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Cell Division of *Escherichia coli*: Control by Membrane Organization

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Cells of certain strains of *Escherichia coli*, after transfer from 37 to 45 C and incubation for 16 min, were observed to swell and subsequently divide synchronously. This swelling and the resulting stretching of the membrane are proposed to be the basis for the synchronous division. Four lines of evidence support this hypothesis. First, osmotic protection by the addition of either sodium chloride or sucrose at the time of heat shock prevents both swelling and synchrony. Second, a mutant neither swelled nor divided synchronously after heat shock. Third, cells grown for several generations with 10% sucrose in the medium swelled and divided synchronously upon transfer to medium without sucrose. Fourth, the mutant not synchronized by heat shock also swelled and underwent synchronous division after the osmotic shift. A tentative model is suggested for the normal control of division, based on membrane configuration at the septation site.

The work reported here represents an attempt to elucidate a particular aspect of cell division in *Escherichia coli*. Synchronous cell division was observed to follow application of a brief heat shock to exponentially growing cells of strains B/r or 15 T^- (15). The heat shock was postulated to inactivate a protein, necessary for division, which accumulated throughout the cell cycle.

Ron and Shani found the enzyme homoserine trans-succinylase to be reversibly inactivated in strain 15 T^- at temperatures of 43 to 45 C (14). Lomnitzer and Ron suggested that the synchrony due to heat shock might result from temporary methionine starvation (11). We have been unable to repeat their results with our strains. Under our conditions, methionine does not seem to be the limiting factor for division, and so we have searched for another basis for the effects of heat shock.

We report here (taken from a Ph.D. thesis: Po C. Wu, Princeton University, Princeton, N.J., 1972) that heat shock caused the cells to swell and divide synchronously. Prevention of this swelling was accompanied by loss of the synchronous division pattern. These observations suggested that swelling might affect cell division. To test this idea, cells were caused to swell by a change in the osmotic pressure of the medium. They were observed to divide synchronously.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains of *E. coli* used in this work are listed in Table 1. The bacteria were grown aerobically in a gyratory shaker (New Brunswick Scientific Co., New Brunswick, N. J.) at 37 C in a modified M-9 medium consisting of (per liter of deionized water): 7.0 g of Na_2HPO_4, 3.0 g of KH_2PO_4, 0.5 g of NaCl, and 1.0 g of NH_4Cl. The pH was 7.1. Added after sterilization were: $MgSO_4 \cdot 7\ H_2O$ to a final concentration of 200 μg/ml, thiamine to 2 μg/ml, and glucose to 0.4%. Carbon sources other than glucose were made to 0.1% (final concentration). Amino acid supplements, when required, were added to a final concentration of 50 μg/ml, and thymidine was added to 10 μg/ml. Pennassay Broth consisting of 17.5 g of antibiotic medium no. 3 (Difco) in 1 liter of deionized water was used. Agar plates contained 15 g of agar per liter.

The basic heat shock experiment. The design of the experiment is essentially the same as that used by Smith and Pardee (15). Bacteria were grown in M-9 medium containing glucose for about 3 h until they reached a density of about 5×10^7 cells/ml. Cultures (15–20 ml) in 125-ml side-arm flasks were quickly transferred from a shaking water bath at 37 C to another one carefully maintained at 45 C. After 16 min they were returned to the 37 C water bath. Turbidity was followed in a Klett-Summerson colorimeter fitted with a blue filter. Turbidity measurements were not usually taken during the period at 45 C to prevent possible cooling.

Cell counts. Samples of 0.2 ml were taken for cell number determination by using a Petroff-Hausser counting chamber and a phase-contrast microscope.

TABLE 1. *Strains and phenotypes*

Strain	Source	Synchrony after heat shock
B/r	Laboratory collection	+
B	Laboratory collection	−
15 T⁻A⁻	Laboratory collection	+
K-12 MX74 T⁻arg1	Laboratory collection	+
K-12 (M-O)	W. A. Newton	+
K-12 M-O-3	Nitrosoguanidine mutagenesis	−
K-12 M-O-7	Nitrosoguanidine mutagenesis	+

Duplicate counts (300–500 cells per sample) were within 5 to 10%. Approximately 7% of the cells had easily visible cross walls and were scored as "twos." This end point gives the most consistent data on cell division. The Coulter electronic particle counter, model B, similarly showed synchrony, but not as clearly since it counts all particles singly. The distribution plotter attachment to the Coulter model B yields histogram graphs of particle number versus particle size. Samples were prepared for the Coulter counter in a solution of 0.9% sodium chloride and 2.5% formaldehyde, which was used as the electrolyte solution for the machine. A 30-nm aperture tube was used with an aperture current of 1/0.707. The amplification factor was 1/8.

