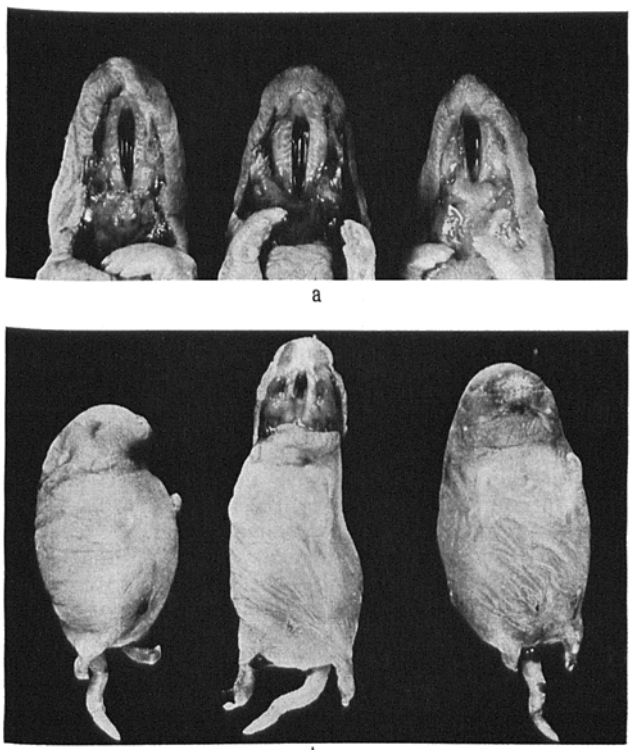
Äthyl-nitrosoharnstoff[2], 1,2-Diäthyl-hydrazin[5], Azo- und Azoxyäthan[6] sind stark wirksame Carcinogene. Nach transplacentarer Einwirkung erzeugten auch sehr geringe Dosen bei fast sämtlichen Nachkommen (insgesamt etwa 600) im Alter von meist 150–300 Tagen maligne Tumoren im Gehirn und Nervensystem[1,2]. Dagegen haben wir nach Gabe von PDMT am 15. Tage post coitum bei den aufgezogenen Nachkommen bis zum Alter von 500 Tagen bisher keinen einzigen Tumor beobachtet. Daher sind für die teratogene und die carcinogene Wirkung des PDMT verschiedene Mechanismen anzunehmen.



Teratogene Wirkungen an BD IX-Ratten. Oben (a) Gaumenspalten nach C_6H_5-N = N-N$(CH_3)_2$, unten (b) Gaumenspalten, Phokomelie, Mikrognatie und Adaktylie nach C_6H_5-NH-N = N-CH_3 jeweils einmal 75 mg/kg am 15. Tage post coitum.

PDMT zerfällt nach Protonierung (pH 6) in Dimethylamin und Phenyldiazonium. Bei der relativ sauren Reaktion im Föt käme dieser Mechanismus für die teratogene Wirkung in Frage. Andererseits konnten wir nachweisen[4], dass PDMT metabolisch z.B. durch Leber-Mikrosomen von erwachsenen Ratten bei Anwesenheit von TPNH und Sauerstoff unter Abspaltung von Formaldehyd zum Phenyl-monomethyl-triazen (PMT) demethyliert wird. PMT verhält sich wie ein stabilisiertes Diazomethan[7,8], das bereits bei pH 9 neben Anilin freigesetzt wird. Hierauf beruht wahrscheinlich die spezifisch neurotrope carcinogene Wirkung des PDMT.

In einem weiteren Versuch, bei dem das PDMT erst am letzten Tage der Schwangerschaft injiziert wurde, haben wir bei Nachkommen bereits die Entstehung von Hirntumoren beobachtet. Danach ist anzunehmen, dass die Fähigkeit zur oxydativen Demethylierung von PDMT am 15. Tage der fötalen Entwicklung noch nicht vorhanden ist, sondern erst später erworben wird.

Zur weiteren Klärung haben wir die Monomethyl-Verbindung (PMT) auf teratogene Wirkung untersucht. Die einmalige s.c. Injektion von 75 mg/kg am 15. Tag post coitum führte bei allen 22 beobachteten Nachkommen zu Gaumenspalten und weiteren Missbildungen, darunter Phokomelie, Adaktylie und Mikrostomie, (Figur b). PMT ist offenbar stärker wirksam, als PDMT. Der Wirkungsmechanismus wird in weiteren Versuchen geprüft.

Summary. 1-Phenyl-3,3-dimethyl-triazene and its monomethyl-derivative, when given as single s.c. injection of 75 mg/kg b.w. to pregnant rats on the 15th day of gestation, proved to be powerful cleft palate teratogens.

