THE EFFECTS OF THE ALKYLATING CYTOSTATIC AGENT, 2,3,5-TRIS-ETHYLENEIMINO-BENZOQUINONE-1,4 (TRENIMON), ON THE PRIMING ABILITY OF DNA FROM MOUSE-ASCITES-TUMOR CELLS IN THE RNA-POLYMERASE-SYSTEM [+)]

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The inhibition of incorporation of radioactive labelled precursors into DNA and RNA by alkylating cytostatic agents has been reported by numerous investigators (for reference see Wheeler 1962). Only little is known, however, about the mechanism of this inhibition. The effect of the alkylating cytostatic agent Trenimon on the priming ability of DNA from mouse ascites tumor cells was investigated in order to gain information on this problem. Trams et al. (1961) and Rutman et al. (1961) showed that only very few alkylations of DNA take place when therapeutic doses of alkylating cytostatic agents are administered in vivo. The priming ability of DNA in the RNA polymerase system, however, was shown to provide a system which was extremely sensitive to very small changes in DNA structure (Zimmermann et al. 1964).

Materials and Methods: Trenimon (2,3,5-tris-ethylene-imino-benzoquinone-1,4) was provided by the Bayer AG, Leverkusen, Germany. All nucleoside-triphosphates, including ^{14}C-ATP, were purchased from Schwarz Bioresearch. Ehrlich ascites tumor cells were collected ten days after inoculation. The cells were separated from serum and contaminating erythrocytes by low speed centrifugation. DNA was extracted by the method of Colter et al. (1962) and

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determined by phosphorous analysis. RNA-polymerase was prepared by the method of Chamberlin and Berg (1962). The $(NH_4)_2SO_4$-fraction III was used in all experiments. Protein was determined according to Warburg and Christian (1942). The priming ability of DNA was assayed as described by Chamberlin and Berg (1962). RNA synthesis was measured by following the incorporation of 8-^{14}C-AMP into the fraction insoluble in cold 0.4 N $HClO_4$. Radioactivity could be rendered acid soluble by treating with RNase (Worthington) thus proving the incorporation of label into RNA. The perchloric acid precipitate was washed twice in 0.4 N $HClO_4$, dissolved in 5 N NH_4OH, transfered to aluminum planchets and the radioactivity measured in a windowless gas flow counter. For treatment with Trenimon 5.75 µmoles DNA, corresponding to 1.76 mg DNA in 2 ml 0.01 M NaCl, were mixed with 3 ml of Krebs-Ringer-phosphate buffer, pH 6.0, containing the desired concentration of Trenimon. Incubations were run at 37°C for 60 min. and thereafter dialysed twice for 12 h against 10 l of 0.01 M NaCl. After dialysis no Trenimon could be detected by UV-absorption measurements. Moreover, it could be demonstrated that Trenimon does not inhibit the RNA polymerase system at a concentration of 0.2 mM.

Results: Fig.1 shows the effect of different concentra-

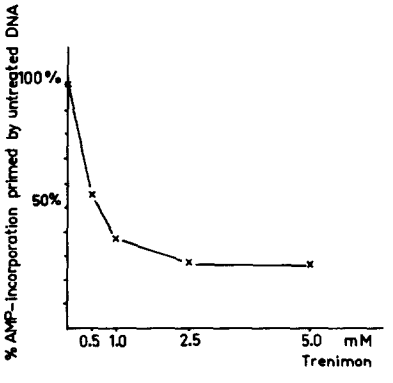

Fig. 1. Priming ability of DNA treated with different concentrations of Trenimon.
Samples of 0.25 ml contained in µmoles: Tris pH 7.9 10.0; $MgCl_2$ 1.0; $MnCl_2$ 0.25; ß-mercaptoethanol 2.0; GTP, CTP, UTP and ATP 0.1 each; specific activity of 8-^{14}C-ATP was 440 cpm/mµmole; 20 µg DNA; 90 µg enzyme protein.

tions of Trenimon on DNA as revealed by DNA-directed RNA-synthesis. 5 x 10^{-4} M Trenimon reduces the priming ability of DNA by 50%. After treatment with 2.5 x 10^{-3} M Trenimon the reduction is 75%. Further increase in concentration does not enhance this effect. Fig.2 shows the relationship of RNA-synthesis to the amount of DNA added. The yield of RNA synthesized increases with increasing concentrations of DNA in the range from 5 up to 30 µg DNA per incubation sample. When the DNA is first incubated with 10^{-3} M Trenimon, RNA-synthesis is greatly reduced, even with the lowest concentration of 5 µg DNA, when compared to RNA-synthesis with the same amount of untreated DNA. When the concentration of Trenimon-treated DNA is increased above 10 µg per sample,