Mutagenesis. The nitrosoguanidine mutagenesis procedure is a modification of the method of van de Putte, von Dillewijn, and Rörsch (16). A 0.2-ml sample of an overnight culture was added to 10 ml of medium M-9 containing thiamine and glucose, and the cells were grown to a density of 5×10^8 cells/ml. A 0.5-ml amount of this culture was added to 2 ml of medium M-9 containing 250 μg of *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine per ml. After 20 min of incubation at 37 C, the cells were harvested by centrifugation for 5 min at 5,000 × *g* and washed twice. The final pellet was suspended in Penassay Broth and grown overnight.

Rationale for selection. When *E. coli* B was found not to be synchronized after heat shock, an attempt was made to find nonsynchronizing mutants derived directly from strains B/r and K-12 (M-O). *E. coli* B has the unusual phenotype of growing as filaments in very rich media, especially at slightly elevated temperatures. This phenotype was used in a selection procedure. After mutagenesis, diluted cultures were grown at 42 C in Penassay Broth for 2 h. Cultures were then filtered through an 8-nm pore size membrane (Millipore Corp.). (This pore size allowed for retention of larger cells only.) After this procedure was repeated several times, the cells were plated out on Penassay Broth agar and allowed to grow overnight at 42 C. Microcolonies, as well as normal-sized cell colonies, were observed to develop. The microcolonies were then purified at 30 and 37 C and tested for various properties.

Protein and DNA assays. Incorporation of uniformly labeled ^{14}C-leucine (specific activity 10 μCi/ml) and ^{3}H-thymidine (specific activity 6.7 μCi/ml) into 5% trichloroacetic acid-insoluble material were used to measure protein and deoxyribonucleic acid (DNA) synthesis, respectively. The isotope was added at the beginning of the experiment, and 0.2-ml samples of the culture were removed at intervals to filter paper disks. The filters were soaked in 5% trichloroacetic acid and dried, and the trichloroacetic acid was removed with acetone washes. The radioactivity was counted in a liquid scintillation system [toluene-1,4-bis-2-(5-phenyloxazolyl) benzene].

Colorimetric methods used for protein and DNA determinations were those of Lowry et al. (12), and of Burton (1), respectively.

RESULTS

Effects of length of shock. One-half of a culture of *E. coli* B/r was subjected to the usual 16-min heat shock at 45 C, and the other half was heated for only 6 min (Fig. 1). Synchronous divisions resulted in both cases. The synchrony after a 6-min shock is not inconsistent with the observation that a 6-min shock on a presynchronized culture of cells late in the growth cycle did not delay the first division (15). Possibly, a brief heat shock might affect mostly younger cells in an exponentially growing culture.

Heat shocks of other durations also were tested on strain B/r. The delay before synchronous division after a shock of 10 min was found to be less than for a 16-min shock and more than for a 6-min shock. At 45 C, periods longer than 20 min did not cause cells of either strain B/r or

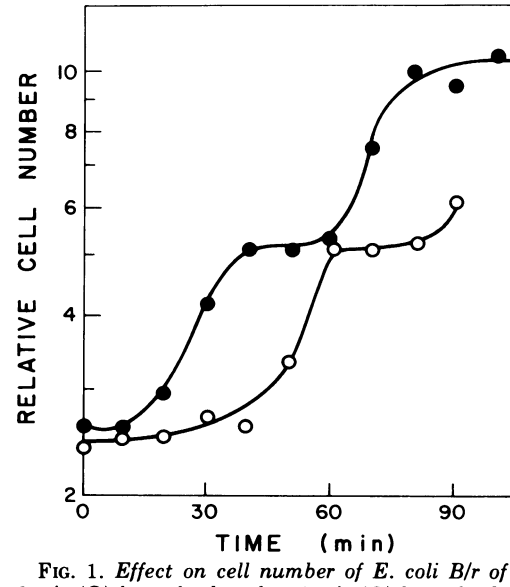

FIG. 1. *Effect on cell number of E. coli B/r of a 6-min (●) heat shock and a 16-min (○) heat shock at 45 C.*

15 T^-A^- to divide synchronously. Since DNA, protein, and ribonucleic acid syntheses are all shut off immediately when the temperature is raised to 45 C (15), the cells must have suffered damage after this treatment.

Effect of various additives. We were unable to repeat the experiments of Lomnitzer and Ron (11) on our standard B/r, 15 T^-A^-, and K-12 strains. Our strains, grown in their medium or ours, did not synchronize after heat shocks at 44 C, and addition of methionine at the time of shock had no effect. Addition of methionine at the time of a 45 C heat shock also did not abolish the synchronous divisions. Finally, temporary methionine starvation induced by a pulse of α-methyl methionine, chased after 15 min by addition of L-methionine, also did not cause synchronous division of our bacteria (Table 2, Fig. 2 and 3). We seem to be studying different effects due to different temperatures and strains.

This work suggested that addition of other substances to the growth media might alter the heat shock effect (Table 2). Very slowly growing cells, as in succinate or acetate-minimal medium, or very rapidly growing cells, as in Casamino Acids-enriched medium, did not synchronize after a 16-min heat shock at 45 C.

