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Low-pH-Induced Effects on Patterns of Protein Synthesis and on Internal pH in *Escherichia coli* and *Salmonella typhimurium*

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***Escherichia coli* and *Salmonella typhimurium* were grown in a supplemented minimal medium (SMM) at a pH of 7.0 or 5.0 or were shifted from pH 7.0 to 5.0. Two-dimensional gel electrophoretic analysis of proteins labeled with $H_2{}^{35}SO_4$ for 20 min during the shift showed that in *E. coli*, 13 polypeptides were elevated 1.5- to 4-fold, whereas in *S. typhimurium*, 19 polypeptides were increased 2- to 14-fold over the pH 7.0 control. Upon long-term growth at pH 5.0, almost double the number of polypeptides were elevated twofold or more in *S. typhimurium* compared with *E. coli*. In *E. coli*, there was no apparent induction of heat shock proteins upon growth at pH 5.0 in SMM. However, growth of *E. coli* in a complex broth to pH 5.0, or subsequent growth of fresh *E. coli* cells in the filtrate from this culture, showed that a subset of five polypeptides is uniquely induced by low pH. Two of these polypeptides, D60.5, the inducible lysyl-tRNA synthetase, and C62.5, are known heat shock proteins. Measurements of the internal pH (pH_i) and growth rates of both organisms were made during growth in SMM at pH 7.0, pH 5.0, and upon the pH shift. The data show that the pH_i of *E. coli* decreases more severely than that of *S. typhimurium* at an external pH of 5.0; the growth rate of *E. coli* is about one-half that of *S. typhimurium* at this pH, whereas the two organisms have the same growth rate at pH 7.0. The two-dimensional gel, growth, and pH_i experiments collectively suggest that, at least in SMM, *S. typhimurium* is more adaptive to low-pH stress than is *E. coli*.**

A considerable effort has been mounted to understand how cells cope with environmental stresses. Experimentally, two microorganisms, *Escherichia coli* and *Salmonella typhimurium*, have been a focus for this quest, and global regulatory systems which respond to heat stress (19, 20, 31), oxidation stress (5, 16, 36), ultraviolet light (36, 37), and aerobic-to-anaerobic shifts (30, 31, 33) have been studied in either one or both of these organisms.

Less information is available on the response of these organisms to changes in external pH (pH_o) or internal pH (pH_i), although it is clear that the regulation of pH_i (pH homeostasis) is critical for cell growth and function (for a review, see reference 4). It has been known since the work of Gale and Epps (7) that certain enzymatic activities can be induced by a change in pH_o. In recent years, Schuldiner et al. (26) and Taglicht et al. (34) have shown, respectively, that in *E. coli*, SOS proteins are induced by alkaline pH_i shifts and heat shock proteins are induced by alkaline pH_o shifts. However, Taglicht et al. (34) did not detect any induction of heat shock proteins by shifting the pH_o from 8.0 to 6.0.

Recently, genetic aspects of pH control have been explored. Slonczewski et al. (28) isolated Mu d(*lacZ*) operon fusions of *E. coli* in which the expression of *lacZ* depended on low external pH (*exa* mutants) or internal acidification (*ina* mutants). Aliabadi et al. (2) have discovered a gene, *earA*, in *S. typhimurium* which is directly involved with the transcriptional control of pH-regulated loci.

The major thrust of this report is to compare the responses of *E. coli* and *S. typhimurium* with a low-external-pH stress of pH_o 5.0. Collectively, the data suggest that upon growth in the minimal-type medium employed in these experiments, *S. typhimurium* is more adaptive to low-pH stress than is *E. coli*. The difference in the ability of these two microorganisms to adapt to low-pH stress is discussed in terms of their ecological habitats.

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MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 (this laboratory) and *S. typhimurium* ATCC 1428 (both prototrophs) were used throughout this study.

Media and monitoring of bacterial growth. AC broth was obtained from Difco Laboratories (Detroit, Mich.) and was made according to the directions of the manufacturer. Supplemented minimal medium (SMM) was prepared as described previously by Hirshfield et al. (11), with 0.4% glucose as the carbon and energy source. In experiments in which proteins were labeled with $H_2{}^{35}SO_4$, the SMM had a reduced sulfur content (0.04 mM K_2SO_4). Phosphoric acid was used to adjust SMM to a pH of 5.0.

All cultures were grown aerobically in a rotary shaker at 37°C at 180 rpm. Growth of cultures in either AC broth or SMM was monitored spectrophotometrically at 580 nm. The pH of the culture medium was analyzed by periodically removing a 1-ml sample and measuring the pH with a model 701A Orion Research pH meter with a Corning semi-micro combination electrode.

