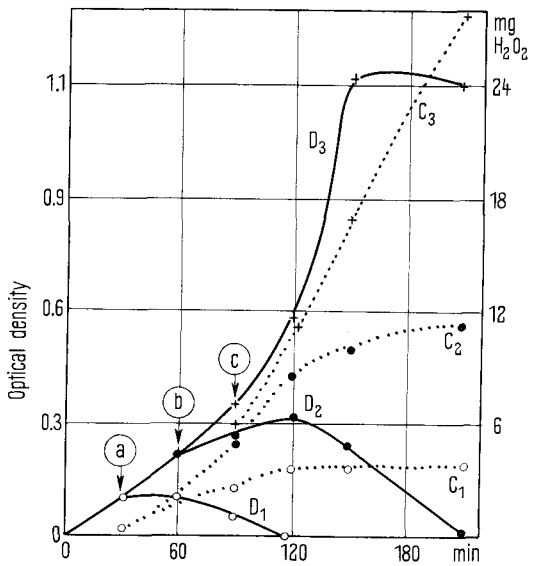
The results of the experiments seem to prove that the catalase adsorbed to the intracellular membrane becomes desorbed, due to the destruction of the cell structure caused by the phage infection. It is manifested in an increase in the catalase activity. From the decrease of density, and the further increase 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

[1] N. D. Harris, J. appl. Bact. *26*, 387 (1963).
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[3] T. Alper and N. E. Gillies, J. gen. Microbiol. *18*, 461 (1958).
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[6] A. C. Baird-Parker and E. Davenport, J. appl. Bact. *28*, 390 (1965).
[7] M. C. Allwood and A. D. Russell, unpublished data.
[8] F. E. Nelson, J. Bact. *48*, 473 (1944).
[9] D. Harries and A. D. Russell, unpublished data.

damaged at higher temperatures also showed a greater revival in media containing this yeast extract. However, significant differences ($P^1 = 0.05$) were noted between Oxoid and Difco yeast extracts in their effect upon the revival of heated *E. coli*: the Difco brand always reviving the greater number of organisms. Analysis of these extracts indicates that, for 'typical batches', the Difco brand contains a higher percentage of amino acids and a greater number of vitamins than does the Oxoid. Certain of these constituents, either singly or in combination, may be necessary for the revival of the heat-damaged organisms.

The failure of the damaged organism to recover in a synthetic medium may be due to inability of the cells to manufacture new cell constituents from simple materials, although other explanations are feasible, e.g. in such a medium there may be unbalanced metabolism in damaged cells resulting in death, or, alternatively, damaged bacteria may be highly sensitive to ions present[10]. Differences in viable counts are not explained by any differences in agglutination, since preliminary experiments with phase contrast microscopy showed that moist heat did not cause agglutination of *E. coli*.

Effect of medium on colony counts of unheated and of heated *E. coli*

Medium	Additions	Unheated bacteria			Heated bacteria		
		Experiment No.			Experiment No.		
		1	2	3	1	2	3
Nutrient agar		182	203	265	60	247	100
Nutrient agar	1% YE (Difco)	185		*262*	173		*320*
Nutrient agar	1% YE (Oxoid)	185		260	82		89
Synthetic agar			202			49	
Synthetic agar	1% YE (Difco)		194			390	

YE = Yeast extract. Figures refer to the number of colonies obtained by the pour-plate technique, and are the mean of 10 plates in each case. Colony counts for unheated bacteria were obtained by plating out 1 ml of a 10^{-4} dilution; for heated bacteria (50 °C, 1 h) by plating out 1 ml of a 10^{-2} dilution.

Résumé. Une recherche a été faite sur quelques facteurs ayant une influence sur le retour à la vie d'*Escherichia coli* endommagé par la chaleur. On a obtenu plus de survivants par la méthode «pour-plate» que par la méthode «surface-viable». C'est l'extrait du ferment Difco, et non celui de l'Oxoid, qui a stimulé la guérison des bactéries chauffées. Dans un milieu synthétique, il y a eu peu de survivants.

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June 7, 1966.

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Dependence of the Cell Morphology of *Vitreoscilla* on the Temperature of Incubation

In descriptions[1,2] of *Vitreoscilla* the diameter and length of single cells as well as of trichomes are reported to be typical for a particular species, but the temperature of incubation was not mentioned. However, the cell size and the trichome size of *Vitreoscilla* were suspected of being related to the incubation temperature, and a study of the dependence was made. Differences found in cellular and colonial morphology of *Vitreoscilla* grown at high and low temperatures, show that the cell length and diameter, as well as the gross morphology and motility, are variable and temperature dependent. The different cell sizes found in *Vitreoscilla* grown at different incubation temperatures may be explained by the dissociative action of high temperature on the normal balance of cell growth and division of these organisms.

2 strains of *Vitreoscilla* (strains 1 and 2), used in the present study, were obtained from G. J. HAGEAGE, University of New Hampshire. They were grown on nutrient agar at room temperature (about 23 °C) and subcultured every 48 h. To test the influence of the incubation temperature, a small inoculum of a 24 h culture was transferred to the whole surface of the following media: 5% horse blood agar, chocolate agar, trypticase soy agar, 10% calf serum agar, and Loeffler's medium. Before inoculation the media were warmed to the temperature at which they were to be incubated. Immediately after the inoculation, i.e. without any preincubation at room temperature, they were placed at 23, 25, 28, 31, 34, 37, and 39 °C for 24 h. During this time they were inspected with the unaided eye and also microscopically at 3 h intervals. On transparent media we used the Örskov direct agar microscopy. The cellular morphology of both strains grown on different media at various temperatures was checked also in smears prepared at the above mentioned time intervals in the usual way, i.e. with the inoculating needle. They were dried in air, fixed in a Bunsen flame, and stained by the Gram technique and with methylene blue.

When cultured at lower temperatures (34 °C and below), the cells were short and uniformly large (Figure 1). At 37 °C, however, they began to grow in length in the second 3 h observation period. Some of the filaments grew also in width. The process of elongation continued rapidly in the ensuing hours of incubation. In 18 h some of the cells attained a length of 30 μ and more (Figure 2), and also the trichomes were much longer. These enlarged cells lost their motility.

As a consequence of the cytological changes and the motility differences at different incubation temperatures, the form of the colonies also changed. At room temperature and up to 34 °C, spreading growth with more or less distinct waves was characteristic. At about 37 °C, individual colonies were formed. They were smaller and scarcer

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