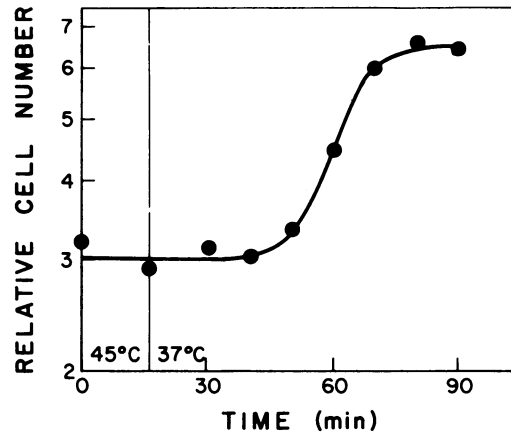

FIG. 2. *Effect on cell division of strain 15 T^-A^- of methionine (100 μg/ml) added at the initiation of a 16-min heat shock.*

TABLE 2. *Effect of various additives*

Strain	Medium	Additives[a]	Synchrony after heat shock
K-12	M-9 + acetate (0.1%)		−
	M-9 + succinate (0.1%)		−
15 T^-A^-	M-9 + glucose (0.04%)		+
	M-9 + acetate (0.1%)	Casamino Acids[b] (15 mg/ml)	−
	M-9 + succinate (0.1%)		−
	M-9 + succinate + glucose		+
	M-9 + glucose	Casamino Acids[b] (5 mg/ml)	−
	M-9 + glucose	Glycine[c], alanine, cysteine, methionine	+
	M-9 + glucose	Tryptophan, phenylalanine, histidine	+
	M-9 + glucose	Isoleucine, leucine, valine	+
	M-9 + glucose	Lysine, arginine, tyrosine, serine	+
	M-9 + glucose	Aspartate[d], glutamate, proline	−
	M-9 + glucose	Aspartate	−
	M-9 + glucose	Glutamate	−
	M-9 + glucose	Proline	−
	M-9 + glucose	Sodium Chloride (1%)	−
	M-9 + glucose	Sucrose (10%)	−
	M-9 + glucose	c-AMP[e] (10^{-2} M)	−
	M-9 + glucose	Palmitic acid (1%)	−
	M-9 + glucose	Putrescine (0.5 mg/ml)	+

[a] No changes were made in the growth medium other than these additives, and the additive was not removed after the heat shock.

[b] Also no synchrony was observed when added after heat shock.

[c] Concentrations of amino acids as in 5% Casamino Acids.

[d] Significant results were tested also on B/r.

[e] Cyclic 3′,5′-adenosine monophosphate.

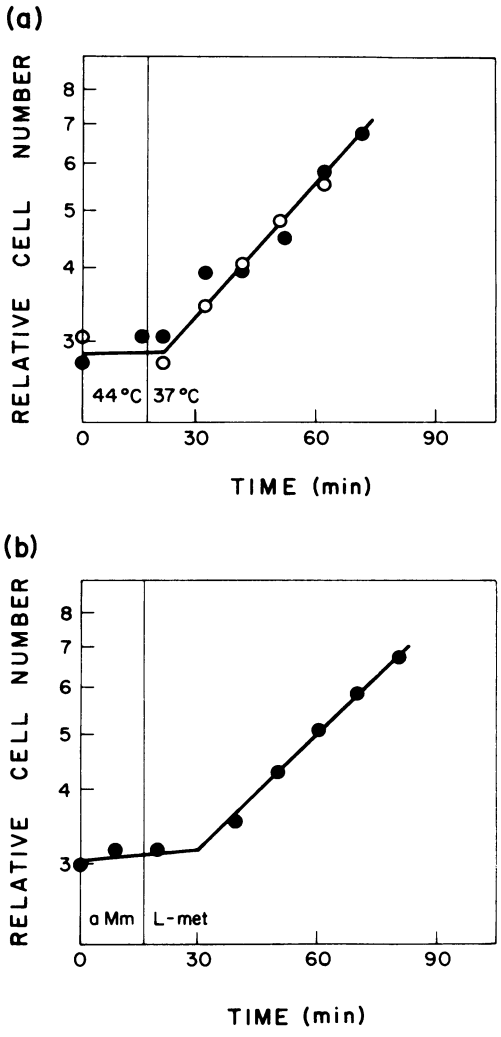

FIG. 3. *Cell division of E. coli 15T⁻A⁻. a, Effect of a 16-min heat shock at 44 C, with* (●) *or without* (○) *addition of 100 μg of* L*-methionine per ml at the time of shock. b, Effect of 16 min of methionine starvation created by addition of 2.5 × 10⁻⁵ M* DL*-α-methylmethionine followed by a chase of 100 μg of* L*-methionine per ml.*

Among the amino acids, aspartic acid, glutamic acid, and proline, even added separately, were able to abolish synchrony when added at the time of shock.