Growth of *E. coli* and *S. typhimurium* in preparation for two-dimensional gel electrophoresis. For experiments with *E. coli* in complex medium (AC broth), cells were grown in 50-ml cultures under the following six conditions: (i) to early log phase, at an optical density (OD) of 0.15 (pH 6.8), referred to as the control; (ii) to late log-early stationary phase until the pH of the culture medium declined to 5.0; (iii) in a cell-free filtrate of the culture described in (ii), referred to as the low-pH filtrate; (iv) in a filtrate of the culture described in (ii) in which the pH was adjusted to 6.8 with saturated KOH, referred to as the neutralized low-pH filtrate; (v) to late log-early stationary phase in a culture maintained at pH 6.8 by continuous administration of saturated KOH (this culture was grown to the same density as

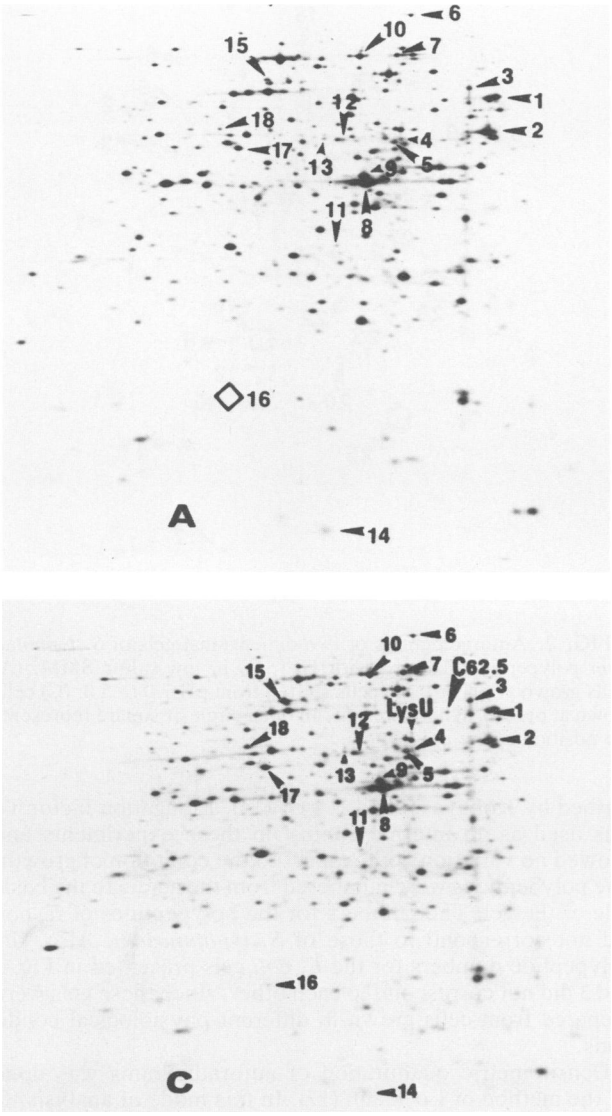


FIG. 1. Autoradiograms of two-dimensional gels of *E. coli* polypeptides labeled with $H_2{}^{35}SO_4$ in low-sulfur SMM. (A) Cells grown at pH 7.0; (B) cells shifted from pH 7.0 to 5.0; (C) cells grown at pH 5.0.

was the culture described in (ii); and (vi) in a cell-free filtrate of the culture described in (v).

Filtrates were prepared from the AC broth cultures by filtering the cells through a sterile 115-ml Nalgene filter-membrane unit with a 0.2-μm pore size. Filtrates were inoculated with *E. coli* from SMM cultures grown to log phase (OD, 0.15 to 0.20) (pH 7.0) and diluted so that the initial OD in the broth filtrate was 0.025 to 0.04. The cells were then grown for one to three doublings. The filtrates were supplemented with glucose to a concentration of 0.4% (vol/vol).

For radiolabeling proteins with $H_2{}^{35}SO_4$, cultures of *E. coli* or *S. typhimurium* were grown in SMM having a reduced sulfur concentration (0.04 mM K_2SO_4) and 30 μCi of isotope per ml. Methionine and cysteine were omitted from the medium so that incorporation of the ^{35}S into these amino acids would not be repressed. Duplicate sets of cultures growing for several generations at an external pH (pH_o) of 7.0 or 5.0 were radiolabeled for one to two cell doublings. These cells were harvested to log phase at an OD of 0.1 to 0.15. For cultures which were shifted from pH_o 7.0 to 5.0, the cells were grown to an OD of 0.1 in low sulfur SMM (pH 7.0), centrifuged, and suspended in the same medium but at a pH_o of 5.0. The radiolabel was added, and the cells were incubated for 20 min, at which point the cells were harvested.

