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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[10] S. E. JACOBS and N. D. HARRIS, J. appl. Bact. *23*, 294 (1960).

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

[1] E. G. PRINGSHEIM, J. gen. Microbiol. *5*, 124 (1951).
[2] R. S. BREED, E. G. D. MURRAY, and N. R. SMITH, *Bergey's Manual of Determinative Bacteriology*, 7th edn. (The Williams & Wilkins Co., Baltimore 1957).

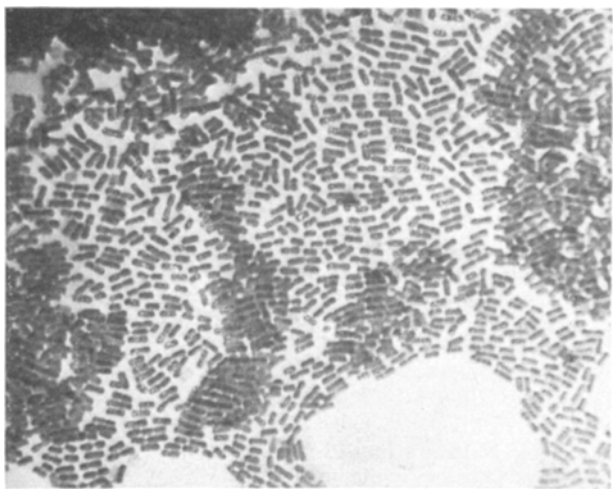

Fig. 1. *Vitreoscilla*, strain 1, grown at room temperature (ca. 23 °C) for 24 h. Gram stained. Magnification ca. × 1200.

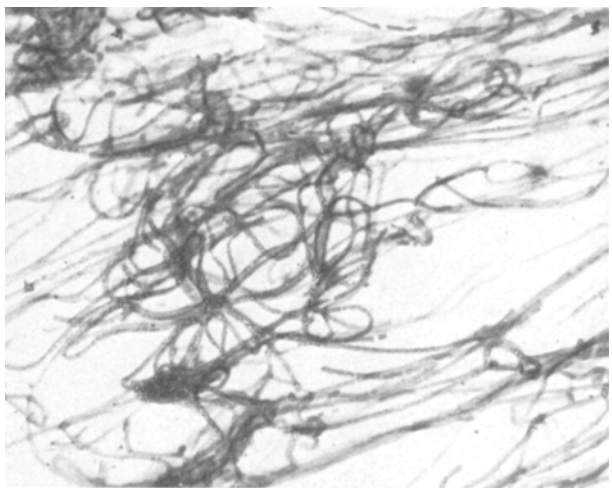

Fig. 2. *Vitreoscilla*, strain 1, grown at 37 °C for 24 h. Gram stained. Magnification ca. × 1000.

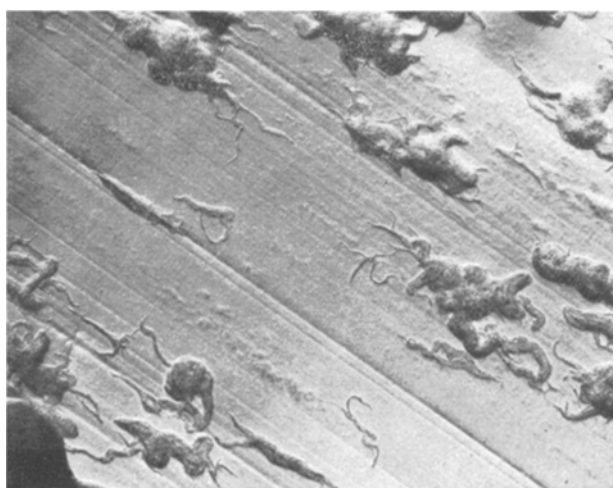

Fig. 3. *Vitreoscilla*, strain 1. Colonial morphology at 37 °C. Magnification ca. × 40.

as the incubation temperature increased. The outline of these colonies was more or less irregular, with bunches of long winding trichomes protruding in different directions out of the border of the colonies (Figure 3).

When the cultures grown at higher temperatures were subcultured at different time intervals (up to 48 h of growth) to the same or other media and incubated at lower temperatures, they again grew in their small form, the growth was spreading, and the cells showed pronounced gliding motility. The new cells showed a normal succession of cell division and cell growth as long as they were not reincubated at an unfavourably high temperature.

At 39 °C, no colonies or other visible growth were observed with the unaided eye in either *Vitreoscilla* strain, even after prolonged incubation (48 h). The individual cells, however, found in the smears prepared from the surface of the media or observed there microscopically, were elongated and enlarged but never to such a degree as in the cultures incubated at 37 °C. These changed cells were irregularly outlined, poorly stained and showed other signs of disintegration as early as in the last hours of a 24 h incubation at this high temperature. When subcultured to fresh media and incubated at lower temperatures, colonies occasionally were produced in the first 24 h of incubation. Some appeared in the second 24 h of incubation at lower temperatures and the growth of some occasional late ones was delayed still more.

The filamentation of the individual cells and the subsequent changes in colony-form at about 37 °C might be explained by the vigorous growth of the individual cells at temperatures where cell division is already stopped or at least inhibited. At higher temperatures also the cell growth is impaired or completely stopped and very big cells cannot be produced.

The observations briefly presented here suggest the usefulness of these organisms in studies on the cytological and colonial manifestations of the action of incubation temperature on the mechanisms of cell growth and division.

The effect of different incubation temperatures on a single culture at short consecutive intervals can be studied by simply transferring the cultures from one temperature to another without interfering by subculturing with the normal growth of a population. The results of more extensive studies on the cytological reactions of these and other strains of *Vitreoscilla* to the temperature of incubation will be presented elsewhere[3,4].

Zusammenfassung. In Temperaturexperimenten hat sich ergeben, dass Zellen und Trichomen zweier *Vitreoscilla*-Stämme in Länge und Diameter temperaturabhängig sind. Ferner erweist sich sowohl die Form der Kolonien als auch die Beweglichkeit der untersuchten Stämme durch die Inkubationstemperatur beeinflussbar.

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[4] This research was supported in part by Grant No. 01FRO5402045, National Institutes of Health, U.S. Public Health Service.