Nachdem nun von MARQUARDT et al.[4] gezeigt wurde, dass die Nitrosamide N,N'-dinitroso-N,N'-dimethyloxamid (DDO) und N,N'-dinitroso-N,N'-dimethylterephtalsäureamid (NMT) bei *Saccharomyces cerevisiae* mutagen wirken, untersuchten wir[5], ob diese Substanzen – die ohne enzymatischen Anstoss hydrolytisch in Diazoalkan überführt werden – *E. coli* K12 (λ)-Zellen induzieren.

Beide Substanzen inaktivieren die behandelten lysogenen Zellen stark (Figur (a) und (b)), während der Titer der freien Phagen nur geringfügig abnimmt, so dass in erster Linie eine Wirkung intrazellulär entstandenen Diazomethans angenommen werden kann. Beide Amide induzieren, aber jeweils nur relativ geringe Anteile der behandelten Zellen. Dies ist zumindest aus den Versuchen mit DDO offensichtlich. Immerhin ist die Tatsache bedeutsam, dass DDO überhaupt induziert, obwohl es – wie wir nach Abschluss unserer Versuche erfuhren – bei Hefe beim pH 7 nur im Temperaturbereich unter 25 °C mutagen wirkt[6,7]. Der Verlauf der mit NMT erhaltenen Induktionskurve schliesst nicht aus, dass in den ersten Minuten zunächst mehr als 8,5% der Zellen induziert, dann aber zunehmend inaktiviert werden.

Unsere Befunde können nicht zur Diskussion über die Beziehungen zwischen cancerogener und induzierender Aktivität herangezogen werden, da DDO bei Ratten kaum cancerogen ist und NMT in dieser Hinsicht zumindest von DRUCKREY et al.[8] noch nicht geprüft wurde. Unsere Befunde stützen aber die oben geäusserte Ansicht, dass die Unempfindlichkeit lysogener Zellen gegenüber Nitrosaminen auf einen Mangel an dealkylierenden Enzymen zurückzuführen sein dürfte und nicht auf eine Resistenz gegenüber Diazoalkanen[9].

Summary. N,N'-dinitroso-N,N'-dimethyloxamide and N,N'-dinitroso-N,N'-dimethylterephtal acid amide, both mutagenic for yeast cells, induce phage production by lysogenic *Escherichia coli* K12 (λ) cells.

G. R. MENZEL und E. GEISSLER[10]

Institut für Krebsforschung der Deutschen Akademie der Wissenschaften zu Berlin, Experimenteller Bereich, *Berlin (DDR), 14. März 1966.*

[1] E. GEISSLER, Naturwissenschaften *49*, 380 (1962).
[2] H. MARQUARDT, R. SCHWAIER und F. K. ZIMMERMANN, Naturwissenschaften *50*, 135 (1963).
[3] L. PASTERNAK, Naturwissenschaften *49*, 381 (1962).
[4] H. MARQUARDT, F. K. ZIMMERMANN und R. SCHWAIER, Z. Vererb-Lehre *95*, 82 (1964).
[5] Bezüglich methodischer Einzelheiten vgl. E. GEISSLER, Biochim. biophys. Acta *114*, 116 (1966).
[6] R. SCHWAIER, F. K. ZIMMERMANN und U. v. LAER, Z. VererbLehre *97*, 72 (1965).
[7] H. MARQUARDT, persönliche Mitteilung (1966).
[8] H. DRUCKREY, persönliche Mitteilung (1966).
[9] Herrn Prof. H. DRUCKREY, Freiburg i.Br., danken wir für die Überlassung der von Herrn Dr. PREUSSMANN synthetisierten Substanzen.
[10] Neue Anschrift: Institut für Mikrobengenetik, Universitätsplatz 5, 25 Rostock, DDR.