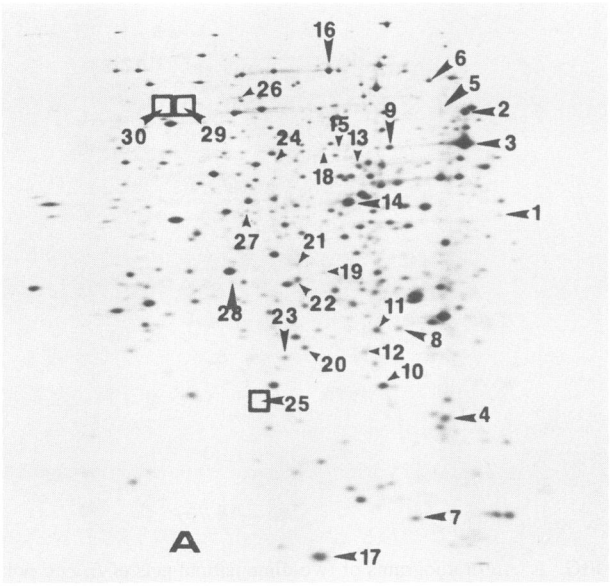
Two-dimensional gel electrophoresis. Cell extracts for two-dimensional gel electrophoresis were prepared by sonic disruption with a Branson Sonifier Cell Disrupter (model 185). All cultures were disrupted in a sonication buffer consisting of 0.1 M Tris (pH 7.3) and 0.01 M $MgCl_2$. Cells obtained from AC broth cultures were disrupted in 1.2 ml of sonication buffer by the method of Hirshfield et al. (11). Radiolabeled cells were disrupted by sonication in a volume

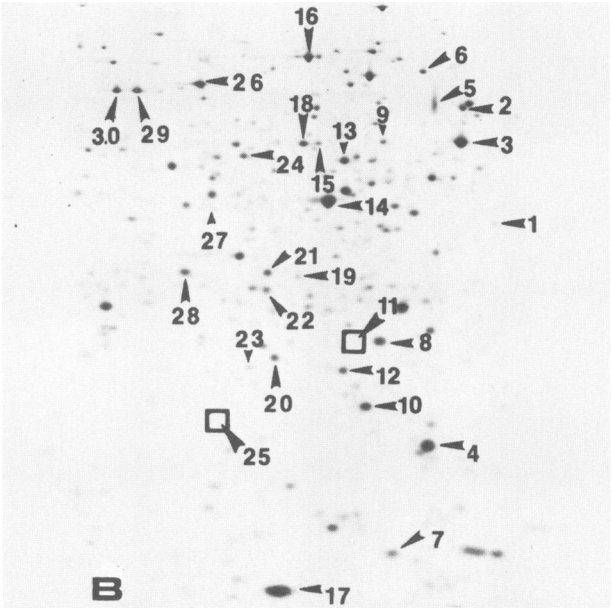
TABLE 1. Polypeptides induced in *E. coli* upon shifting cells from pH_o 7.0 to 5.0 or upon long-term growth at pH_o 5.0 in SMM

Polypeptide[a] no.	Induction in shifted cultures (pH 7.0 to 5.0)[b]	Induction in long-term cultures (pH 5.0)[b]
3	2.8	2.6
4	2.9	1.0
5	2.4	1.7
6	1.5	1.3
7	1.5	2.0
9	1.5	1.9
10	3.3	3.3
11	2.6	2.1
12	2.4	1.4
13	4.1	1.8
14	2.4	0.8
15	1.3	3.6
16	1.0	2.3
17	1.6	1.9
18	1.5	2.4

[a] The numbers of the polypeptides correspond to those on the gels (Fig. 1) and are presented sequentially from the acidic to the basic side of the gel. Polypeptides 1, 2, and 8 were omitted from Table 1 since they showed no induction under either condition.

[b] The induction values represent the quantity of each polypeptide relative to the control (cells grown at pH 7.0 in SMM). Each value represents an average of two trials; the maximum variance from the average was ±0.3.





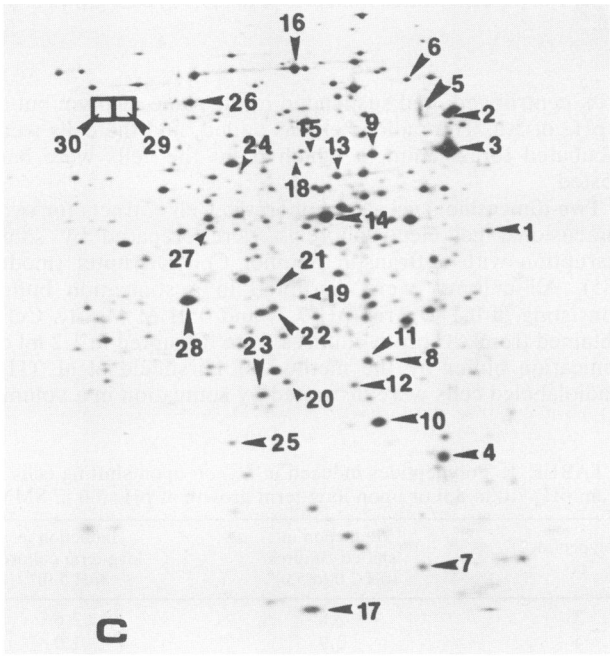


FIG. 2. Autoradiograms of two-dimensional gels of *S. typhimurium* polypeptides labeled with $H_2{}^{35}SO_4$ in low-sulfur SMM. (A) Cells grown at pH 7.0; (B) cells shifted from pH 7.0 to 5.0; (C) cells grown at pH 5.0. Where present, an open circle or square represents the position of the polypeptide.