A number of other compounds were added to see whether they would affect division. Addition of 1% sodium chloride or 10% sucrose to increase the osmotic pressure of the growth medium has been found to reverse the temperature-sensitive DNA phenotypes (7) and to abolish the heat shock synchrony (Fig. 4). Cyclic 3′,5′-adenosine monophosphate (10^{-2} M) and 1% palmitic acid, added separately, both abolished synchrony. On the other hand, putrescine had no effect here, unlike in the experiments of Inouye and Pardee (10).

Effect of heat shock on different strains of E. coli. Several other strains in the laboratory collection were treated for their response to the heat shock treatment: 15 T⁻A⁻, B, K-12 (M-O), K-12 (MX74 T2 arg1), and some mutants derived from these strains by nitrosoguanidine mutagenesis (Table 1). Not all of these strains were synchronized as a result of the heat shock. Strain B, in particular, showed very poor synchrony.

Mutants derived as described in Materials and Methods, from M-O, a K-12 strain, were found to have different phenotypes One, M-O-3, was not synchronized after heat shock. Another, M-O-7, was capable of growing at 45 C but was synchronized after the brief heat shock as well (Fig. 5). The delay before division was particularly short with this mutant.

As an independent test of the synchrony with mutant M-O-7, the percentage of double cells showed a peak that corresponded nicely with the time at which the cells doubled in number (Fig. 6). On the other hand, strain M-O-3, which did not show synchronous division, also did not show significant variation in the percentage of double cells.

Effect of heat shock on DNA synthesis. No incorporation of ^{3}H-thymidine into cold trichloroacetic acid-precipitable material occurred at 45 C during the course of a heat shock experiment on either strain B/r or M-O-3, despite the difference in the division phenotype. On the other hand, the mutant M-O-7, resistant

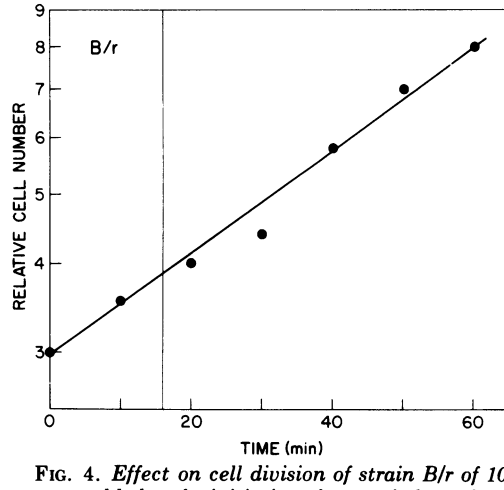

FIG. 4. *Effect on cell division of strain B/r of 10% sucrose added at the initiation of a 16-min heat shock.*

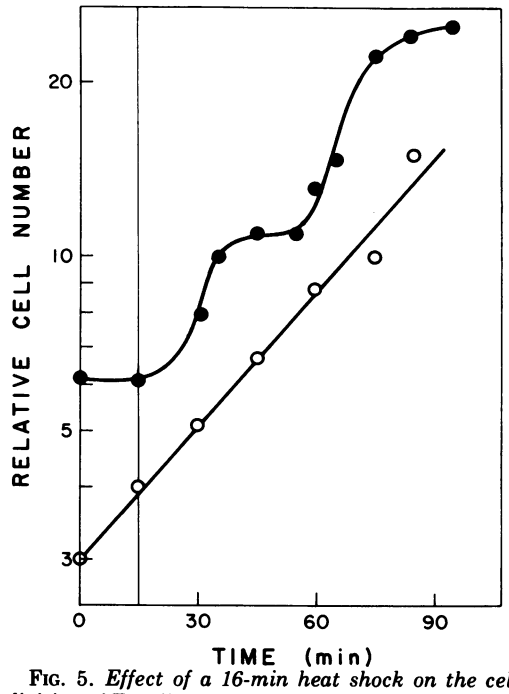

FIG. 5. *Effect of a 16-min heat shock on the cell division of E. coli K-12 strains M-O-7 (●) and M-O-3 (○).*

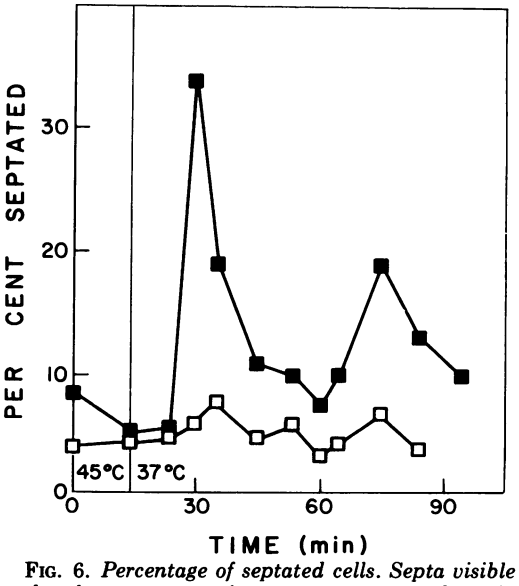

FIG. 6. *Percentage of septated cells. Septa visible under phase-contrast microscopy were counted; strain M-O-7 (■), M-O-3 (□). The total cell counts of this experiment are shown in Fig. 5.*

to 45 C, unlike the wild-type M-O, continued to synthesize DNA at 45 C as determined by the diphenylamine assay (Table 3). Thus, the effect of heat shock on cell division appears to be independent of its effect on DNA synthesis.

