyses the compounds were found to contain, respectively, 1, 2, and 3 molecules of phosphate per molecule of adenine. They did, however, not

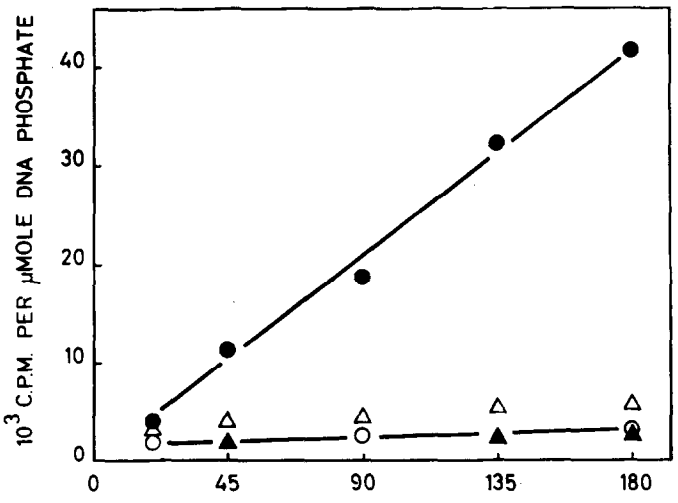

Fig. 1. Effect of iso-deoxyadenosine on incorporation of P^{32}-orthophosphate into DNA of Ehrlich ascites tumor cells.
The reaction mixtures contained: Tumor cells (200 mg, wet weight), glucose (50 μmoles), sodium succinate (33 μmoles), P^{32}-orthophosphate (15 μC) and Tyrode's solution (1.2 ml). The total volume of each flask was 1.5 ml.
Additions: ● none; ○ iso-deoxyadenosine (3.3 μmoles); ▲ iso-deoxyadenosine (3.3 μmoles) plus deoxyguanosine (4.5 μmoles); △ iso-deoxyadenosine (3.3 μmoles) plus deoxyguanosine (4.5 μmoles) plus deoxyadenosine (3.0 μmoles).
Abscissa: Incubation time at 37° C in minutes.

give any positive reaction either with the diphenylamine reagent for deoxyribose (Burton 1956) or with the anthrone reagent (Kredich and Guarino 1960). The monophosphate was found to be dephosphorylated by snake venom phosphomonoesterase at the same rate as deoxyadenosine monophosphate. The product reacted with the anthrone reagent and gave an absorption spectrum identical with that obtained with iso-deoxyadenosine. On the basis of these findings it is assumed that the isolated monophosphate is iso-deoxyadenosine monophosphate and that, by analogy, the two other compounds are the corresponding di- and triphosphates.

Recent studies have indicated that deoxyadenosine is partial-

ly converted to deoxyATP in Ehrlich ascites tumor cells in vitro (Munch-Petersen 1960) and that this latter compound or one closely related to it inhibits DNA synthesis when present in a certain concentration (Coddington and Bagger Sørensen 1961). Adenosine appears to prevent not only the inhibiting effect of deoxyadenosine and iso-deoxyadenosine, but also prevents the accumulation of deoxyATP and iso-deoxyATP, respectively. Also for iso-deoxyadenosine the inhibiting effect on nucleic acid synthesis may, therefore, be due to the formation of the corresponding triphosphate or a closely related compound.

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