Effects of Temperature Shift on Acid and Heat Tolerance in *Salmonella enteritidis* Phage Type 4

TOM J. HUMPHREY,¹* NIGEL P. RICHARDSON,¹ KATE M. STATTON,² AND ROBIN J. ROWBURY³

*Food Unit, Public Health Laboratory, Church Lane, Exeter EX2 5AD,¹ Department of Biological Sciences, Hatherley Laboratories, University of Exeter, Exeter EX4 4PS,² and Department of Biology, University College London, London WC1E 6BT,³ United Kingdom

Received 8 March 1993/Accepted 9 June 1993

The transfer of cells of *Salmonella enteritidis* phage type 4 from 28 to 37–46°C resulted in marked increases in acid and heat tolerance. The former was maximized within 5 to 15 min of the shift and was largely independent of protein synthesis. In contrast, induction of increased heat tolerance was slower, requiring more than 60 min to be completed, and was prevented by inhibition of protein synthesis. When cells were transferred to medium at temperatures between 47 and 50°C, the kinetics of induction of heat tolerance were essentially the same as at the lower temperatures. In contrast, the cells became more acid sensitive. The results of these studies clearly show that although both acid and heat resistance can be enhanced by preexposure to high incubation temperatures, the mechanisms involved are different.

*Salmonella enteritidis* phage type 4 (PT4), in common with *Salmonella typhimurium* (5) and *Escherichia coli* (8), can become markedly more acid tolerant following exposure to mild acid conditions (11). Such a response is one of many that microorganisms can mount to a variety of potentially damaging conditions. It is also becoming clear that exposure to one stress stimulus can afford protection against another, often unrelated, hostile environment. Thus, exposure to alkali will increase heat resistance (3, 10) and starvation has been shown to protect cells of *E. coli* against subsequent challenge with heat or H₂O₂ (12).

A variety of cooked and processed foods have been implicated in outbreaks of human salmonellosis (2). Cross-contamination from raw to cooked foods is clearly important (17). There is also the possibility that the conditions to which organisms are exposed during either food processing or cooking will enhance their subsequent ability to cause infection. Important in this respect may be increased acid tolerance.

As part of a wide-ranging investigation into the impact of food processing and storage systems on heat and acid tolerance in *S. enteritidis*, the impact of growth temperature and temperature shift were studied. The results of these preliminary investigations are reported.

A strain of *S. enteritidis* PT4, which had been isolated from egg contents, was used in all experiments. Stock cultures were maintained by daily subculture onto blood agar incubated at 37°C. Colonies from the above culture were inoculated into lemco broth (Oxoid) and incubated at either 20°C for 24 h, 37°C for 16 h, or 44°C for 12 h. A 1-ml volume of each culture was then transferred to 9 ml of fresh medium at the same temperature and incubated for a further 3 h. These cultures were used to measure death curves in lemco broth either adjusted to pH 2.6 ± 0.02 with 1 M HCl and held at 37°C or adjusted to pH 7.0 ± 0.02 and incubated at 56 ± 0.2°C.

Previously published techniques (10, 11) were used, and, in essence, this involved the removal of 1-ml volumes from the acidified or heated medium at intervals which varied according to the preexposure conditions of the salmonellas. The removed broth was added immediately to 9 ml of buffered peptone water, and the cells were enumerated by plating on blood agar incubated in air at 37°C for 24 h. *D* (time to kill 90% of the population) values were calculated from the death curves.

Cells of *S. enteritidis*, grown for 24 h at 20°C, were also transferred to lemco broth prewarmed to temperatures between 30 and 50°C. Cultures were maintained at the higher temperatures for up to 60 min. At 0, 5, 15, 30, and 60 min, samples were removed and death rates at pH 2.6 ± 0.02 and 56 ± 0.2°C were estimated as above. In parallel experiments, cells grown at 20°C were transferred to lemco broth at 46°C and either with or without 200 μg of chloramphenicol per ml.

---

* Corresponding author.
Acid tolerance was assessed after 10 min of incubation, and heat tolerance was assessed after 60 min. All experiments were repeated on at least three occasions. The significance of the various differences in heat or acid tolerance was measured by using paired t tests.

