which had previously been used in 0.5-percent concentration for isolation of nuclei (*19*), was, in concentrations of 0.5 to 2 percent, effective in removing the outer nuclear membrane without further disruption of the nuclei (Fig. 1B). There was only a small (4 to 7 percent) loss of nuclear RNA and virtually no loss of DNA (Table 2). It is not clear whether the loss of RNA after treatment with detergent can be attributed solely to removal of the outer nuclear membrane and its attached ribosomes. It is conceivable that some of the nuclear RNA is made soluble by the detergent and no longer sediments with the nuclei. Most of the nuclear RNA, however, is bound by a mechanism which is clearly resistant to the tested concentrations of Triton X-100. This is distinctly different from the situation in the cytoplasm, where 60 percent of the RNA (*5*) is bound to membranes as ribosomes and can be released by 2 percent Triton solutions.

Our method avoids use of citric acid completely and avoids use of detergent until the nuclei have been separated from the cytoplasm and thus permits further fractionation of the cytoplasm, as will be shown elsewhere (*5*). Removal of traces of the endoplasmic reticulum from nuclei by means of Triton X-100 can be carried out optionally, and further studies on the advantages or disadvantages in each specific application may be necessary.

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26 September 1966 ■

Interaction of the Water-Soluble Carcinogen 4-Nitroquinoline N-Oxide with DNA

Abstract. *Thin-layer chromatography revealed that 4-nitroquinoline* N-*oxide interacts with native DNA, but not with denatured DNA's or the following macromolecules and nucleic acid derivatives: soluble RNA from* Escherichia coli *and from yeast, polyguanylate-cytidylate, and precursors of DNA and RNA. Polydeoxyadenylate-thymidylate, polyadenylate, and histone interacted to a lesser degree than native DNA's did. That magnesium ion, sodium ion, and proflavine did not interfere with interaction of carcinogen and DNA suggests that this interaction is not an intercalation. Interaction of 4-nitroquinoline* N-*oxide with DNA may be related to its carcinogenicity.*

Proximate derivatives of such carcinogens as the amino-azo dyes, aminofluorenes (and perhaps of the naphthylamines and polycyclic benzenoid hydrocarbons) are usually more polar than the parent carcinogens (*1*). This increases interest in the polar, water-soluble carcinogen 4-nitroquinoline *N*-oxide (NQO) (Fig. 1a) as a model compound for investigating the carcinogenic process (*2*). It has recently been suggested that a reduced form of NQO, 4-hydroxyaminoquinoline *N*-oxide, may be the proximate form of this carcinogen (*3*).

The manifold carcinogenicity of NQO rivals that of the most potent polycyclic hydrocarbons (*4*). Its carcinogenic properties are expressed after its administration by skin painting, ingestion, or various routes of injection (*5*). It is carcinostatic and also mutagenic for bacteria and yeast (*6*). In Chang liver cells and in the protozoan *Tetrahymena pyriformis*, NQO elicits visible intranuclear inclusion bodies (*7*), sharing with mitomycin C an ability to induce nucleolar caps (*8*). It also bleaches *Euglena*, induces chromosomal aberrations in Yoshida sarcoma cells, and diminishes uptake of ^{32}P into nucleic acid fractions of the Ehrlich ascites carcinoma (*9*). We therefore studied the interaction of NQO with total extracted *Euglena* DNA as well as with DNA from other sources. The choice of *Euglena* DNA might, moreover, permit insight into the bleaching process because of its relation to the autonomy of organelles, such as the chloroplast, whose DNA's differ from nuclear DNA's (*10*). Our results show that NQO does interact with native DNA from *Euglena* and other sources.

The spectrum of NQO (Fig. 1b) has a sharp peak at 250 mμ and a broad peak at 370 mμ. The overlapping spectra of NQO and DNA at 250 mμ preclude accurate spectrophotometric analysis at this wavelength. Use of the peak at 370 mμ as an index of binding is not a conclusive means of demonstrating interaction between NQO and either DNA or RNA (*11*). To surmount the problem posed by overlapping spectra, we used thin-layer chromatography (TLC): Eastman Chromagrams with distilled water or a mix-