of 0.25 ml of buffer contained in a 1.5-ml Beckman microfuge tube. The sonication was performed at a low intensity for three or four 15-s bursts, with cooling in an ice-water bath between bursts.

Protein was determined by the method of Lowry et al. (18) with crystalline bovine serum albumin as the standard. For gels of extracts from broth-grown cells, 10 to 15 μg of protein was loaded. One to four micrograms of protein from radiolabeled cell extracts, equivalent to 1.0 to 1.5 million counts, was loaded on the gels in order to compare the extracts on a count-to-count basis. The two-dimensional gels were run by the method of O'Farrell (22).

Polypeptides on the gels were detected either by silver staining (38; AC broth extracts) or by Coomassie blue staining and autoradiography (22; radiolabeled cell extracts). The $H_2{}^{35}SO_4$-labeled polypeptides were quantitated as described by Kobayashi and Harris (14). Elongation factor Tu was used as an internal control in these experiments and showed no variation with respect to the condition of growth. The polypeptides were numbered from the acidic to the basic side of the gel. The numbers for the polypeptides of *E. coli* did not correspond to those of *S. typhimurium*. Also, the polypeptide numbers for the *E. coli* gels presented in Fig. 1 and 3 did not correspond to each other, since these gels were prepared from cells grown in different physiological conditions.

Densitometric quantitation of autoradiograms was done by the method of Lockshin (17). In this mode of analysis, G factor was used as a reference and did not vary in amount in any of the three growth conditions.

Internal pH determination. The rapid filtration technique described by Kashket (12, 13) and Rottenberg (24) was used for estimating the internal pH (pH_i). The method utilizes a double-labeling technique which employs [^{14}C]benzoic acid as the pH_i probe and [^{3}H]sorbitol as the excluded probe for the estimation of the filter-trapped label. The internal volume was estimated by the methods of Kashket (12, 13) and Rottenberg (24), as adapted from that of Stock et al. (32).

RESULTS

Pattern of $H_2{}^{35}SO_4$-labeled polypeptides in *E. coli* grown in SMM. Upon visual inspection of the autoradiograms prepared from extracts of cells shifted from pH 7.0 to 5.0, approximately 18 polypeptides appeared to be enhanced in amount. Quantitation of these polypeptides showed that 13 of the 18 polypeptides were evaluated 1.5- to 4-fold when compared to those of the pH 7.0 control (Fig. 1B compared with Fig. 1A; Table 1). The synthesis of many other polypeptides apparently declined during the shift, as judged by their intensity on the autoradiograms, a phenomenon seen upon the imposition of other environmental stresses such as

TABLE 2. Polypeptides induced in *S. typhimurium* upon shifting cells from pH_o 7.0 to 5.0 or upon long-term growth at pH_o 5.0

Polypeptide[a] no.	Induction in shifted cultures (pH 7.0 to 5.0)[b]	Induction in long-term growth (pH 5.0)[b]
1	1.0	2.1
4	3.2	2.4 (1.3, 3.5)
5	2.9	2.0 (1.3, 3.5)
6	2.2 (1)[c]	0.9 (1)[c]
7	2.2	1.6
8	3.9 (1)[c]	1.2 (1)[c]
9	0.8	2.2
10	1.9 (1)[c]	2.0 (1)[c]
11	0.6 (1)[c]	2.0 (1)[c]
12	2.0	1.3
13	2.3	1.2
15	3.3 (1)[c]	1.0 (1)[c]
16	2.2	2.4
17	2.5 (1)[c]	0.7
18	5.2 (2.9, 7.5)	1.5
19	2.0	2.7
20	2.1	1.4
21	2.6	1.6
22	1.6 (1)[c]	2.5 (1)[c]
23	1.5	2.6
24	6.4 (3.7, 9.0)	2.7 (1.5, 3.9)
25	1.3	5.1
26	3.3	2.5
27	ND[d]	4.7 (1)[c]
28	0.9 (1)[c]	2.6 (1)[c]
29	14.0	1.0
30	7.0	1.0

[a] The numbers of the polypeptides correspond to those on the gels (Fig. 2) and are numbered sequentially from the acidic to the basic side of the gel. Polypeptides 2, 3, and 14 were omitted since they showed less than a 1.5-fold increase in amount in both experimental conditions.