H. Druckrey, S. Ivankovic,
R. Preussmann und U. Brunner

Forschergruppe Präventivmedizin am Max-Planck-Institut für Immunbiologie, Freiburg i.B. (Deutschland), 27. September 1967.

[5] H. Druckrey, R. Preussmann, F. Matzkies und S. Ivankovic Naturwissenschaften *21*, 557 (1966).
[6] H. Druckrey, R. Preussmann, S. Ivankovic, C. H. Schmidt, B. T. So und C. Thomas, Z. Krebsforsch. mikrosk. Anat. *67*, 31 (1965).
[7] O. Dimroth, Chem. Ber. *38*, 670 (1905).
[8] E. H. White und H. Scherer, Tetrahedron Lett. 758 (1961).

The Enhancing Effect of EDTA on the Antibacterial Activity of Nalidixic Acid against *Pseudomonas aeruginosa*

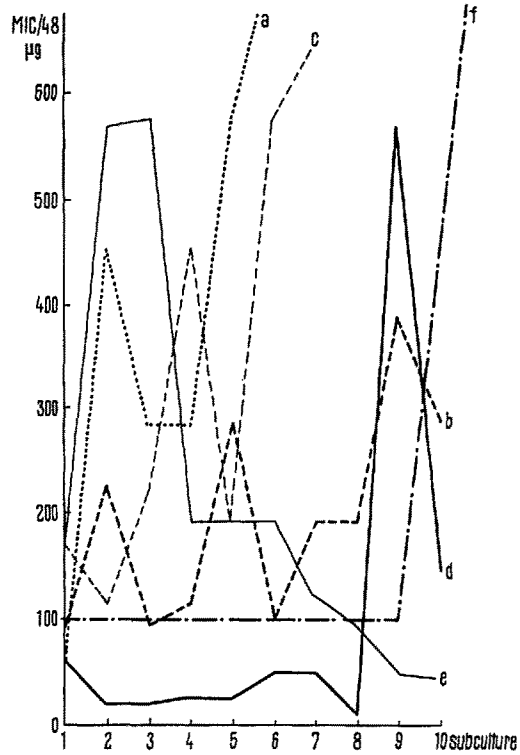
Nalidixic acid, one of a series of 1,8-naph-thyridine derivatives, was first synthesized by Lesher et al.[1] and introduced into general clinical use in 1964. In vitro and in vivo experience suggests that the medication has many advantages over other available agents (McDonald[2]). However, as in other antibacterial agents, the microbial resistance is induced relatively quickly, especially in the case of *Pseudomonas aeruginosa* (Buchbinder et al.[3]). During treatment or during performance of in vitro studies, susceptible microorganisms are eradicated, leaving their resistant counterparts to emerge. The epidemiological importance of these organisms is unknown, but it is conceivable that they may become prevalent in a hospital environment in which nalidixic acid is used extensively. Besides this epidemiological hazard, the chance that resistant organisms will develop in 25% of patients treated with nalidixic acid sharply limits the usefulness of the drug (Ronald et al.[4]). Therefore we attempted, in

[1] G. Y. Lesher, E. J. Froelich, M. D. Gruett, J. H. Bailey and R. P. Brundage, J. mednl pharm. Chem. *5*, 1063 (1962).
[2] D. F. McDonald and H. B. Short, in *Antimicrobial Agents and Chemotherapy* (Ed. J. C. Sylvester; New York 1965), p. 628.
[3] M. Buchbinder, J. C. Webb, La V. Anderson and W. R. McCabe, in *Antimicrobial Agents and Chemotherapy* (Ed. J. C. Sylvester; New York 1963), p. 308.
[4] A. R. Ronald, M. Truck and R. G. Petersdorf, New Engl. J. Med. *275*, 1081 (1966).

vitro only, to influence this undesirable phenomenon with the combination of nalidixic acid and ethylene diamine tetraacetic acid (EDTA).