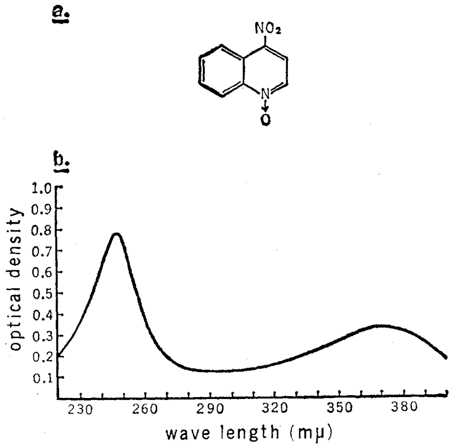

Fig. 1. (a) Structure of 4-nitroquinoline *N*-oxide, molecular weight 191.1. (b) Absorption spectrum of NQO in 0.1 times SSC buffer, *p*H 7.2, 8.1 μg/ml.

ture of 0.015*M* NaCl + 0.0015*M* sodium citrate (0.1 times SSC buffer) as solvent. In this system an interaction between NQO and DNA was inferred from the failure of the 4-nitroquinoline *N*-oxide to attain its normal R_F value. The bulkiness of the DNA molecule completely prevents its movement from the origin (*12*) (Fig. 2a). At a ratio of 10 μg of DNA to 1 μg of NQO, NQO interacted with DNA for it did not move from the origin; the carcinogen alone had an R_F of 0.33. No ultraviolet-absorbing material was seen in the band containing only buffer.

To ascertain the ratio for maximum interaction, the total DNA extracted from *Euglena* at various concentrations was incubated with a fixed concentration of NQO, then chromatographed. Maximum interaction occurred at ratios of DNA to NQO greater than 7 to 1 (Fig. 2b). As the ratio of DNA to NQO decreased, more NQO was released from the DNA. This was manifested by streaking of the NQO and by increases in its R_F (as measured at that part of the band farthest from the origin). At a ratio of 1 to 1, no NQO seemed bound. To verify this, the origins of a number of such bands were eluted and spectrophotometrically analyzed for NQO at 370 mμ. Control bands of DNA and the buffer were also eluted in equivalent amounts. Analysis of these bands demonstrated that NQO was present at the origin and therefore bound to the DNA. The amount was small, approximately 5 to 10 percent of the total NQO originally in the band. This small amount of tightly bound NQO may indicate the presence of a second, stronger binding site on the DNA molecule or it may simply indicate the tenacity of the NQO for DNA present in relatively small amounts. Because of streaking and contaminating NQO, it was not possible to elute the origins at the other ratios to see whether similarly bound NQO was present.

