DEOXYADENOSINE 1-N-OXIDE AND ITS EFFECT ON THE INCORPORATION IN VITRO OF P^{32}-ORTHOPHOSPHATE INTO DNA OF EHRLICH ASCITES CELLS

Sune Frederiksen and Hans Klenow

The Fibiger-Laboratory
Biochemical Section
University of Copenhagen
Denmark

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It has previously been shown both in this laboratory (Klenow 1959) and independently by Prusoff (1959) that deoxyadenosine inhibits the synthesis in vitro of DNA in Ehrlich ascites tumor cells. It has, furthermore, been found that the compound has an antimitotic effect on cultures of embryonic rat tissues (Kieler 1959). Stevens et al. (1958 and 1959) have synthesized the 1-N-oxides of adenine, adenosine and of adenosine-5'-monophosphate. Brown et al. (1958a) have found that the 1-N-oxides of adenosine and of adenosine-5'-phosphate inhibit the growth of tissue cultures.

In this communication the synthesis of deoxyadenosine 1-N-oxide and its effect on ascites tumor cells in vitro is reported. Deoxyadenosine 1-N-oxide was prepared by treating deoxyadenosine with a molar excess of monoperphthalic acid in aqueous solution at pH 5. Monoperphthalic acid was prepared by the procedure of Linholter and Sørensen (1958). After incubation for two hours at room temperature almost all of the deoxyadenosine had reacted and the reaction mixture was passed through a column of Dowex-1 formate. The column was washed with water until the effluent showed almost

no absorbency at 233 mμ. The pooled effluents were taken to dryness *in vacuo* and the residue was recrystallized twice from water.

The isolated compound had the following properties: The absorption spectrum in ultraviolet light had the same characteristics as that given by Stevens and Brown (1958) for adenosine 1-N-oxide. It had absorption maxima at 233 and about 262 mμ, and a minimum at 250 mμ ; the ratio E_{233}/E_{260} was 4.4 . The spectrum did not change after xanthine oxidase, nucleoside phosphorylase and adenosine deaminase had been added either separately or together. After weak acid hydrolysis of the compound, however, addition of xanthine oxidase caused a change in the absorption spectrum characteristic for the conversion of adenine 1-N-oxide to 8-hydroxy-adenine 1-N-oxide (Brown *et al.* 1958b). It was concluded therefore, that the compound contained adenine 1-N-oxide. It also gave a positive reaction for deoxyribose. If it is assumed that the compound has the same molar extinction coefficient as adenosine 1-N-oxide, i.e. 40,800 at pH 5.3, then the ratio of adenine 1-N-oxide to deoxyribose was found to be 1:1. In paper chromatograms using two different solvent systems, the compound migrated as a single ultraviolet absorbing compound giving a positive reaction for deoxyribose. After weak acid hydrolysis of the compound the chromatogram showed an ultraviolet absorbing spot with the Rf value of adenine 1-N-oxide, and a deoxyribose positive spot with the Rf value of authentic deoxyribose. From these data it was concluded that the compound was deoxyadenosine 1-N-oxide. Assuming 2 molecules of water of crystallization the product was at least 96 per cent pure.

Effect on Ehrlich cells

The incorporation of P^{32}-labelled orthophosphate into DNA in Ehrlich ascites hypotetraploide tumor cells *in vitro* was measured

as described previously (Klenow 1959). It appears from Fig. 1

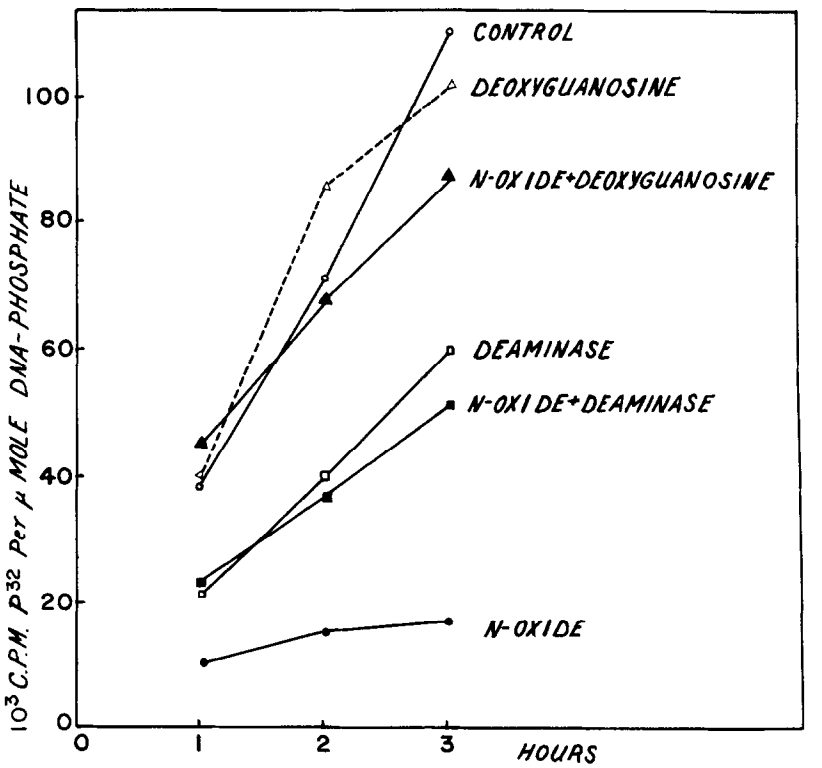

Fig. 1. Effect of deoxyadenosine 1-N-oxide (abbreviated, N-oxide) on incorporation of P^{32}-orthophosphate into DNA of Ehrlich ascites tumor cells in vitro. Concentration of N-oxide, 2.2 μmoles per ml. Concentration of deoxyguanosine, 3.0 μmoles per ml. The deaminase added in two of the vessels was purified adenosine deaminase from intestinal mucosa.

that in the presence of 2.2 μmoles of deoxyadenosine 1-N-oxide per ml. cell suspension the rate of incorporation of P^{32} is reduced to 10-15 per cent of that of the control. When deoxyguanosine (3.0 μmoles/ml.) is added together with the 1-N-oxide of deoxyadenosine the inhibiting effect is almost completely abolished. This finding is similar to that previously reported (Langer and Klenow 1960) for deoxyadenosine and may suggest, therefore, that the same mechanism is responsible for the inhibition in the two cases. It is further seen that addition of adenosine deaminase together with deoxyadenosine 1-N-oxide essentially reverses the inhibitory effect. In other experiments, not shown here, it was found that

preincubation of the deoxyadenosine 1-N-oxide with adenosine deaminase for 3 hours followed by heat inactivation of the enzyme, had no influence on its inhibitory effect.

These observations led to the assumption that deoxyadenosine 1-N-oxide is not deaminated by adenosine deaminase and that the compound in itself is not an inhibitor. In the presence of the cells it is, however, probably being reduced to deoxyadenosine, which has the observed inhibiting effect. In order to investigate this possibility, aliquots of an incubation mixture of tumor cells and deoxyadenosine 1-N-oxide were analyzed for purine compounds. It was found that the deoxyadenosine 1-N-oxide concentration decreased slowly and simultaneously corresponding amounts of hypoxanthine and deoxyinosine appeared. The amounts of deoxyadenosine present were extremely small.

The mechanism of inhibition of DNA synthesis by deoxyadenosine 1-N-oxide is subject to further investigation.

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