Effect of heat shock on protein synthesis. Incorporation of radioactive leucine into cold trichloroacetic acid-precipitable material in strains B/r and M-O-3 during heat shock nearly ceased immediately upon raising the temperature and then resumed on the return to 37 C. On the other hand, strain M-O-7, the heat-resistant strain, unlike the wild-type M-O, continued to synthesize protein normally at 45 C as determined by the Lowry assay (Table 3). Thus, although protein and DNA syntheses usually can be inhibited by the heat shock, this does not correlate with the synchronous divisions that follow.

Swelling. Heat shock caused wild-type cells to swell. The first observation was that, during the period at 45 C, the turbidity was seen to increase (Fig. 7). This increase of about 20% for the wild-type B/r could not be caused by an increase of cell number or by macromolecular

TABLE 3. *Macromolecular syntheses at 45 C*[a]

Strain	DNA synthesis	Protein synthesis	Synchrony
B/r, M-O	None[a]	None	Yes
M-O-3	None	None	No
M-O-7	Normal[b]	Normal	Yes

[a] DNA synthesis was measured as ^{3}H-thymidine uptake into cold trichloroacetic acid-precipitable material. Protein synthesis was measured as ^{14}C-leucine incorporation into cold trichloroacetic acid-precipitable material.

[b] DNA synthesis in this strain was measured by the diphenylamine assay (17), and protein synthesis by the Lowry (Folin) assay (16).

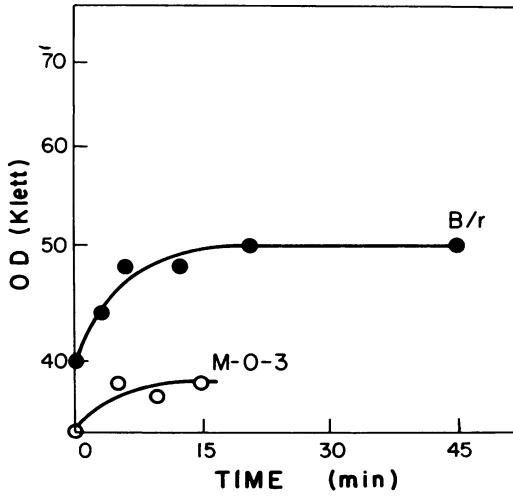

FIG. 7. *Optical density (Klett) (OD) change in strains B/r and M-O-3 on a shift from 37 to 45 C.*

syntheses, all of which are blocked at 45 C, and so is most likely due to a size increase.

As an independent, quantitative measure of the swelling, size distributions of the cells were measured by using a Coulter counter model B with a distribution plotter attachment. A definite shift in size after heat shock was observed in strain B/r (Fig. 8). Figure 9 shows the percent increase in the average volume per sample during and after heat shock in the presence and absence of 10% sucrose. Clearly, heat shock caused a large (14%) increase in volume; this could be partially counteracted by the addition of sucrose at the time of heat shock. Thus, prevention of swelling by the addition of sucrose or sodium chloride is seen to correlate with prevention of the synchronous divisions after heat shock (Table 2 and Fig. 4). Other tests for swelling, as by photomicrography, would be hard to interpret because of the variable sizes of the cells and the small size change.

In striking contrast, the non-heat-synchronizable strain, M-O-3, was found not to swell if heated for 16 min at 45 C; the size distributions were identical before and after heat shock was administered to M-O-3 (Fig. 10). Turbidity measurements of strain M-O-3 showed an insignificant increase of 5 to 7% during the period at 45 C (Fig. 7). Thus, again, when the cells did not swell as a result of heating, the division pattern was hardly disturbed.

As a final test of this hypothesis a means was sought whereby cells could be caused to swell by some agent other than heat. Cells were grown with 10% sucrose in M-9-glucose for several hours and then were harvested by either centrifugation or membrane filtration. They were suspended in either fresh or preconditioned glucose M-9 medium and allowed to grow with no change in temperature (37 C). The result confirmed the hypothesis. The cells of strain B/r swelled rapidly after the resuspension, stopped dividing, and then, after a delay, divided synchronously (Fig. 11 and 12). Readdition of sucrose (to 10%) 16 min after this sucrose osmotic shock merely increased the division delay slightly; synchronous divisions could still

