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.

MARTIN F. MALKIN
Haskins Laboratories, New York
ARTHUR C. ZAHALSKY
Queens College, Flushing, New York

References and Notes

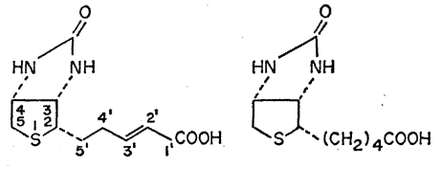
1. E. C. Miller and J. A. Miller, *Pharmacol. Rev.* **18**, 805 (1966); H. Uehleke, *Progr. Drug Res.* **8**, 195 (1965).
2. Abbreviations used in this work: sRNA, soluble RNA; poly-dAT, alternating deoxyadenylate-thymidylate copolymer; polyGC, copolymer of guanylatecytidylate in random sequence; polyA, homopolymer of adenylate; polyC, homopolymer of cytidylate; polyG, homopolymer of guanylate; poly-dG:dC, double chain copolymer of deoxyguanylate-deoxycytidylate; dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate; dCTP, deoxycytosine triphosphate; TTP, thymidine triphosphate; UTP, uridine triphosphate.
3. T. Okabyashi and A. Yoshimoto, *Chem. Pharmaceut. Bull.* **12**, 262 (1964).
4. W. Nakahara and F. Fukuoka, *Gann* **51**, 125 (1960).
5. ———, and T. Sugimura, *ibid.* **48**, 219 (1957); C. Huggins, in *Horizons in Biochemistry*, M. Kasha and B. Pullman, Eds. (Academic Press, New York, 1962), p. 497; A. Lacassagne, N. Buu-Hoï, F. Zajdeta, *Compt. Rend.* **155**, 44 (1961).
6. F. Fukuoka, T. Sugimura, S. Sakai, *Gann* **48**, 65 (1957); T. Okabayashi, M. Ide, A. Yoshimoto, M. Otsubo, *Chem. Pharmaceut. Bull.* **13**, 610 (1965); I. Mifuchi, M. Hosoi, Y. Yangihara, M. Otsubo, *Gann* **54**, 205 (1963).
7. H. Endo, M. Aoki, Y. Aoyama, *Gann* **50**, 209 (1959); T. Mita, R. Tokyzen, F. Fukuoka, W. Nakahara, *ibid.* **56**, 293 (1965).
8. L. Lapic and W. Bernhard, *Cancer Res.* **25**, 628 (1965).
9. A. C. Zahalsky, M. M. Keane, S. H. Hutner, K. J. Lubart, M. Kittrell, D. Amsterdam, *J. Protozool.* **10**, 421 (1963); T. Yosida, Y. Kurita, K. Moriwaki, *Gann* **56**, 523 (1965); F. Fukuoka and H. Naora, *ibid.* **48**, 271 (1957).
10. G. Brawerman and J. M. Eisenstadt, *Biochim. Biophys. Acta* **91**, 477 (1964); D. Humm and J. Humm, *Proc. Nat. Acad. Sci. U.S.* **55**, 114 (1966).
11. H. Endo, *Gann* **49**, 151 (1958).
12. This, incidentally, is a good test of purity; most commercial DNA preparations show streaking, presumably because of low-molecular-weight contaminants.
13. Total extracted DNA from *Euglena gracilis* was prepared from cultures in the log phase of growth according to the method of Marmur as revised by J. Leff, M. Mandel, H. T. Epstein, J. A. Schiff, *Biochem. Biophys. Res. Commun.* **13**, 126 (1963).
14. E. Reich, *Science* **143**, 684 (1964).
15. L. Lerman, *Proc. Nat. Acad. Sci. U.S.* **49**, 94 (1963).
16. S. Cohen and K. Yielding, *J. Biol. Chem.* **240**, 3123 (1965).
17. C. Nagata, M. Kodama, Y. Tagashira, A. Inamura, *Biopolymers* **4**, 409 (1966).
18. E. H. J. O'Donnell, personal communication.
19. Aided by Damon Runyon Memorial Fund grant 827A and in part from a grant from the Loomis Institute. Poly-dAT was generously provided by Drs. A. Kornberg and S. Pestka; polyGC, by Dr. M. Nirenberg. PolyA was purchased from Miles Chemical Co. Calf thymus DNA, histone, and DNA and RNA precursors were purchased from Sigma Chemical Co.; DNA and sRNA from *E. coli, from* GBI; and yeast sRNA, from Calbiochem. Actinomycin D was provided by Merck Sharp, and Dohme; NQO was purchased from Beacon Chemical Industries, Boston, Massachusetts.

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).



I 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

Table 2. Nuclear magnetic resonance spectra of biotin and α-dehydrobiotin. With structure I, only differences are shown; the remainder of the spectrum was essentially identical with that of biotin.