Cystamine Inhibition of Enzyme Induction and Acetate Oxidation in *Pseudomonas aeruginosa* and Reversal by High Salt Concentrations

Cystamine, the disulfide form of 2-mercapto-ethylamine (MEA), inhibits the entry of sodium ions into cells of a strain of *Pseudomonas aeruginosa*[1]. The evidence for this consists of the inhibition of cell swelling when washed cells are suspended in sodium phosphate buffer. No inhibition of swelling occurs in potassium phosphate buffer. The percentage inhibition is inversely proportional to the buffer concentration, which suggests a competitive inhibition between sodium ions and cystamine. MEA is inactive. In the absence of sodium ions organic acid substrates are not oxidized by this organism (unpublished observation). It follows, therefore, that cystamine should inhibit the oxidation of such substrates and that the percentage inhibition should depend on the buffer concentration. To test this possibility the following experiments were performed.

A strain of *P. aeruginosa* maintained in this laboratory for 18 years was grown at 34 °C for 24 h in Difco nutrient broth. The cells were spun down and then washed twice with water by centrifugation. They were then suspended in water, adjusted to a standard optical density, and added to Na-K phosphate buffers pH 7.7 of various molarities in Warburg vessels which had a final fluid volume of 2.0 ml. Freshly dissolved MEA, which had been allowed to autoxidize until the nitroprusside test was negative, was added, followed by the substrates. These were Na benzoate, which requires enzyme induction as shown by the fact that its oxidation is inhibited by streptomycin and other drugs which inhibit protein synthesis, and Na acetate, which is oxidized without a lag phase. An increase in the molarity of the buffer from 0.025–0.1 caused a small increase in the rate of benzoate oxidation and a small decrease in the rate of acetate oxidation.

CLELAND[2] has shown that dithiothreitol (DTT) reduces S–S linkages and protects –SH groups from oxidation. Table I shows that 15 μg/ml DTT had no effect on the

Table I. The effect of 10 μg/ml cystamine. HCl in the presence or absence of 15 μg/ml DTT on the oxidation of 0.5 mg/ml Na acetate (anhydrous). 0.1 *M* Na-K phosphate buffer pH 7.7, 37 °C. The figures ar μl O_2 uptake

Time (min)	Control	Cystamine	Inhibition %	Cystamine + DTT	Inhibition %	DTT
30	58	34	38	59	0	57
45	86	55	36	91	0	87
60	116	78	36	121	0	117
75	141	98	30	145	0	142

[1] F. BERNHEIM, Biochem. Pharmac. *15*, 1105 (1966).
[2] W. W. CLELAND, Biochemistry *3*, 480 (1964).

Table II. The effect of the molarity of the buffer on the inhibition of benzoate oxidase induction by cystamine HCl. 0.25 mg/ml Na benzoate, pH 7.7, 37 °C. Cystamine concentration in μg/ml. O_2 uptake in μl.

Time	0.025 *M* Buffer			0.05 *M* Buffer			0.1 *M* Buffer			0.025 *M* Buffer			0.05 *M* Buffer			0.1 *M* Buffer		
(min)	[b]	1.5[c]	[a]	[b]	1.5[c]	[a]	[b]	1.5[c]	[a]	[b]	1.5[c]	[a]	[b]	3.0[c]	[a]	[b]	6.0[c]	[a]
30	6	6	–	6	6	–	7	6	–	13	12	–	17	12	–	13	11	–
60	43	19	56	44	29	34	53	44	17	43	25	42	35	22	37	37	23	38
75	96	49	48	96	73	24	110	98	11	64	36	44	54	30	44	61	31	49
90	119	66	45	118	93	21	138	125	9	91	55	40	83	40	47	93	45	52
Average [a]			50			26			12			42			43			46

[a] % inhibition. [b] control uptake. [c] μg/ml cystamine.

oxidation of acetate but completely antagonized the inhibition by cystamine. Similar results were obtained with benzoate. This indicates that only oxidized MEA is active. Table II shows the effect of buffer molarity on the inhibition of benzoate oxidase induction by cystamine. In this Table the decrease in inhibition is almost directly proportional to the increase in molarity, but as the molarity of the buffer was doubled and the concentration of cystamine was doubled the inhibition of benzoate oxidase induction did not stay strictly constant. Cystamine added after the enzyme was induced did not inhibit the oxidation. Other diamines such as putrescine, cadaverine and spermine do not inhibit these reactions but cysteine does. Its inhibition is not affected by buffer concentration and cystine is inactive.