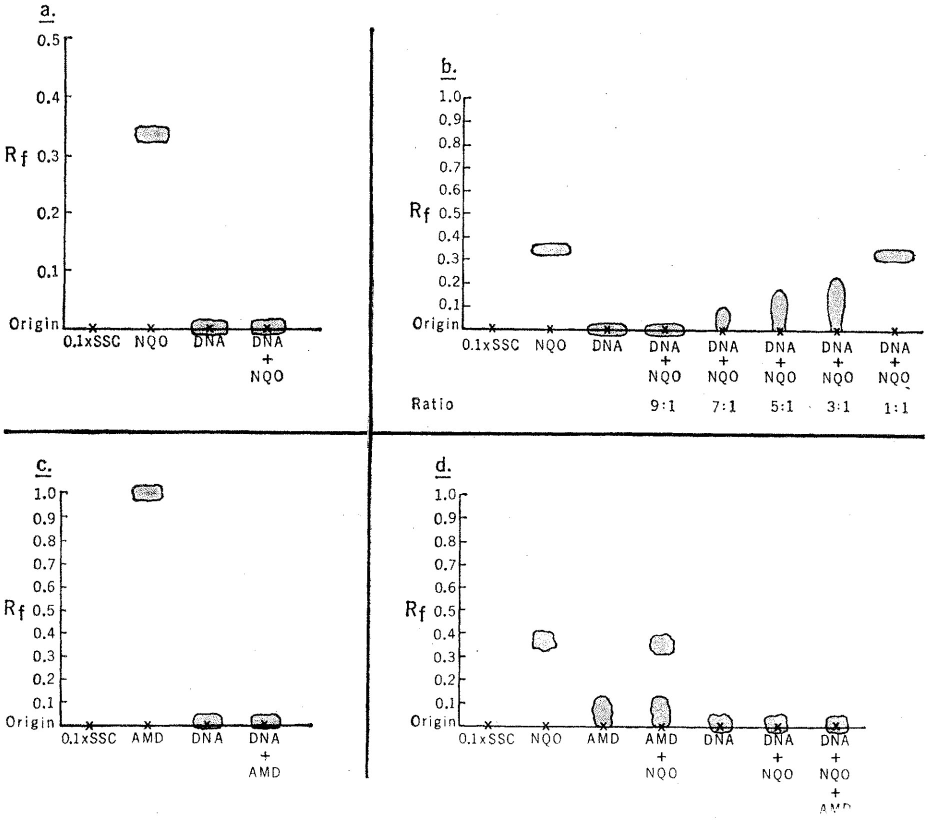

Fig. 2. Chromatograms of interaction of NQO and actinomycin D with DNA. (a) *Euglena* DNA (*13*) (500 μg/ml) and NQO (50 μg/ml), dissolved in 0.1 times SSC at *p*H 7.2, were incubated in the dark for 10 minutes and stirred; then 30 μl of the mixture was applied to a Chromagram (Type K301R) sheet; the chromatogram was developed in tanks with distilled water or 0.1 times SSC as solvent. Controls of NQO and DNA alone, at the same concentrations, were also prepared. After about 90 minutes, the chromatograms were dried in air, and the spots were examined under ultraviolet light (2537 Å) for calculation of R_F's. The method was sensitive to at least 1.0 μg of NQO. (b) Varying concentrations of *Euglena* DNA were incubated with NQO (50 μg/ml) under the same experimental conditions as in part (a) and then chromatographed. (c) Actinomycin D (100 μg/ml) was added to calf thymus DNA (1 mg/ml) and incubated with controls for 10 minutes in the dark. After incubation, 30 μl of each reaction mixture was applied to the chromatogram which was developed in methanol. After drying, the yellow actinomycin D could be seen directly, and its R_F was noted. (d) The *Euglena* DNA (500 μg/ml) and actinomycin D (50 μg/ml) were incubated together in the dark for 10 minutes and then NQO (50 μg/ml) was added. After another 10-minute incubation period in the dark, the mixtures of DNA and NQO and the controls were chromatographed and analyzed for interaction.

Similar results were obtained with highly polymerized DNA's from calf thymus and *Escherichia coli*. Heat-denatured, single-stranded DNA showed no interaction at a ratio of macromolecule to NQO of 4 to 1 by weight. Soluble RNA's from *Escherichia coli* and yeast also showed no binding at ratios of RNA to NQO of 10 to 1. Histone had a small effect on the R_F of NQO, decreasing it to 0.28; but this decrease was only at very high concentrations (500 μg/ml) of histone. As further evidence of the efficacy of TLC for demonstrating binding, actinomycin D bound to DNA (Fig. 2c).

To discern the site or sites of attachment of NQO to the DNA molecules, DNA and RNA precursors and synthetic polynucleotides were incubated with NQO, then chromatographed. None of the DNA and RNA precursors (dATP, dGTP, dCTP, TTP, UTP and the five common bases) reacted strongly with the NQO at ratios of precursor to NQO of 10 to 1. Of the synthetic polynucleotides tested, poly-dAT and polyA decreased the R_F of NQO by 20 percent; polyGC failed to do so. The polynucleotides were used at concentrations of polymer to NQO of 10 to 1. Incubation of DNA with actinomycin D, which binds to guanine (*14*), and subsequent addition of NQO to the reaction mixture, gave no evidence of interference of actinomycin D on the interaction of NQO with DNA, suggesting that the guanine residues do not act as attachment sites for NQO (Fig. 2d).

Magnesium ion (as 0.25*M* $MgCl_2$) had no effect on the interaction, nor was it inhibited by concentrations of sodium up to 1.1*M*. Presumably, then, the intercalation mechanism proposed by Lerman (*15*) for proflavine does not hold for NQO in that intercalated compounds such as chloroquine dissociate at high ionic strengths (*16*). This conclusion was supported by an experiment like the one conducted with actinomycin D; NQO interacted with DNA that had been incubated with proflavine.

