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.

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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).

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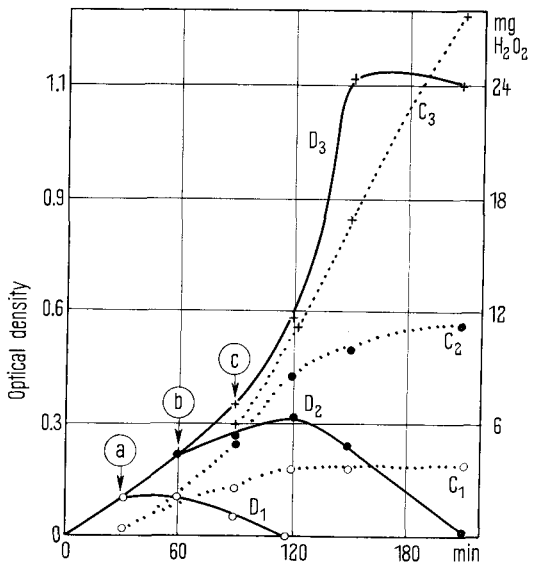
The results of the experiments seem to prove that the catalase adsorbed to the intracellular membrane becomes desorbed, due to the destruction of the cell structure caused by the phage infection. It is manifested in an increase in the catalase activity. From the decrease of density, and the further increase of the catalase activity, we may conclude that the infection does not damage the protein of the catalase and consequently does not influence the enzyme activity. This is supported also by the fact that the maximum enzyme activity obtained at complete clearing of the culture persists for a longer while. This activity maximum reflects the total amount of the catalase of the cells, corresponding to the maximum density value of the infected culture.



Infection of *Staphylococcus aureus* 162 culture with phage 7 at different densities (a, b, c) during its developmental stages. With decreasing density values (D_1, D_2, D_3) after the infection, the catalase activities (C_1, C_2, C_3) increase and reach a maximum value.

In the course of the development of the cells, the catalase activity increases gradually but not proportionally with the density; this means that the ratio of the bound and soluble catalase changes during growth. At the beginning of the culture, the balance is shifted towards the structurally bound catalase; while later, under the influence of the changed conditions, a soluble enzyme with a different configuration prevails. The decrease of the electrode potential may well play a decisive role in this shift of balance[4].

Zusammenfassung. Bei Phaginfektion erhält man totale Auflösung der *Staphylococcus aureus*-Kultur, bei gleichzeitiger Steigerung der sich durch H_2O_2-Degradation manifestierenden Aktivität der Katalase. Somit wird durch die unter Phageinwirkung erfolgende Zerstörung der Bakterienzellen strukturell gebundene Katalase befreit und die Menge des löslichen Enzyms, welches grosse katalytische Aktivität aufweist, vermehrt.

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Revival of Heat-Damaged *Escherichia coli*

Recently, several studies of the factors influencing the revival of chemically- and physically-damaged bacteria have been published[1,2]. It is known that the composition of the recovery medium affects the revival of bacteria damaged by phenol[1], irradiation[3] and heat[4].

In the present study, washed suspensions of a laboratory strain of *Escherichia coli* Type 1 were obtained by centrifugation and washing of the growth at 37 °C of an 18 h culture in nutrient broth (Oxoid Laboratories, London, England). 1 ml of this suspension was added to, and mixed well with, 99 ml of sterile water held in a thermostatically-controlled water bath at 50, 55 or 60 °C (± 0.1 °C) to give approximately $2–3 \cdot 10^6$ viable organisms/ml. When required, aliquots were removed, and after serial dilution the numbers of viable survivors were made by the pour-plate and surface-viable methods, using nutrient agar (Oxoid) or a synthetic agar, alone or supplemented with 1% yeast extract (Oxoid; or Difco Laboratories, Detroit, Michigan, USA). The synthetic medium contained per litre: potassium dihydrogen phosphate 20 g, ammonium sulphate 1 g, magnesium sulphate 0.4 g, glucose 3.6 g; it was solidified with 1% Ionagar No. 2 (Oxoid). All plates were incubated at 37 °C for 48 h.

With unheated bacteria, there was no significant difference ($P^1 = 0.05$) between viable counts obtained by the pour-plate and surface-viable methods. When heated bacteria were used, however, viable counts obtained by the former procedure were some 20% higher than those obtained by the surface-viable technique. This result is in contrast to that obtained with phenol-damaged bacteria[5], but supports the findings made with heat-damaged *Staphylococcus aureus*[6,7]. Also, the incorporation of sodium thioglycollate into the recovery medium increases the number of reviving cells of heat-damaged *E. coli*[8,9], and it is thus conceivable that heated *E. coli* revives better under anaerobic or semi-anaerobic than under aerobic conditions.

The incorporation of Difco yeast extract in the recovery medium increased the colony counts of heat-damaged (50 °C, 1 h), but not of unheated, cells (Table); cells

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