NNMG-Behandlung 15 – 20% der überlebenden Zellen TTC nicht zu reduzieren vermögen gegenüber 1 – 4% bei unbehandelten Zellen. Ein Anteil von 15 bis 20% TTC-negativer Zellen fand sich jedoch auch nach Behandlung mit salpetriger Säure und Methansulfonsäureäthylester, so daß man nicht von einer außergewöhnlich starken Induktion atmungsdefekter Mutanten durch NNMG sprechen kann. Es sei angemerkt, daß sich unter den auxotrophen Mutanten aller drei Agenzien TTC-negative Stämme befanden. Der Grund für das schlechte Wachstum vieler NNMG-Mutanten bleibt also unklar.

Insgesamt gesehen scheinen uns die erwähnten Nachteile den Vorteil der hohen Mutationsrate des NNMG z. T. wieder aufzuwiegen. Wir halten es daher für mindestens ebenso vorteilhaft, Methansulfonsäureäthylester anzuwenden, der bei etwa gleich hohen Mutationsraten [13] im Durchschnitt vitalere Mutanten liefert.

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Photodynamic Effects on the Template Activity of Nucleic Acids

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The photodynamic inactivation of nucleic acids with pyronin, methylene blue, thiopyronin and furocoumarines has been studied. The template efficiency of DNA in RNA-Polymerase reaction was found to be decreased after the treatment of DNA with these compounds. However, the magnitude of their inhibiting capacity varied from one compound to the other. Psoralen and thiopyronin were found to be the most active inhibitors followed by xanthotoxin and methylene blue respectively. At a lower temperature the inhibiting capacity of thiopyronin was considerably decreased but that of psoralen remained nearly unaffected. We have also tried to show evidence for a complimentary code in t-RNA through a specific destruction of guanine with thiopyronin.

According to present concepts genetic information in the cell is borne and transmitted by the nucleic acids. For the undistorted functioning of these macromolecules, there apparently exist mechanisms that within certain limits protect the cell from damaging influences or repair such damages upon their occurences. However, pysical and chemical influences on the cell may lead to alterations of the genetic material, which result in mutation or even cell death. An example is the action of radiation, for instance u.v.-light, which can lead to genetically manifested changes. The relationship between the template activity and the structural damage of nucleic acids through u.v.-light is now well established [1–5].

The present report provides in-vitro evidence that the photodynamic effect of certain compounds influences specifically the template activities of desoxyribonucleic acid (DNA) and ribonucleic acid (RNA). We report here on the photodynamic action on the DNA-template activity in RNA-polymerase reaction and m-RNA template activity on the cell-free protein synthesis. From a number of compounds, that we have investigated we will compare in the following the photodynamic activities of two classes of compounds; to one class belong methylene blue, pyronin and thiopyronin and to the other structurally related furocoumarines.

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Material and Methods

The cell-free extracts of *E. col* B were prepared according to the methods described [3]. Amino acid incorporation system was used as described by NIRENBERG and MATTHAEI [6], except that UTP and CTP were omitted. Incorporation of amino acids was measured by the method of MANS and NOVELLI [7]. Poly-AG and Poly-UC were polymerized from diphosphates, using polynucleotide phosphorylase from spray dried cells of *Micrococcus lysodeictikus*. „DNA-dependant" incorporation of triphosphates was studied by the method of CHAMBERLIN and BERG [8].

Nucleoside di- and tri-phosphates were obtained from Schwarz Biochemicals, Polyuridylic acid (Poly-U), Polyadenylic acid (Poly-A) and Polycytidylic acid (Poly-C) from Miles Chemical Co., radioactive amino acids from Amersham, England. Furocoumarines were kindly supplied by Dr. C. H. KRAUCH of the Max Planck Institut für Kohlenforschung, Abteilung Strahlen-Chemie, Mühlheim. All other chemicals were bought from C. F. Boehringer u. Soehne, Mannheim.

Results and Discussion

The photodynamic effect of methylene blue, pyronin and thiopyronin on the template efficiency of DNA in RNA-polymerase reaction ist shown in table 1.

DNA treated with	Visible light 6,6 $\cdot 10^6$ ergs/mm^2	^{3}H-AMP incorporation [Ipm]	[%] Inhibition
Control	—	1788	0
without DNA	—	249	86
Pyronin	—	1683	6
	+	1411	21
Methylene	—	1696	5
blue	+	1261	29
Thiopyronin	—	1552	13
	+	762	57

Table 1. Photodynamic action of pyronin, methylene blue and thiopyronin on the template activity of DNA in RNA-polymerase reaction.

The experiments were performed in such a way that for each dye a control was made where the treated samples were kept in dark. As follows from the results of table 1 the incorporation of ^{3}H-AMP into RNA under the influence of different photosensitizing agents is inhibited. The inhibition obtained by these dyes in the presence of light is considerably higher than the controls where no light was given. This shows that the effects observed in our experiments are specific depending on the type of dye used. Although the concentration of all these dyes used was the same the range of inhibition differs remarkably from one dye to the other. Thus for example, pyronin at a concentration of 10 μg/ml shows an inhibition of 21% but thiopyronin at the same concentration inhibits three times more the uptake of ^{3}H-AMP. The earlier experiments in this laboratory [9] and from others [10] have shown that on irradiation of a DNA solution containing methylene blue with a daylight source in the presence of oxygen, guanine is preferentially destroyed, whereas the other bases are nearly unaffected. Thiopyronin at the same concentration reacts similiarly, but with a considerably higher efficiency [9]. In contrast no destruction of any base has been detected after irradiation of DNA in the presence of pyronin. Our present results on the effects of methyleneblue and thiopyronin on the template activity of DNA stand in good agreement with the photodynamic destruction of guanine and the photodynamic inactivation of bacteria as observed by WACKER et al. [9]. The inhibition of DNA-template activity caused by pyronin does not favour the observation that no guanine is destructed by the photodynamic action of pyronin. However, one could consider that the amount of guanine destroyed was so low that it could not be detected. Then it is known that the priming ability of DNA in the RNA-polymerase reaction provides a system which is extremely sensitive to very small changes in DNA.