Cells of *S. enteritidis* grown at 20°C were significantly (P < 0.001) more heat or acid sensitive than those cultured at either 37 or 44°C. For example, the *D*(56°C) values for organisms grown at 20, 37, or 44°C were 0.91 ± 0.03, 2.84 ± 0.14, and 14.4 ± 1.44 min, respectively. The corresponding *D*(pH 2.6) values were 5.4 ± 0.6, 9.2 ± 0.8, and 12.0 ± 1.1 min. Tolerance to heat or acid was also increased as a result of a sudden shift from 20 to 37-46°C (Fig. 1 and 2). Results indicate, however, that two separate systems are involved. Acid tolerance is maximized rapidly (Fig. 1) and is almost entirely independent of protein synthesis (Fig. 3b). Cells were most acid tolerant following preexposure to 40 to 44°C (Fig. 2), but a shift to temperatures above 46 to 47°C resulted in acid sensitivity (Fig. 2). In contrast, cells of *S. enteritidis* were at their most heat tolerant when held at 46 to 48°C before heat treatment (Fig. 2). Induction of increased heat tolerance was also considerably slower than acquisition of acid resistance (Fig. 1) and required protein synthesis (Fig. 3a). The results for increased heat tolerance are in general agreement with those of other investigations (1, 14, 15). There has been little previous research, however, on the interrelationship between elevated temperatures and acid tolerance, although related work (9) has demonstrated that synthesis of some potentially protective mechanisms in cells of *E. coli* cultured to pH 5.5 is much greater at 37°C than at 24°C.

It has not yet been possible to fully identify the cellular mechanisms responsible for enhanced acid tolerance. Its occurrence as a result of exposure to mild acid has been studied extensively in *S. typhimurium* (4-7) and in less detail in *S. enteritidis* (11). Two processes are involved in both organisms (4, 11). In *S. typhimurium* the response involving protein synthesis has been defined as preshock and is induced by exposure to mild acid (pH 5.5 to 6.0). The second system, which is independent of protein synthesis, becomes active in *S. typhimurium* following exposure to pH values of 4.5 or below. This has been termed acid shock (6). Both systems are required to be in operation for the survival of *S. typhimurium* at pH values below 3.0.

**FIG. 2.** Impact of shift temperature on acid or heat tolerance in *S. enteritidis*. Symbols are as in Fig. 1. *D*(56°C) values were measured after 60 min at the shift temperature. *D*(pH 2.6) values were measured after 5 min. The data are mean values from four separate experiments. Vertical lines show standard deviation.

**FIG. 3.** Inhibition of protein synthesis and temperature shift-induced heat or acid tolerance. (a) Impact on heat resistance. Symbols: □, culture exposed to 46°C for 60 min before transfer to 56°C; ■, culture exposed to 46°C for 60 min in the presence of 200 μg of chloramphenicol per ml; △, control culture grown at pH 7.0 at 20°C. (b) Impact on acid resistance. Symbols: □, culture exposed to 46°C for 10 min before transfer to pH 2.6; ■, culture exposed to 46°C in the presence of chloramphenicol; △, control culture. The data are mean values from three separate experiments. Vertical lines show standard deviation.
S. enteritidis PT4 would appear to be inherently more acid tolerant than S. typhimurium, and increased tolerance which is independent of protein synthesis is induced at pH values of 4.0 and below (11) and is solely responsible for enhanced survival under conditions with pH values as low as 2.5 (11). This or a similar protective mechanism would also appear to be activated in response to temperature shift (Fig. 3b). This may be helpful in elucidating the nature of increased tolerance. Alterations in outer membrane permeability may be involved, and it has been shown that cells of E. coli cultured at 42°C have markedly different fatty acid profiles from those of cells grown at 40°C (16). Different growth temperatures have also been shown to result in different soluble-protein profiles (18). Such responses can be rapid, and cells of E. coli showed measurably different protein patterns within 3 min of a temperature shift from 28 to 42°C (13). Proteins synthesized in response to such stimuli may have a direct protective effect on vital cellular components such as DNA or may bring about an alteration in membrane permeability. It is also possible that entirely different processes, such as a Mg²⁺-dependent proton-translocating ATPase, are involved in facilitating the survival of salmonellas in low-pH environments (7). The ferric uptake regulator system (fur) would also appear to have a central role in survival strategies (7). Whatever mechanisms are involved, the interrelationship between temperature, acid, and increased tolerance to these challenges is of clear practical importance and is worthy of further study.

We thank G. Broom for typing the manuscript.

We thank the PHLS for financial support.

REFERENCES