[b] The induction values represent the quantity of each polypeptide relative to the control (cells grown in SMM at pH 7.0). Duplicates were within ±20%, except for those values shown in the parentheses.

[c] (1), Only one trial was done.

[d] ND, Not done.

heat shock on *E. coli* (19, 20). A few of the polypeptides for which an apparent increase in the rate of synthesis was observed were identified from the *E. coli* gene-protein index (21). These were no. 3, dihydrolipoamide acetytransferase; no. 6, RNA polymerase B subunit; and no. 10, DNA polymerase I. However, major heat shock proteins, such as DnaK, GroEL, GroES, C62.5, or F84.1, were not elevated in amount. Labeling of *E. coli* at pH 5.0 during long-term growth showed that 7 of the 13 polypeptides (no. 3, 7, 9, 10, 11, 17, and 18, which were apparently induced during the pH shift), were maintained at levels either close to or higher than those observed in the shift. Polypeptides 15 and 16 were elevated only upon long-term growth at pH 5.0 (Fig. 1C; Table 1).

Pattern of $H_2{}^{35}SO_4$-labeled polypeptides in *S. typhimurium* grown in SMM. The response to the pH 7.0 to 5.0 shift was more dramatic in this organism than it was in *E. coli*. Nineteen polypeptides displayed a relative increase in amount from 2- to 14-fold compared with the pH 7.0 control (compare Fig. 2B with Fig. 2A; Table 2). During long-term growth at pH 5.0, many of the polypeptides, which are elevated during the pH shift, were maintained at levels two to five times higher than those found in the pH 7.0 control (Fig. 2C; Table 2). Five polypeptides (no. 1, 9, 11, 25, and 28) were elevated only during long-term growth at pH 5.0. There is no obvious relation based on isoelectric point or molecular weight between these five polypeptides and polypeptides 15 and 16 from *E. coli*, which exhibited similar behavior. Two other polypeptides, 22 and 23, displayed a modest increase in their relative amount during the pH shift in *S. typhimurium* but were present at a higher level upon long-term growth at pH 5.0 (Table 2).

Polypeptide patterns in *E. coli* grown in AC broth. Prior experiments in this laboratory have shown that growth of *E. coli* in a complex medium (AC broth) to late log-early stationary phase, where the external pH drops to 5.5 or less, results in the increased formation of the LysU heat shock protein (6). In the experiments presented here, *E. coli* was grown to a low pH to examine more carefully the synthesis of polypeptides under this condition. (The details of the growth conditions are described in Materials and Methods.) Also, as noted above, the polypeptide numbers on these gels did not correspond to those shown in Fig. 1. Of the 25 polypeptides that were tracked on the two-dimensional gels, 22 clearly displayed higher levels upon growth of the bacterium to late log-early stationary phase (pH 5.0) in comparison to those in the log-phase control (compare Fig. 3B to 3A). These polypeptides can be divided into two major groups. The first group, designated Group I, is comprised of five polypeptides (no. 7, 9, 11, 15, and 20), which increase in the stationary-phase culture grown to pH 5.0 and in the low-pH filtrate from this culture. Their levels were either not enhanced or were elevated less significantly in the neutralized low-pH filtrate. The levels of these polypeptides were not strongly influenced in *E. coli* grown to late log-early stationary-phase in a culture maintained at neutral pH or in the filtrate obtained from this culture. Hence, this group of five polypeptides, which includes the LysU protein (no. 7), seems to be induced optimally at a low pH in this medium.

By the two-dimensional gel analysis, there was a second group of polypeptides, designated Group II, composed of polypeptides 4, 8, 10, 12, 13, 21, and 25, which behaved similarly to the Group I polypeptides, except that their levels were as high or higher in the neutralized low-pH filtrate (Fig. 3D) than in the late log-early stationary phase culture grown to pH 5.0 or in the low-pH filtrate. These, like the Group I polypeptides, showed little if any change in the culture maintained at neutral pH or in the neutral pH filtrate. These results suggest that the apparent induction of the Group II polypeptides in the neutralized low-pH filtrate relied on the release of a factor(s) into the medium upon growth of *E. coli* to low pH_o.

Internal pH measurements of *E. coli* and *S. typhimurium* grown in SMM. The pH_i values for *E. coli* and *S. typhimurium* were assessed upon their growth at pH_o 7.0 and 5.0 and upon shifting from pH_o 7.0 to 5.0. Growth rates were also determined for both organisms under these three conditions (Table 3). The pH_i of *E. coli* was estimated to be 7.73 during growth at pH_o 7.0; it decreased to 7.28 during the pH shift and declined further to 6.75 during long-term growth at pH_o 5.0. The generation time for *E. coli* was 40 min upon growth at pH_o 7.0, 114 min during the pH shift, and 140 min upon growth at pH_o 5.0.