While the photodynamic action of dyes mentioned above is oxygen-dependent that of furocoumarins does not require oxygen. The experiments made in this laboratory did not show any furocoumarin-mediated destruction of purines or pyrimidines [11]. However, it has been observed that the radioactive furocoumarines under the influence of light get bound to nucleic acids, preferentially to thymine and uracil [12]. The photodynamic effects of psoralen, xanthotoxin and xanthotoxol on the DNA-template activity are shown in table 2.

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DNA treated with	visible light 6,6 $\cdot 10^6$ ergs/mm^2	^{3}H-AMP Incorporation [Ipm]	[%] Inhibition
Control	—	1788	0
Control	+	1711	4
without DNA	—	249	86
Xanthotoxol	—	1335	25
	+	1230	31
Xanthotoxin	—	1332	25
	+	860	48
Psoralen	—	1265	19
	+	605	66

Table 2. Photodynamic action of furocoumarines on the template activity of DNA in RNA-polymerase reaction.

These three furocoumarines are structurally very similar and differ only at C-8 substitution. If the molecular structure of psoralen is altered. e. g. by substitution of the hydrogen at C-8 by a methoxy group one gets xanthotoxin; a hydroxy group at the same carbon atom gives xanthotoxol. As follows from results of table 2 the incorporation of ^{3}H-AMP into RNA under the influence of all the furocoumarines is inhibited. The concentration of compounds used was same but the extent to which they inhibit differs. The maximum inhibition is shown by psoralen followed by xanthotoxin and xanthotoxol respectively. This difference in their magnitude of action is due to the difference in their binding capacity to DNA [12]. The photoinactivating effect of furocoumarines in bacteria also showed that the most pronounced effect was with psoralen followed by xanthotoxin and xanthotoxol respectively [9].

It has been reported in literature that the furocoumarin-mediated inactivation of bacteria is independant of temperature [13, 14]. Experiments in this laboratory have shown that the photoinactivation of bacteria with methylene blue, pyronin and thiopyronin is a temperature-dependant reaction and at low temperatures no inactivation was achieved [9].

We have tested this temperature effect on the photodynamic activity of thiopyronin and psoralen. The samples of DNA and dyes were irradiated in an ice bath (2 °C).

As can be seen from table 3 the inhibition of the uptake of ^{3}H-AMP caused by psoralen is twice as much as by thiopyronin. As follows from results of tables 1 and 2 one notices that the photoinactivating effects of thiopyronin and psoralen at room

DNA treated with	Visible light 6.6 $\cdot 10^6$ ergs/mm^2	^{3}H-AMP Incorporation [Ipm]	[%] Inhibition
Control	—	1647	0
	+	1566	6
Thyopyronin	—	1411	14
	+	1177	29
Psoralen	—	1374	16
	+	746	55

Table 3. Photodynamic action of thiopyronin and psoralen at 2 °C.

temperatures are not much different from one another. These results suggest that the photoinactivation of DNA with thiopyronin and psoralen is based on different mechanisms.

The photochemical alteration of guanine in the presence of thiopyronin has been shown to be very specific one. Thus WACKER et al. [15] found that after irradiation of radioactively labeled bacteria in the presence of thiopyronin, and perchloric acid hydrolysis, only guanine degradation can be detected by paper chromatography. At very high doses of visible light they found that 8% of thymine and 4% of cytosine were destroyed as well but no destruction of adenine or uracil was noticed. Making use of this reaction the coding of different polynucleotides and transfer RNA (t-RNA) were studied. The template activity of different polynucleotides and t-RNA was measured in the in-vitro protein- synthesizing system as developed by NIRENBERG and MATTHAEI [6].

As follows from results in table 4 the template activities of Poly-U, Poly-A and Poly-UC remain unchanged but the template activities of Poly-AG and Poly-C were decreased to 44% and 11% respectively in the presence of thiopyronin and visible light. The inhibition of leucine and proline incorporations caused by the photoinactivation of t-RNA with thiopyronin suggests the requirement of guanine as one of the bases in their complimentary codes in t-RNA. Since the m-RNA codes used for leucine and proline are Poly-UC and Poly-C, one would expect that the complimentary codes in t-RNA must contain guanine. If this is so, then one should expect such an inhibition as shown by our results. The photosensitizing action of thiopyronin on the biological activity of ribosomes is

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Amino acid	Phenyl-alanine	Lysine		Leucine	Proline
Polynucleotide	Poly-U	Poly-A	Poly-AG [5:1]	Poly-UC [4:1]	Poly-C
System Complete	100	100	100	100	100
minus m-RNA	4	15	15	3	3
Nucleic acids treated with thiopyronin and visible light $6{,}6 \times 10^6$ ergs/mm^2					
m-RNA	110	98	56	96	89
t-RNA	94	96	96	78	20
Ribosomes	34	65			

Table 4. Photodynamic effect of thiopyronin on different RNA fractions in cell-free protein synthesis. Numbers indicate the percent incorporation of amino acids.

very striking. To what extent thiopyronin is responsible for the alteration of nucleic acids or protein components of ribosomes is not yet clear, but we have found that histidine, tyrosine and methionine under the influence of light and thiopyronin are photo-oxidised [15].

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