Nagata *et al.* (*17*), using flow dichroism and equilibrium dialysis, demonstrated an interaction between NQO and DNA from calf thymus and showed that the spectrum of DNA-bound NQO was obscured by the absorption of free NQO. This would explain why interaction of NQO with DNA was not optically detected (370 mμ) earlier (*11*). By equilibrium dialysis, then by charting difference spectra of the inner and outer compartments, Nagata *et al.* noted binding in the following order: native DNA > polyA > apyrimidinic acid ≅ denatured DNA > apurinic acid. They suggested that NQO attaches at the adenine or guanine residues (or both), and that NQO is oriented parallel to the planes of the bases. Our results with actinomycin D suggest that guanine is not involved; our results obtained with poly-dAT and polyA neither prove nor disprove theirs with polyA. Such polymers as helical poly-dG:dC, polyC, and polyG should be tested before attachment sites can be specified. A discrepancy between our findings and those of the Japanese workers resides in their claim that 1*M* NaCl inhibited binding by NQO to DNA; in our system there was no inhibition.

A conspicuous property of NQO is that it induces chromosomal aberrations—for example, in Yoshida sarcoma cells (*9*), where NQO behaves like a radiomimetic agent. The interaction of NQO with DNA should be taken into account in defining its mechanism of carcinogenesis. Such binding might interfere with base pairing or DNA replication. NQO alters the chromosome morphology of *Euglena gracilis* (*18*)—which favors the idea that DNA is a target of NQO in *Euglena*.

Our results on binding may not be comparable to those for native benzenoid carcinogens inasmuch as NQO may be close to the primary carcinogenic agent. In contrast, binding of the hydrocarbons to nucleic acids may reflect only the initial attachment which permits later transformation to a proximate carcinogen. The low direct cellular toxicity (possibly due to poor solubility) of the benzenoid hydrocarbons and of the unaltered azo-amino dyes and aminofluorenes supports this idea of the necessity of metabolic alteration for these compounds to become carcinogens. On the other hand, cellular poisons that act immediately would be eligible to be proximate carcinogens. The 2-alkyl-4-hydroxyquinoline *N*-oxides come to mind; unfortunately information is lacking with regard to their carcinogenicity.

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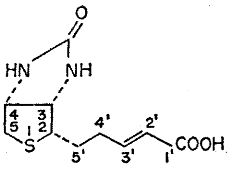
19 September 1966

Naturally Occurring Antimetabolite Antibiotic Related to Biotin

Abstract. *A crystalline antibiotic produced by* Streptomyces lydicus *has been isolated and shown to be the hitherto unknown* α*-dehydrobiotin (I). This is active against a variety of gram-positive and gram-negative bacteria and fungi. Its antimicrobial activity is reversed by the presence of biotin in growth media.*

During an investigation of antimicrobial activity produced by a strain of *Streptomyces lydicus*, we isolated a crystalline antibiotic which we have shown to be the hitherto unknown α-dehydrobiotin (I) which has a double bond conjugated with the carboxyl group. This antibiotic is active in vitro against a variety of gram-positive and gram-negative bacteria and fungi (*1*). Of special interest, however, is its antimetabolite relationship to biotin in that its antibacterial properties are reversed by the presence of biotin in synthetic media. (Table 1).



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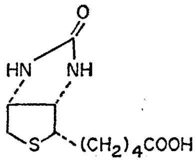

II
(Numbering as in I)

Fermentation was carried out at 32°C in a medium consisting of 1 percent each of glucose, Pabst yeast, cottonseed meal, and lard oil; and 2 percent of dextrin in water. Peak antimicrobial activities were attained in 5 to 6 days with the medium remaining es-

Table 1. Inhibition by α-dehydrobiotin of *E. coli* in synthetic medium supplemented with various concentrations of biotin. The data in the body of the table refer to diameters (millimeters) of inhibition zones around a paper disc diameter, 13 mm) treated with α-dehydrobiotin as indicated. The composition of the medium was as follows: $Na_2HPO_4 \cdot 7H_2O$, 2.2 g; KH_2PO_4, 1.0 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4$, 0.1 g; glucose, 2.0 g; distilled water, 1 liter.

Biotin in medium (μg/ml)	Amount of α-dehydrobiotin (μg) on disc				
	20.0	10.0	5.0	2.5	1.25
0.100	0	0	0	0	0
.050	tr	0	0	0	0
.025	18	tr	0	0	0
.012	26	20	tr	0	0
.006	28	25	18	0	0
(control)	39	36	32	29	25