The pH_i of *S. typhimurium* grown at pH_o 7.0 was determined to be 7.62; this value declined to 7.15 during the pH shift and to 7.03 upon long-term growth at pH_o 5.0. The generation times for this organism were 40 min at pH_o 7.0, 103 min during the shift, and 80 min during long-term growth at pH_o 5.0 (Table 3).

The internal volume (V_i) was assessed for both organisms under the three conditions in which pH_i was measured. It was found that, at all three growth conditions, the V_i of *E. coli* was larger than that of *S. typhimurium* and that, in both

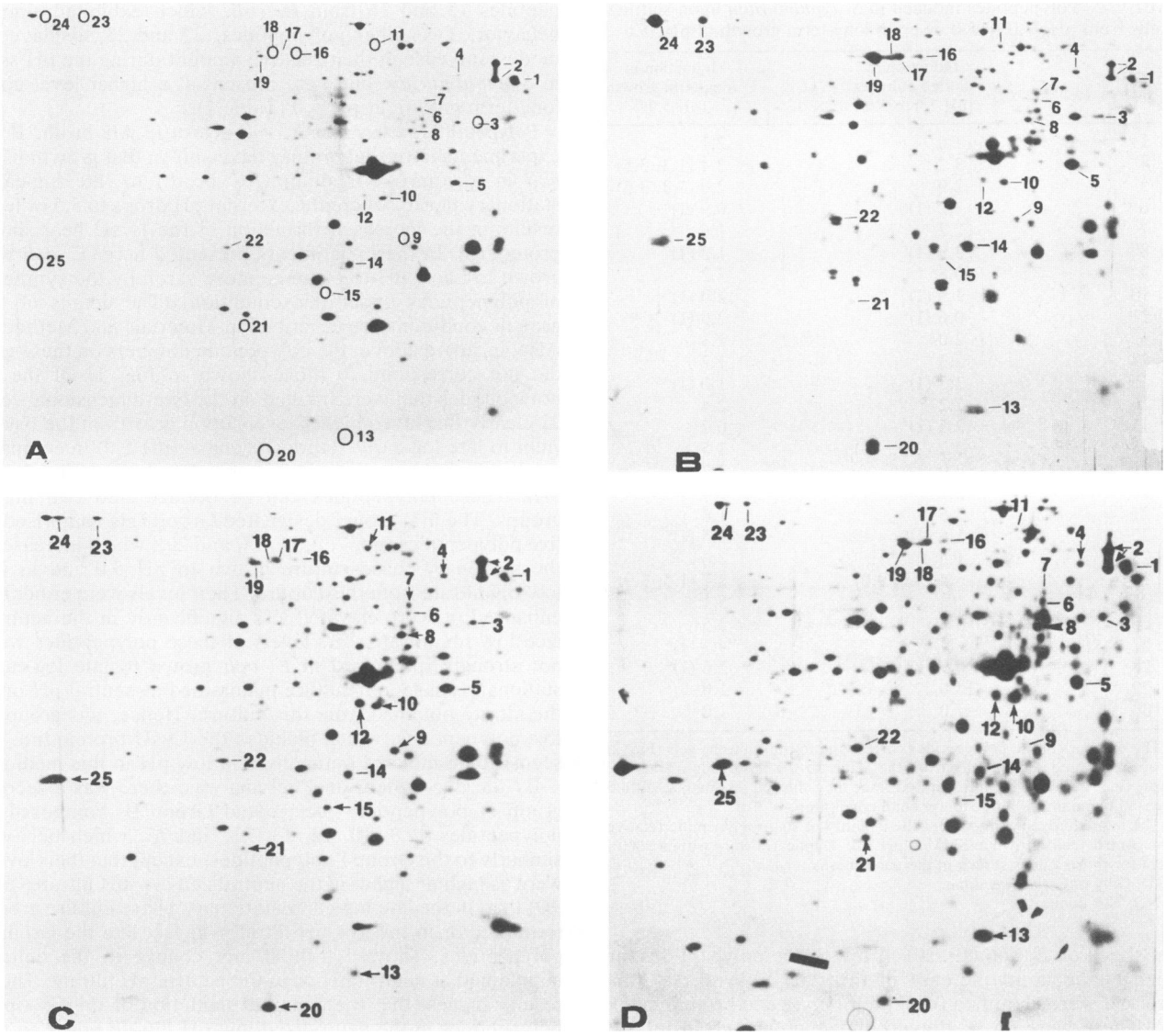


FIG. 3. Silver-stained two-dimensional gels of *E. coli* polypeptides from cells grown in AC broth. (A) Cells grown to an OD of 0.15 (pH 6.8) (the control); (B) cells grown to late log-stationary phase until the pH of the medium declined to 5.0; (C) cells grown in the low-pH filtrate; (D) cells grown in the neutralized low-pH filtrate. See Materials and Methods for a description of preparation of the filtrates. The polypeptide numbers on these gels do not correspond to those shown in Fig. 1. Where present, an open circle or square represents the position of the polypeptide.

organisms, the V_i declined as the pH_o (and pH_i) decreased (Table 3).

