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Metabolic Events Occurring During Recovery from Prolonged Glucose Starvation in *Escherichia coli*

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The effects of starving *Escherichia coli* for glucose and required amino acids were determined. The disappearance of the majority of the ribosomal population, particularly of the ribonucleic acid (RNA) moiety, was noted. The events during recovery are detailed, with emphasis placed on the timing, requirements, and control of deoxyribonucleic acid, RNA, and protein synthesis and on ribosome reassembly. Finally, the applicability of the system to the study of temperature-sensitive mutants is documented and discussed.

Under certain nongrowing conditions, for example, during long-term magnesium (11) or phosphate (8) starvation, bacteria catabolize their ribosomes. This is usually reflected by a marked degradation of the ribonucleic acid (RNA) component, but not necessarily of the protein constituents.

Kinetic analysis of cells recovering from starvation has allowed insight into the sequence of synthesis of constituents of the protein-synthesizing machinery (8, 15). This communication reports a third starvation procedure, glucose starvation, which also results in the degradation of the ribosomal population.

The system was developed as a tool for the study of temperature-sensitive bacterial mutants, specifically mutants that are unable to synthesize biologically active macromolecules at high temperatures. In logarithmically growing cells, the rates of synthesis of different macromolecular species, e.g., RNA, deoxyribonucleic acid (DNA), and protein are interdependent, so that if a mutant is unable to make one species at a high temperature, the synthesis of other species is secondarily affected. This interdependence makes the classification of most macromolecular mutants difficult, if not impossible. In the study of such mutants, then, it is desirable to work under conditions where many of the control mechanisms do not operate. Cells recovering from glucose starvation satisfy this condition.

It should be remembered that what we are calling glucose starvation actually represents the withdrawal of several required amino acids as well as glucose, and that we have no clues concerning the individual role of these molecules in the events reported here.

The subsequent article (1) illustrates the use of the glucose-starvation system for screening temperature-sensitive mutants and points out additional advantages of the procedure in this respect. The mutants, in turn, have been used to shed light on the nature of the events occurring during recovery from glucose starvation.

MATERIALS AND METHODS

Bacteria and media. The bacterial strains used in this communication were *Escherichia coli* A232, *arg*$^-$, *trp*$^-$, *this*$^-$, B_1^-, and temperature-sensitive derivatives. Detailed characteristics of these strains and methods for their culture are described in the following communication (1).

Glucose starvation. Cells were grown at 25 C to late logarithmic phase, harvested by centrifugation, washed once with SC medium lacking glucose (SC-glu), and resuspended in the same medium at an optical density (660 mμ) of 0.7 to 0.9. Cultures were starved with gentle shaking for 18 hr at 30 C, and then for 2 hr at 43 C. At this point (designated hereafter as zero-time), recovery was initiated by the addition of 0.1 volume of 10-fold concentrated Nutrient Broth. Incubation was continued at 43 C with vigorous shaking.

The identity of the bacterial culture was verified by nutritional tests before starvation, after starvation, and after recovery.

β-Galactosidase assay. The method used for the determination of β-galactosidase activity is described in the following communication (1).

Macromolecule analysis—chemical assays. For the determination of DNA and RNA, 10-ml samples of recovering cultures were placed in 0.15 ml of cold 50% perchloric acid (PCA). The samples were briefly stored in an ice bath and then centrifuged. The pellets

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were stored at −20 C overnight, and were then fractioned by a modification of the Schneider procedure (14), as follows.

The pellet was resuspended in 3 ml of 0.01 M tris (hydroxymethyl)aminomethane (Tris), *p*H 7.3, and 1 ml of 1 N PCA, and was incubated at 4 C for 30 min. The particulate fraction was collected by centrifugation, resuspended in 4 ml of 75% ethyl alcohol, and incubated at 45 C for 30 min. The suspension was centrifuged, and the resulting pellet was thoroughly resuspended in 2 ml of ethyl ether and 2 ml of 75% ethyl alcohol and incubated at 45 C for 15 min. The particulate fraction was again collected by centrifugation, and excess ether and alcohol were removed from the precipitate with a stream of air.

Nucleic acids were solubilized by treatment with 4 ml of 0.5 N PCA at 70 C for 15 min. The suspension was centrifuged, the pellet was re-extracted with 3 ml of 0.5 N PCA, and the two PCA solutions were combined. All centrifugations used in this procedure were at 10,000 rev/min for 10 min in a SS34 Servall rotor.

Estimations of the amounts of DNA and RNA were made on the final PCA solution by the diphenylamine (2) and orcinol (3) procedures, respectively. Results were compared to standard curves obtained with purified *E. coli* DNA or RNA. Deoxyribonucleotides interfere with the orcinol reaction, and appropriate corrections were made to compensate for this interference.