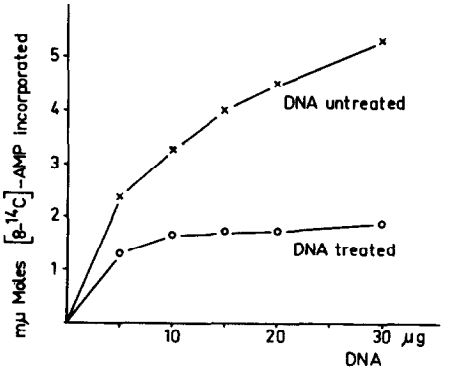

Fig. 2. Dependence of RNA-synthesis on the amount of DNA untreated (x-x), and treated with 1.0 mM Trenimon (o-o).
Incubation mixture as described in the legend of figure 1.

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there is no further increase in AMP-incorporation. Experiments with DNA which had been exposed to x-rays and UV-light (Zimmermann et al. 1964, Hagen et al. 1964) showed that the treated DNA not only displayed a reduced priming ability but also inhibited RNA-synthesizing activity of untreated DNA. As demonstrated in Table 1, treatment of DNA with 2.5 x 10^{-3} M Trenimon does not exert a considerable inhibitory effect.

Table 1

The effect of DNA treated with 2.5×10^{-3} M Trenimon on RNA-synthesis directed by untreated DNA.
Incubation mixture as described in the legend of figure 1.

µg DNA added		mµmoles AMP incorporated
untreated DNA	treated DNA	
-	-	0.17
10.0	-	4.76
-	10.0	1.34
10.0	5.0	5.25
10.0	10.0	4.95
10.0	15.0	4.66
10.0	20.0	4.66

In addition to studying the effects of Trenimon on the priming ability of DNA we tested whether treatment of DNA with Trenimon caused any extensive denaturation or degradation. We performed viscosity measurements and followed the change in extinction at 260 mµ during thermal denaturation. The viscosity of the DNA was assayed with a Couette-viscosimeter. The intrinsic viscosities were 104 for untreated DNA and 90 and 88 for DNA treated with 2.5×10^{-3} and 5.0×10^{-3} M Trenimon, respectively. These slight differences have not been checked for significance. It can be stated however, that at least considerable changes in viscosity cannot be detected under the conditions used. Fig.3 shows the increase in extinction at 260 mµ during thermal denaturation for untreated DNA and DNA treated with 5.0×10^{-3} M Trenimon. There is hardly any difference in the extinction curves of DNA although the priming ability of the treated sample shows only 27% of the activity of the untreated one.

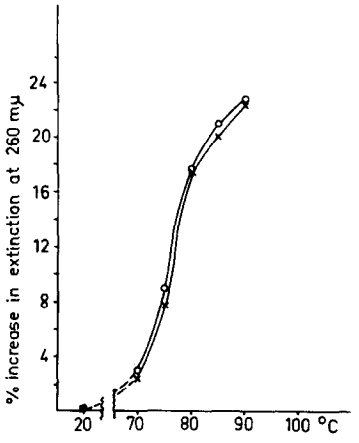

Fig. 3. Thermal denaturation curves of DNA untreated (x-x) and treated with 5.0 mM Trenimon (o-o). 0.025 μg/ml DNA in 0.01 M NaCl were heated at the desired temperature for 10 min, quenched in ice and the extinction at 260 mμ was read with a Zeiß-spectralphotometer.

Discussion: The experiments reported here demonstrate that an interaction of Trenimon with DNA causes an impairment of the priming ability of DNA for RNA-synthesis. A clearcut reduction of priming ability however, can only be achieved with very high concentrations of Trenimon. Thus in order to obtain a 50% reduction a ratio of about 1 mole Trenimon per 2 moles DNA-P is required. Therefor the inhibition of RNA-synthesis observed with the much lower therapeutic doses in vivo cannot be explained by a direct interaction of Trenimon with DNA. Preliminary experiments with DNA isolated from Ehrlich ascites cells which had been treated with therapeutic doses of Trenimon in vivo show that, under these conditions, a strong reduction of the priming ability of DNA can be obtained (Grunicke et al.). Presumably DNA-priming-ability is affected in vivo by mediation of some still unknown cellular mechanisms.

Summary: Concentrations in the range from 10^{-4} to 10^{-3}M of the alkylating, cytostatic agent Trenimon produce a strong reduction of the priming ability of DNA for RNA synthesis.

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