DISCUSSION

When both *E. coli* and *S. typhimurium* are subjected to the pH_o 7.0-to-5.0 shift in SMM, two-dimensional gel analysis indicates that not only are more polypeptides found in elevated amounts in *S. typhimurium* but also that the relative increases in the amounts of the polypeptides are higher in this organism. Consequently, it appears that *S. typhimurium* responds qualitatively and quantitatively more decisively to the pH shift than does *E. coli*. However, this conclusion should be tempered by the realization that only one time point was analyzed upon shifting and it is possible that *E. coli* might have a kinetically different response than did *S. typhimurium* to the pH shift. Nevertheless, even upon long-term growth at pH_o 5.0, *S. typhimurium* had more polypeptides which were elevated twofold or higher compared with the pH_o 7.0 control than did *E. coli* (15 compared with 7, respectively) and the polypeptides with the highest relative levels were found in *S. typhimurium*.

As a backup measure to the method of Kobayashi and Harris (14), densitometric analysis by the method of Lockshin (17) was performed directly on the autoradiograms. In general, the data obtained by the two methods are in good agreement, and all polypeptides which were shown to be elevated in amount by the technique of Kobayashi and Harris (14) were also elevated by the densitometric technique (data not shown). In addition, it is evident from examination of the autoradiograms that a select group of polypeptides are elevated in amount when the cells are subjected to low-pH stress. Thus, the low value of each set

TABLE 3. Effect of external pH (pH_o) on the growth rate and internal pH (pH_i) of *E. coli* and *S. typhimurium*

Organism	Parameters	Growth rate (min)[a]	pH_i	V_i[b]
E. coli	pH_o 7.0	40	7.73 (0.05)[b]	2.30 (0.10)[c]
	Shifted culture (pH 7.0 to 5.0)	114	7.28 (0.00)[b]	1.80 (0.10)[c]
	Steady-state culture (pH 5.0)	140	6.75 (0.05)[b]	0.90 (0.05)[c]
S. typhimurium	pH_o 7.0	40	7.62 (0.08)[d]	2.00 (0.00)
	Shifted culture (pH 7.0 to 5.0)	103	7.15 (0.01)[d]	1.00 (0.05)
	Steady-state culture (pH 5.0)	108	7.03 (0.05)[d]	0.75 (0.00)

[a] Growth rate is expressed as the doubling time in minutes.
[b] Values represent the average of duplicate trials.
[c] V_i is expressed as microliters of internal water space per milligram of dry cell weight.
[d] Values represent the average of triplicate trials.

for polypeptides 4, 5, 9, and 24 from *S. typhimurium* grown at pH_o 5.0 (Table 2) is probably the result of incomplete cutting or elution of the polypeptides from the gel or both since it is clear from the autoradiogram (Fig. 2C) that their levels have increased over those in the control (Fig. 2A). We found with *S. typhimurium* that in over 80% of the duplicate trials, the variation from the average value was within ±20% and that in half of the duplicate trials, the variation from the average value was 10% or less.

The data obtained in these experiments also indicate that, at least in *E. coli*, the polypeptide patterns obtained for growth at low pH_o are not similar to those observed for heat shock proteins. This is in agreement with the report of Taglicht et al. (34), although they tested the response of *E. coli* in a less acidic medium of pH_o 6.0.

Although the response of *E. coli* to low pH_o in SMM was apparently weak, a more definitive response was observed when this organism was grown in AC broth to late log-early stationary phase until the pH_o declined to 5.0. As noted in Results, a group of five polypeptides (Group I) was apparently optimally elevated in cells grown under this condition or grown in the filtrate from this culture. The fact that these polypeptides not only increased in amount in the stationary-phase cells, where the cell density was high, but also in cells grown in the filtrate, where the cell density was low (OD, 0.06 to 0.1), indicates that the increase in their amount is due primarily to the low external pH, rather than to a reduction in the supply of oxygen. This conclusion is reinforced by the finding that growth of *E. coli* in the neutral culture to late log-early stationary phase [the condition described in Materials and Methods in (v)] results in little or no synthesis of the Group I polypeptides. One polypeptide in this group, the inducible lysyl-tRNA synthetase (LysU protein, no. 7), and one in Group II, C62.5 (no. 4), are known heat shock proteins (19, 20). The increase in the amount of the LysU protein at low pH_o in broth appears to be considerably greater than that observed in heat shock experiments. However, in these experiments, major heat shock proteins, such as DnaK or GroEL, showed no apparent increase in their levels. The observation that the LysU and C62.5 polypeptides are elevated in amount in AC broth, but not in SMM, is analogous to recent results reported by Aliabadi et al. (2) with *S. typhimurium*. They showed that the expression of the *aniG* gene is pH_o dependent, being optimally expressed at a low pH. In addition, they showed that at a given pH_o, the expression of this gene was markedly higher in a complex, rich medium than it was in minimal medium. Slonczewski et al. (28) have isolated *E. coli* Mu d(*lacZ*) fusions in which genes termed *exa-1* and *exa-2* are induced 300-fold and 90-fold, respectively, by low pH_o in a complex, rich medium. In contrast, these genes were only weakly expressed at pH_o 7.0. The identity of the products of these genes was not reported, but the expression patterns of the Group I polypeptides identified in our work are consistent with those of *exa-1* and *exa-2*.