We have used in experiment 3 strains of *Ps. aeruginosa* (1 of them was isolated from clinical material – diagnosis: haematuria) and 2 strains from the Czechoslovak collection of microorganisms, Brno. As the test substances we used: nalidixic acid, Penbritin (Beecham Res. Lab., Brentford), calcium dinatrium ethylene diamine tetraacetate (Léčiva, Praha), neomycin sulphate (VUA, Roztoky; 684 IU/mg), Ajatin (lauryldimethylbenzylammonium bromide, Slovakofarma, Hlohovec). The test substances were dissolved in distilled water, while the cultivated medium used was the beef extract, Oxoid Lab-Lemco 1%. First we effected in 10 test-tubes an arithmetic line titration of the test substances and their combinations (1:1). Then the inoculation of 3000 ml of bouillon basis was performed with the respective bacterial strain (in suspension of 10^{2-3} bacterial cell in 1 ml). After a 2-h incubation time at a temperature of 37 °C, the bouillon was transferred from the common inoculation basis to test-tubes, into which later also the antiseptics were put in corresponding concentrations. The cultivation time was 48 h, the temperature 37 °C; during this interval the values of minimum inhibition concentration (MIC) were also deducted. As inoculum for the succeeding procedures, we used 1 drop (0.02 ml) of bacterial suspension from the last test-tube before that which contained the MIC of the antibacterial substances.

The present study gives results obtained with strain N/1; this strain was isolated from clinical material. The courses of MIC values of the collection strains were in conformity within the frame of biological variations. Strain N/1 showed a relatively considerable resistance to antibiotics. As seen in the Figure, the initial MIC of neomycin attained as much as 170 µg/ml in contrast to the 2 collection strains: 9 and 14 µm/ml. The MIC values went up in the succeeding subcultures so that in the 7th passage they already attained 1152 µg/ml of neomycin (curve c). In identical experimental conditions the MIC combinations of neomycin + EDTA were essentially lower (except the 9th subculture; curve d). The antibacterial activity of the nalidixic acid alone was appreciable in the first 5 subcultures only (curve a), while in the 6th passage the values exceeded 1000 µg/ml and in the 11th passage 2300 µg/ml. Through the combination of this substance with EDTA, the values also attained an appreciable therapeutic level (curve b). Favourable results were likewise repeatedly obtained with other bacterial strains. Penbritin alone was, under these experimental conditions, comparatively without antibacterial effect. Substantially better results were obtained with the combination of this antibiotic with EDTA (curve e). In contrast to the other antibacterial substances, the MIC values display in the course of repeated passages a decreasing tendency. Ajatin alone showed a comparatively good effect on the tested strain in a series of subcultures, and also the course of the MIC curve is somewhat different, when compared to the foregoing substances (curve f).



The enhancing effect of EDTA on the antibacterial activity of nalidixic acid, neomycin, Penbritin and Ajatin against *Ps. aeruginosa*. Growth curves of (a) nalidixic acid, (b) combination nalidixic acid + EDTA, (c) neomycin, (d) combination neomycin + EDTA, (e) combination Penbritin + EDTA, f) Ajatin. All combinations in weight ratio 1:1.

Mechanism of action of nalidixic acid on *E. coli* was studied by Goss et al.[5]. Cultures treated with nalidixic acids were osmotically stable; lethality was observed in the presence of stabilizers. Only deoxyribonucleic acid (DNA) levels were markedly lowered in drug-treated cells. These facts are consistent with the view that nalidixic acid interferes with the synthesis of *E. coli* DNA. MICHAELS and EAGON[6] suggested that EDTA acted through its chelation on metal cations and that these metal cations were essential to the structural integrity of cell of *Ps. aeruginosa*. This material is capable of causing with this mechanism a non-specific increase in bacterial permeability (LEIVE[7]). It may be that EDTA also interferes with the divalent cations of subcellular structures, especially with Mg^{2+} of ribosomes. The enhancing effect of EDTA with nalidixic acid on *Ps. aeruginosa* appears very promising, and has many practical aspects, especially in the treatment of urinary tract infections; it has not yet been tested experimentally.

Zusammenfassung. Die antibakterielle Wirkung von Nalidixinsäure und vergleichsweise die von Penbritin, Neomycin und Ajatin gegen *Pseudomonas aeruginosa* wurde durch Subkulturen untersucht. Die Nalidixinsäure allein zeigte nach einigen Passagen eine nur sehr geringe antibakterielle Wirkung, in Kombination mit EDTA wurde diese Wirkung aber wesentlich erhöht. Gleichartig konnte bei den andern mit EDTA kombinierten Stoffen eine markant gesteigerte antibakterielle Aktivität gegen 3 geprüfte mikrobielle Stämme nachgewiesen werden.

J. NEZVAL and K. HALAČKA

Department of Hygiene and Epidemiology, Medical Faculty, University of J. Ev. Purkyně, Brno (Czechoslovakia), 2 June 1967.

[5] W. A. Goss, W. H. DEITZ and T. M. COOK, J. Bact. *88*, 1112 (1964).

[6] G. B. MICHAELS and R. G. EAGON, Proc. Soc. exp. Biol. Med. *122*, 866 (1966).

[7] L. LEIVE, Proc. natn. Acad. Sci. USA *53*, 747 (1965).