Protein was determined by the Lowry procedure (10) on 2 ml of washed cells that had been resuspended in 1 N NaOH. Bovine serum albumin was used as a standard in this assay.

Macromolecule analysis—crude radioactivity. Crude radioactivity is defined here as that radioactivity incorporated into cellular material precipitated by cold 1% trichloroacetic acid.

Radioactive materials were added to starved cells with the Nutrient Broth. Nonradioactive uridine at 10 μg/ml was also added routinely, because the exogenous supply of this molecule is exhausted early in the recovery period. During recovery, 0.5-ml samples were removed and placed in 0.2 ml of cold 10% trichloroacetic acid. The precipitate was collected on nitrocellulose membrane filters (type B6, Schleicher & Schuell Co., Keene, N.H.), washed with cold 1% trichloroacetic acid, and dried. Radioactivity was determined by liquid scintillation counting.

Uridine-5-^{3}H (25 c/mmole), uridine-2-^{14}C (43 mc/mmole), ^{3}H-methyl-thymidine (12 c/mmole), ^{14}C-L-leucine (200 mc/mmole), ^{3}H-L-alanine (4.8 c/mmole), and ^{14}C-L-phenylalanine (355 mc/mmole), were purchased from Schwarz BioResearch, Orangeburg, N.Y. ^{14}C-L-proline (uniformly labeled, 208 mc/mmole) and a uniformly labeled ^{14}C-amino acid mixture (1 mc/0.67 mg) were products of New England Nuclear Corp., Boston, Mass.

Macromolecule analysis—purified radioactivity. Purified radioactivity is defined here as that radioactivity incorporated into DNA and RNA, specifically. Cells were labeled as in the crude radioactivity assay, and 10-ml samples were fractioned as described in the "Chemical assays" section. Radioactivity in the final PCA supernatant fraction was determined by counting 0.1 ml of the supernatant fluid in 7 ml of absolute ethyl alcohol and 8 ml of toluene-Liquiflour. Counting by this method is about 50% as efficient as counting on membrane filters.

All of the radioactivity appeared in the supernatant fraction of the final PCA step; less than 5% was found in the pellet.

Ribosome analysis. About 15 optical density units (660 mμ) of cells were made 20 μg/ml in chloramphenicol, harvested by centrifugation, and resuspended in 0.5 ml of TKCA buffer (0.01 M Tris, *p*H 7.3; 0.5 M KCl; 20 μg/ml of chloramphenicol). The suspension was then made 1 mg/ml wth lysozyme and incubated for 5 min at 30 C. The presence of magnesium should be avoided during the lysozyme treatment, and the KCl concentration should be kept high; otherwise, lysis is incomplete. Magnesium acetate was then added to a final concentration of 0.03 M, and the cells were subjected to a single freeze-thaw in a methanol-dry ice bath. Electrophoretically pure deoxyribonuclease and sodium deoxycholate (DOC) were added to final concentrations of 100 μg/ml and 0.5%, respectively, and complete lysis was achieved during incubation at 4 C for 10 min. When indicated, ribonuclease was added at 20 μg/ml prior to the 4 C incubation.

The crude lysate was clarified by centrifugation at 8,000 rev/min for 15 min in an SS34 Servall rotor unless otherwise indicated. About 2.0 optical density units (260 mμ) were obtained in the lysate for every optical density unit (660 mμ) of cells.

A 0.5-ml sample of the clarified lysate was quickly layered on a linear 10 to 30% sucrose gradient (in 0.01 M Tris, *p*H 7.3; 0.01 M magnesium acetate; 0.1 M KCl) and centrifuged at 25,000 rev/min and 5 C. When unclarified lysates were analyzed, 2 ml of saturated CsCl in 50% sucrose was included as a cushion under the normal gradient. This cushion prevents pelleting of the cell debris.

RNA analysis. RNA was purified from whole cells by phenol extraction (6) and analyzed on linear 15 to 30% sucrose gradients in 0.01 M Tris, *p*H 7.3; 0.1 M NaCl; and 0.5% sodium dodecyl sulfate. Centrifugation was carried out for 19.5 hr at 22 C and 23,000 rev/min.