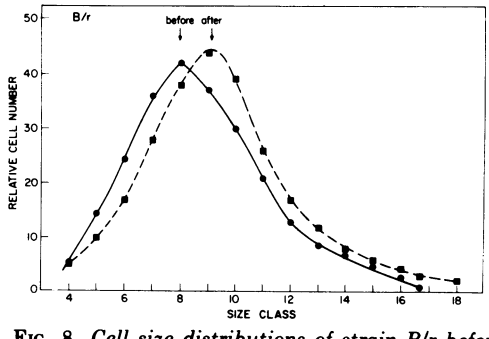

FIG. 8. *Cell size distributions of strain B/r before and immediately following heat shock. Measurements were made with a Coulter counter size-distribution plotter.*

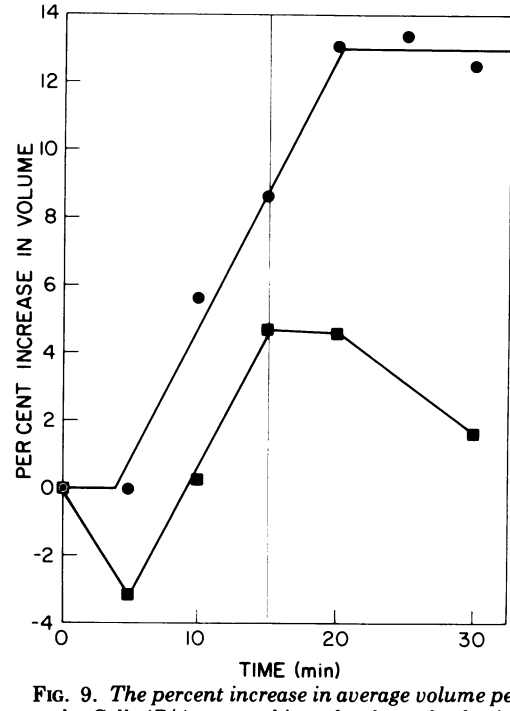

FIG. 9. *The percent increase in average volume per sample. Cells (B/r) were subjected to heat shock with (■) or without (●) the addition of sucrose at the time of shock.*

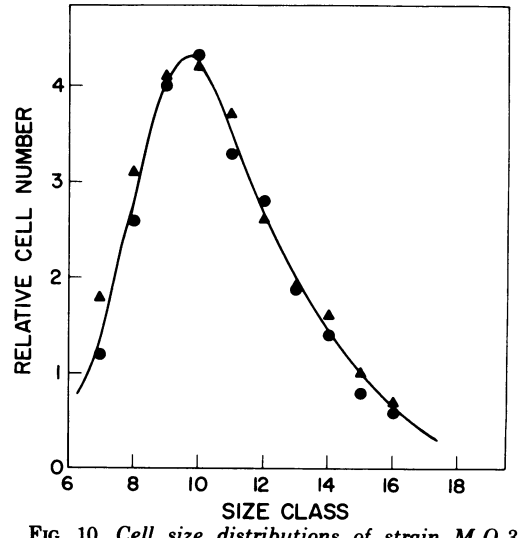

FIG. 10. *Cell size distributions of strain M-O-3 before (▲) and after (●) heat shock, as in Fig. 8.*

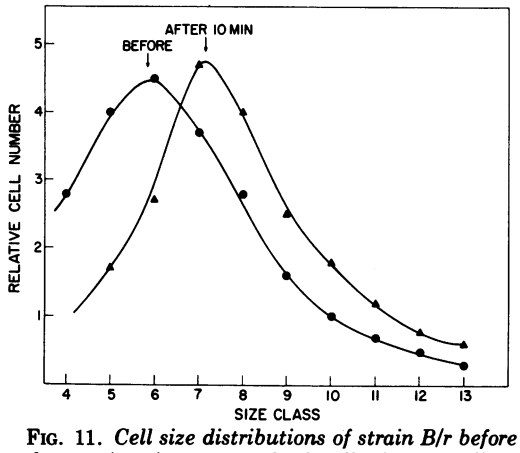

FIG. 11. *Cell size distributions of strain B/r before and 10 min after removal of cells from medium containing 10% sucrose to medium without sucrose.*

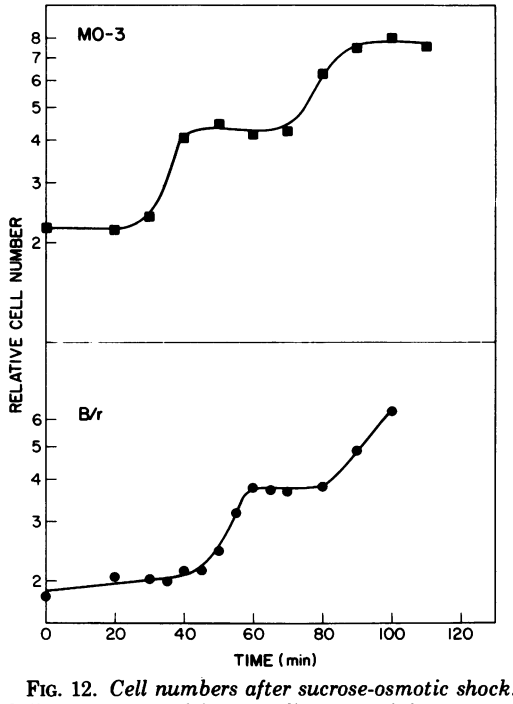

FIG. 12. *Cell numbers after sucrose-osmotic shock. Cells were removed from medium containing 10% sucrose at time zero.*

be observed (Fig. 13). Clearly, the important event was the swelling (following removal of sucrose) and not a shrinkage during recovery.