Riassunto. L'inibizione della ossidazione di acetato e della induzione di benzoato-ossidasi da parte della cistamina in un ceppo di *Pseudomonas aeruginosa*, varia colla concentrazione del mezzo nel quale le cellule sono sospese. L'effetto inhibitorio diminuisce con l'aumentare della concentrazione del buffer. Altre diamine e cistamina in forma ridotta sono inattive. La cistenia inibisce le medesime reazioni ma il suo effetto è indipendente della concentrazione del mezzo di sospensione. La cistina è priva di effetto.

F. BERNHEIM

Department of Physiology and Pharmacology, Duke University Medical Center, Durham (North Carolina, USA), March 4, 1966.

The Effect of Phage Infection on the Catalase Induction of the *Staphylococcus aureus* Culture

The catalase demonstrable in the different cell fractions is formed in the ribosome[1], and it is supposed that it is primarily attached to the membrane of the cell structure[2]. This has an inhibitory effect, from the point of view of the enzyme activity, and in such a condition the catalase is inactive, unfolded on the intracellular membrane. Its attachment to the structure may be prevented by detergents, organic solvents and physical factors[3]. Then the catalase becomes desorbed from the structure, its unfolded state ceases and its very active H_2O_2-decomposing property is attributed to its changed configuration.

However, this desorption may occur not only under artificial but also under normal conditions. Our previous investigations also proved that the increase of the catalase activity under normal growing conditions occurs in the final, stationary phase, when the electrode potential of the culture decreases[4]. The fact that the structural attachment of the catalase ceases under the influence of physical and chemical factors, manifests itself in an increase in activity. So it may also be supposed that the biological destruction of the structure – which can also be a consequence of phage infection – has an influence on the development of the catalase activity of the system.

We conducted our experiments with a culture of *Staphylococcus aureus* 162 isolated from human purulence, showing phage sensitivity of a very wide spectrum. For infection, phage 7 – belonging to the typing basic set of *S. aureus* phage – was used. The change of the catalase activity was measured using the iodometric method[5], and the proliferation and cell lysis by the optical density. If the *S. aureus* culture developing in the logarithmic phase is infected at different optical densities with the same number of phage particles (Figure, a, b, c), the lysis of the culture occurs at different periods of time.

The lysis of the culture with a lower density (smaller cell number) when infected with the same phage particle evidently occurs sooner (Figure, curve D_1) than the lysis of the culture with a higher density which contains more cells; consequently more phage proliferation cycles must occur up to the lysis of all the cells (Figure, curves D_2, D_3). From the time of the phage infection, the catalase activity gradually increases and attains its maximum at the time of complete clearing. Obviously the activity of the catalase will be lower at the lysis of a culture with a smaller number of cells, whereas at the lysis of a culture of higher density, i.e. with a larger number of cells, the catalase activity will be much higher (Figure, curves C_1, C_2, C_3).

[1] T. HIGASHI JR. and T. PETERS, J. biol. Chem. *238*, 3952 (1963).
[2] A. L. MILLER, Exp. Cell Res. *34*, 283 (1964).
[3] M. J. FRASER and J. G. KAPLAN, J. gen. Physiol. *38*, 515 (1955).
[4] E. KOVÁCS, H. H. MAZAREAN, and J. LANTOS, Zentbl. Bakt. ParasitKde *198*, 427 (1965).
[5] H. H. MAZAREAN and E. KOVÁCS, Z. anal. Chem. *211*, 358 (1965).