The results of the measurements of pH_i in our work at a pH_o of 7.0 were within the anticipated range of 7.5 to 7.8 on the basis of previous studies on *E. coli* (12, 29) and *S. typhimurium* (27). That there is a marked reduction in the growth rate of *E. coli* upon long-term growth at a pH_o of 5.0 has been demonstrated by others (4, 25). In the work of Salmond et al. (25), a decline in the pH_i to 6.8 from 7.4 resulted in a 75% reduction in the growth rate. This compares closely with our results in which the pH_i declined to 6.75 from 7.7 with a concomitant 72% reduction in the growth rate. However, Salmond et al. (25) did not measure V_i in their experiments. To our knowledge, measurement of pH_i in *S. typhimurium* at a low external pH has not previously been reported.

As discussed by Ahmed and Booth (1) and by Kroll and Booth (15), V_i measurements vary considerably, depending on conditions of cell growth. Therefore, in these experiments, V_i determinations were made at every external pH for which the pH_i was estimated. The results indicate that, in both *S. typhimurium* and *E. coli*, the V_i values decline as the pH_i decreases. A potential explanation for this observation can be gleaned from the work of Kroll and Booth (15) with *E. coli*. Their studies indicate that acidification of the cytoplasm is associated with the net loss of potassium ions and also that potassium-depleted cells have a smaller V_i than do potassium-replete cells, all other parameters being constant. Perhaps their observations could explain the finding in our work of a smaller V_i with a decrease in pH_i (a smaller V_i may correlate with a net decline in cytoplasmic potassium ion content).

The results presented in this work also indicate that at all pH_o values examined, *S. typhimurium* has a smaller V_i than does *E. coli*. Interestingly, Pritchard (23) has reported that in log-phase cultures growing at the same rate, *Salmonella* has a smaller cell size than does *E. coli*. The cell size of both organisms decreased 5- to 10-fold upon growth to stationary phase, but it was not evident whether this was due to an increased cell density or a reduction in the external pH of the medium or both. On the basis of plasmid incompatibility studies with the H incompatibility complex, it also has been concluded that *S. typhimurium* is smaller in size than most other genera of enteric bacteria (10).

The data from the pH_i, growth, and two-dimensional gel experiments, when examined collectively, suggest that *S. typhimurium* adapts better to low-pH stress than does *E.*

coli, at least in SMM. At pH_o 5.0, *S. typhimurium* exhibits faster growth and maintains pH_i at a higher level than does the *E. coli*. Moreover, *S. typhimurium* appears to have a stronger polypeptide response to low-pH stress than does *E. coli*. It is noteworthy in this respect that Spector et al. (31) have reported finding 28 heat shock polypeptides in *S. typhimurium*, whereas in *E. coli*, 17 are typically reported.

A more complete understanding of the differences in the responses of these two microorganisms to low-pH stress will depend at least in part on a better comprehension of their ecology. Both organisms can inhabit the vertebrate gut, but *E. coli* seems to have adapted more specifically to this environment than has *Salmonella* and it is in the lower intestine that *E. coli* is usually found in the greatest numbers (9). In this environment, *E. coli* would be likely to encounter a rich complex medium as the result of the feeding of the host and would be expected to ferment sugars to acid, acidifying the pH of the microenvironment. On the basis of the data presented here, this organism is equipped to react to this type of environmental flux.

On the other hand, the general consensus is that *S. typhimurium* is hardier outside of the gut than is *E. coli*, having a better survival rate in natural, nutrient-dilute surroundings (8, 35). In addition, *Salmonella* but not *E. coli*, is among the major genera of the family *Enterobacteriaceae* able to generate H_2S gas from sulfur-containing substrates, such as tetrathionate and thiosulfate, which would tend to depress the external pH of the medium in which the organism was growing (3). Perhaps, the superior ability of *S. typhimurium* to produce H_2S gas and its apparent lack of a specialized habitat are related to its better response to low-pH stress than *E. coli* in a minimal-type medium.

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