Finally, when the non-heat-synchronizable strain M-O-3 was tested by this method, it also was found to swell and divide synchronously. Two waves of synchronous divisions following this sucrose-shock are shown in Fig. 12. This last test is most important for it shows that the mutational event stabilized both swelling and division against heat shock but affected neither against osmotic changes.

DISCUSSION

The results presented here suggest that a special membrane (envelope) configuration, which could be altered by swelling of the cell, might be critical for cell division. Preliminary experiments that suggested that hypothesis was supported by a variety of independent tests. Heat shock caused wild-type cells to swell and divide synchronously. Prevention of this swelling by an osmotic pressure change in the medium was accompanied by loss of synchrony. A mutant has been found which neither swelled nor divided synchronously after heat shock. Finally, both mutant and wild-type cells divided synchronously after being caused to swell by an osmotic pressure change in the medium. The simplest conclusion based on these very different kinds of results is that swelling somehow was responsible for the synchrony and was not merely coincidental.

Controls of cell division at many levels of metabolism and morphology have been suggested previously. Completion of DNA replication is normally required (2, 3, 7). At least some protein synthesis is also required (13, 15) as are the major multiply charged cations (10, 17). The environment in which the cells grow affects their metabolism and this, in turn, affects their morphology. The importance of morphology is emphasized by the work of Donachie and Begg (5) and Higgins and Shockman (8).

Swelling might cause cells to "erase" the first critical sites postulated by Donachie and Begg (5), thus effectively blocking division. Division could then occur only as the new sites for septation have matured. Cells beginning this

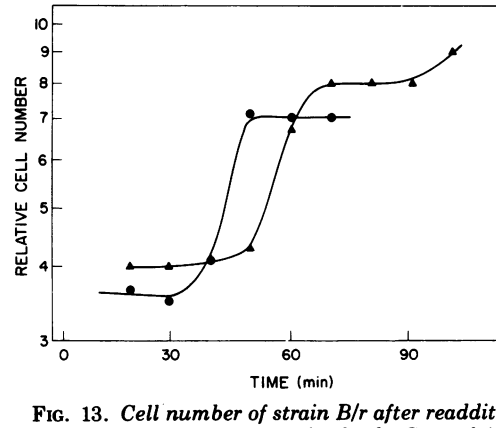

FIG. 13. *Cell number of strain B/r after readdition of sucrose after sucrose osmotic shock. Control (●); sucrose added after 16 min (▲).*

maturation process at the same time, as a result of the swelling, would then divide synchronously. One can combine these ideas and suggest that the "protein" of Smith and Pardee (15) actually represents the septum (or some part of it) and that selling might "push" the septum out to be part of the longitudinal wall membrane.

The phenotypes of the mutants M-O-3 and M-O-7 present strong arguments against the inhibition of macromolecular syntheses as a basis for the heat shock effect. Strain M-O-3, like the wild type, is observed not to synthesize DNA or protein at 45 C. Unlike the wild type, this mutant is not synchronized by the heat shock. On the other hand, strain M-O-7, unlike strain M-O-3 or the wild type, can synthesize DNA and protein normally at 45 C and yet is still synchronized by a brief heat shock. Thus, cessation of DNA or protein synthesis does not seem to be either a necessary or a sufficient condition for the heat shock synchrony. The reversal of the synchrony of the wild type by addition of sodium chloride or sucrose also supports this conclusion.

Memorcon hypothesis. We offer a speculative model of the structure of the membrane septation site to suggest a mechanism for the swelling effect on cell division. Let us assume a local "ordered" structure, a section of which is represented in Fig. 14. We will call this a memorcon: membrane-morphology-control site. The proteins and enzymes involved in septum formation are postulated to have a relatively fixed orientation to each other at this site. Thus, enzymes with related functions can be self-regulating multienzyme complexes, and enzymes may be more closely associated with substrates and products, permitting further control.

We propose configuration (i) in Fig. 14 of a cell just beginning its growth cycle. The enzymes are not in close contact and function independently to provide membrane longitudinal growth. As this extension proceeds, contact between enzymes is increased; that is, a gradual transition to configuration (ii) of the memorcon occurs. When configuration (ii) is reached, the interaction between the complexes shifts membrane synthesis in favor of septum formation. On completion of septation and cell division, the configuration returns to (i), thus defining the division cycle.