Chemical shift* (δ in ppm)	Protons (No.)	Type of signal	Assignment†
		Biotin (II)	
1.45	6	Broad methylene envelope	Methylenes at C-3′, C-4′ and C-5′
2.23	2	Asymmetrical triplet	Methylene at C-2′
~ 2.75	2	Apparent singlet with doublet	Methylene at C-5
3.10	1	Broad multiplet	Methine at C-2
4.25	2	Broad multiplet	Methines at C-3 and C-4
6.37	2	Broad singlet	Protons on nitrogens
		α-Dehydrobiotin (I)	
~ 1.70	2	Broad complex multiplet	Methylene at C-5′
~ 2.20	2	Broad multiplet	Methylene at C-4′
5.78	1	Doublet (J = 17 cy/sec)	Vinyl proton at C-2′
6.83	1	Sextet, doublet or triplets (J_{AB} = 17 cy/sec; J_{AX} = 7 cy/sec)	Vinyl proton at C-3′

* Determined at 60 mc/sec in dimethylsulfoxide-d_6 and relative to tetramethylsilane as internal standard. †See structures I and II.

sentially at *p*H 6.5. The antibiotic from the filtered broth (adjusted to *p*H 4.0) was absorbed onto carbon and eluted with a mixture of acetone and water. It was further purified by partition chromatography on a column of diatomaceous earth. The lower phase of the solvent system, composed of a mixture (16:4:1) of ethyl acetate, cyclohexane, and McIlvaine's buffer (*p*H 3.0) *(2)*, served as the stationary phase while the upper phase of the same system was used as the eluting solvent. Concentration in a vacuum of the active fractions from the column resulted in an aqueous solution (*p*H 3.0) which, after being cooled, yielded the crystalline antibiotic. Recrystalled from methanol, the antibiotic had the following physical properties: m.p., 238° to 240°C; molecular weight, 242 (mass spectrum); $[\alpha]_D^{25} = +92°$ (0.1*N* NaOH); pK_a, 4.32. Elemental analyses were in accord with the formula $C_{10}H_{14}N_2O_3S$.

Structure I for the antibiotic was deduced from a comparison of its infrared and nuclear magnetic resonance (NMR) spectra *(3)* with those of biotin. The infrared spectrum indicates a close structural relationship. However, the infrared spectrum of the antibiotic has sharp, strong bands at 1645 cm^{-1} and 985 cm^{-1} which are absent in the spectrum of biotin. These are attributable to a *trans*-conjugated ($\lambda_{max}^{H_2O}$=203 mμ, ε=15,000: λ, wavelength and ε, extinction) double bond. The outstanding differences between the NMR (Table 2) and the infrared spectra are: (i) the methylene envelope (δ=about 1.45 ppm) was reduced from six protons in biotin to two protons in the antibiotic and (ii) the appearance in the spectrum of the latter of an AB part of an ABX_2 system comprising two *trans*-vinyl protons with a methylene group next to the double bond. The low-field position of the signals for the vinyl protons and the magnitude of the coupling constant (J_{AB} = 17 cy/sec) indicates conjugation of the double bond with the carboxyl group and *trans* orientation.

The above data lead to structure I for the antibiotic, and the validity of this structure was proved by reduction (10 percent palladium on charcoal) of the antibiotic to the naturally occurring *d*-biotin. The identity of the reduction product with biotin was established by the following criteria; there was no depression of melting point on admixture with biotin, the infrared spectrum and optical rotation were identical with those of biotin, and the compound did not separate from biotin on papergrams *(4)*.

It has been established *(5)* by x-ray crystallography that structure II represents the absolute configuration of biotin. Consequently, the absolute configuration of α-dehydrobiotin is as depicted by structure I.

The role of antimetabolites such as the sulfa drugs in the treatment of bacterial infections is well known. More recently, however, antimetabolites have received considerable attention in the chemotherapy of neoplastic diseases. For example, aminopterin is now used in the treatment of acute leukemia in children and of choriocarcinoma in women, and 6-mercaptopurine is used in the treatment of acute leukemia in both adults and children *(6)*.

L. J. HANKA
M. E. BERGY
R. B. KELLY
Research Laboratories, Upjohn Company, Kalamazoo, Michigan

References and Notes

1. For example, activity against the following microorganisms has been detected: *Staphylococcus aureus, Sarcina lutea, Streptococcus pyogenes, Escherichia coli, Proteus vulgaris, Salmonella pullorum, Candida albicans, Saccharomyces cerevisiae* and *Penicillium oxalicum*.
2. T. C. McIlvaine, *J. Biol. Chem.* **49**, 183 (1921).
3. We thank Dr. G. Slomp and his associates, of these laboratories, for determining the spectra.
4. For paper chromatography the following two solvent systems were used: 1-butanol, methanol, benzene, water (2 : 1 : 1 : 1); and 1 butanol, acetic acid, water (2 : 1 : 1).
5. J. Trotter and J. A. Hamilton, *Biochemistry* **5**, 713 (1966).
6. Chemotherapeutical aspects of antimetabolites are discussed by J. A. Stock in *Exp. Chemotherap.* **4**, 79 (1966).

31 Octobe 1966

Reproduction in Lizards: Influence of Temperature on Photoperiodism in Testicular Recrudescence

Abstract. *The photoperiodic response of the testis in* Anolis carolinensis *is very temperature-sensitive. Body temperatures must be elevated to near preferred levels (about 32°C) during at least part of the daily light period for long day-lengths to be effective in stimulating testicular recrudescence. High temperatures during the night, with cool days (20°C), may retard testicular growth more than do continuously low temperatures.*

Annual testicular cycles have been described for many species of reptiles, but the control of the timing of these cycles is poorly understood. Experimentally increased day-lengths stimulate testicular recrudescence in several species of lizards *(1, 2)*; however, interpretation of the importance of such photoperiodic responses is hindered by the paucity of information on thermal influences *(3)*. Studies on other poikilotherms have demonstrated that both the level of temperature and the nature of daily thermal fluctuations may modify the influence of photoperiod on seasonal physiological cycles *(4, 5)*. In-