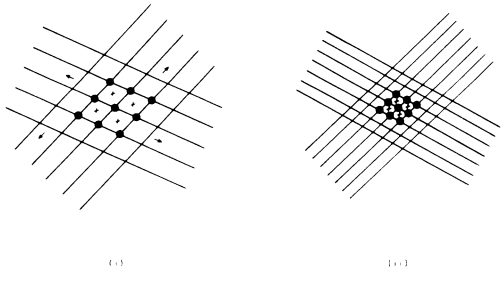

FIG. 14. *Memorcon hypothesis: proposed molecular interactions at the septum site in the membrane. Enzyme complexes (●); effectors, substrates, products, etc. (×). (i) Configuration in young cell; arrows indicate growth; (ii) configuration in old cell ready for septum formation.*

Heat shock and swelling are proposed to cause a shift in the reverse direction, from (ii) to (i), corresponding to a stretching of the membrane. The sudden, dramatic shift, apparently the same amount for cells of all ages (from the Coulter distribution plots), is much more than would occur naturally during growth; thus all cells begin, in effect, a new "division cycle" of longitudinal extension followed by septum formation and division. The resulting synchronous cell divisions could extend through another growth cycle before random fluctuations cause cells to return to exponential divisions.

Implicit in this argument (as in reference 15) is the assumption that no other component in the system, such as DNA, is limiting for division after shock. Completion of DNA replication might trigger cell division, but this model suggests a level of control that can be invoked after the triggering. In this way, synchrony of cell division need not require synchrony of chromosome replication.

The delicate mechanism proposed here is consistent with the ease of disruption of the cell division process. Virtually any metabolic imbalance could affect some component of the system. An alternative explanation for these results is that division is triggered by some cytoplasmic component which leaks out of the cell on heating or swelling. This idea is very hard to disprove. The observation that shorter heat shocks lead to shorter delays before division does not support this hypothesis, however. If less material leaked out, one would expect poorer synchrony to follow a shorter delay. Experiments designed to test for material leaking out of the cells in the various types of experiments in this study might not be conclusive because one would have difficulty identifying these components with the process of cell division.

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LITERATURE CITED

1. Burton, K. 1968. Determination of DNA concentration with diphenylamine, p. 163-166. *In* L. Grossman and K. Holdane (ed.), Methods in enzymology, vol. 12B. Academic Press Inc., New York.
2. Clark, D. J. 1968. The regulation of deoxyribonucleic acid replication and cell division in *Escherichia coli* B/r. J. Bacteriol. **96**:1214-1224.
3. Clark, D. J. 1968. The regulation of DNA replication and cell division in *E. coli* B/r. Cold Spring Harbor Symp. Quant. Biol. **33**:823-838.
4. Donachie, W. D. 1969. Control of cell division in *Escherichia coli*, experiments with thymine starvation. J. Bacteriol. **100**:260-268.
5. Donachie, W. D., and K. J. Begg. 1970. Growth of the bacterial cell. Nature (London) **227**:1220-1224.
6. Gross, J. D. 1971. DNA replication in bacteria. Curr. Top. Microbiol. Immunol. **57**:39-74.
7. Helmstetter, C. E., and O. Pierucci. 1968. Cell division during inhibition of deoxyribonucleic acid synthesis in *Escherichia coli*. J. Bacteriol. **95**:1627-1633.
8. Higgins, M. L., and G. D. Shockman. 1971. Procaryotic cell division with respect to wall and membrane. CRC Crit. Rev. Microbiol. **1**:29-72.
9. Inouye, M. 1969. Unlinking of cell division from deoxyribonucleic acid replication on a temperature-sensitive deoxyribonucleic acid synthesis mutant of *Escherichia coli*. J. Bacteriol. **99**:842-850.
10. Inouye, M., and A. B. Pardee. 1970. A requirement of polyamines for bacterial division. J. Bacteriol. **101**:770-776.
11. Lomnitzer, R., and E. Z. Ron. 1972. Synchronization of cell division in *Escherichia coli* by elevated temperatures: a reinterpretation. J. Bacteriol. **109**:1316-1318.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
13. Pierucci, O., and C. E. Helmstetter. 1969. Chromosome replication, protein synthesis and cell division in *E. Coli* B/r. Fed. Proc. **28**:1755-1770.
14. Ron, E. Z., and M. Shani. 1971. Growth rate of *Escherichia coli* at elevated temperature: reversible inhibition of homoserine trans-succinylase. J. Bacteriol. **107**:397-400.
15. Smith, H. S., and A. B. Pardee. 1970. Accumulation of a protein required for division during the cell cycle of *Escherichia coli*. J. Bacteriol. **101**:901-909.
16. Van de Putte, P., J. van Dillewijn, and A. Rorsch. 1964. The selection of mutants of *Escherichia coli* with impaired cell division at elevated temperature. Mutat. Res. **1**:121-128.
17. Webb, M. 1953. Effects of magnesium on cellular division in bacteria. Science **118**:607-611.