RESULTS

After 20 hr of glucose starvation, the optical density at 660 mμ, the number of viable cells, and the number of total particles dropped to half of the original value. The amount of DNA per cell remained unchanged, while the amount of protein per cell and the dry weight per cell substantially increased (Table 1). Judging from the orcinol reaction, about 70% of the RNA disappeared during starvation, but analysis of the surviving RNA by sucrose density gradient centrifugation revealed that the destruction of RNA was even more severe (Fig. 1). Only 15% of the original RNA was recoverable by phenol extraction, and of this RNA 50% was of low molecular weight. The surviving high molecular weight

TABLE 1. *Effect of prolonged glucose starvation on Escherichia coli*

Determination	Amt per ml[a]		Amt per OD_{660} of culture	
	Before starvation	After starvation	Before starvation	After starvation
OD_{660}	0.669	0.309	—	—
Total no. of cells	4.7×10^8	3.2×10^8	7.1×10^8	10.1×10^8
No. of viable cells	4.9×10^8	3.2×10^8	7.4×10^8	10.3×10^8
DNA, μg	25.1	11.2	37.6	36.5
RNA, μg	46.3	7.4	69.3	23.9
Protein, μg	73.4	71.8	110	233
Dry weight, μg	193	148	289	477

[a] All values have been normalized to correspond to a culture with an optical density at 660 mμ (OD_{660}) of 0.669 before starvation and 0.309 after starvation.

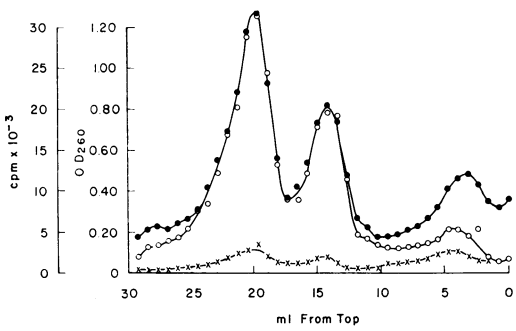

FIG. 1. *Sedimentation properties of RNA before and after starvation. Symbols:* ●, *optical density at 260 mμ of marker RNA from logarithmically growing cells;* ○, *³H-uridine radioactivity of RNA before starvation;* ×, *³H-uridine radioactivity of RNA after starvation, from an equivalent volume of culture. The RNA preparations isolated before and after starvation were centrifuged simultaneously in different tubes, and the profiles have been superimposed.*

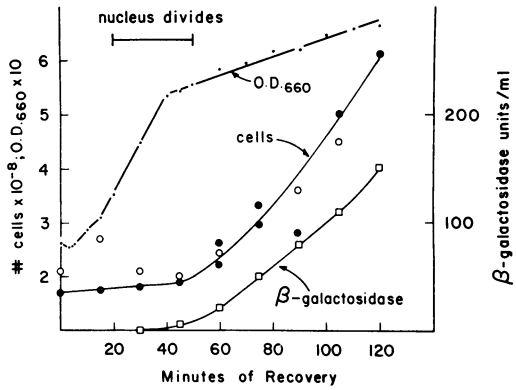

FIG. 2. *Changes in optical density, number of cells, and β-galactosidase in recovering cells. Symbols:* ●, *number of cells per milliliter observed with a Petroff-Hausser counting chamber under a light microscope;* ○, *number of viable cells per milliliter as colony-forming units.*

RNA had sedimentation values of 23 and 16*S* and occurred in the 2:1 ratio characteristic of ribosomal RNA.

The surviving RNA was tightly bound to the cell membrane and could not be released by either DOC or ribonuclease. Concomitant with the destruction of RNA was a loss of at least 95% of the *detectable* ribosomes (*see* Fig. 15 and 16).

The remainder of this communication is concerned with the nature of the events occurring during recovery from glucose starvation.

Biological events. The optical density of a recovering culture increased rapidly for the first 40 to 50 min, then proceeded at a slower linear rate (Fig. 2). This response was paralleled by the increase in dry weight (*see* Fig. 5). No cell division occurred for the first 40 to 50 min, and the increase in optical density at 660 mμ during this period probably reflects cell enlargement and the repair of structural elements. The time of change in cell number agreed well with the time of "nuclear" division observed under the microscope with cells treated with polyvinylpyrrolidone (5). Starved cells were uniformly small and contained two nuclear bodies. For the first 20 min of recovery, the cells increased considerably in size, but still contained only two nuclear bodies per cell. By 35 min, half of the cell population was ready to divide; these cells were very long rods with four nuclear bodies, but lacked septa. At 48 min of recovery, the septa had formed, and 75% of the cells had four nuclei. At later times, the pattern resembled that found in logarithmically growing cells.

The slower optical density increase after 45 min resulted from a depletion of the required amino acids from the growth medium. The strain of *E. coli* used (A232) requires arginine, tryptophan, and histidine for growth, and when these amino acids were added to the recovery medium the sec-

ond optical density increase was magnified (Fig. 3). Note that the addition of these amino acids to the medium did not alter any of the early events that occurred during recovery, either with respect to rate or timing; in fact, there was still a transition in the optical density curve at 45 min. On the other hand, the late events reported here were considerably accelerated. The only possible exception was the synthesis of DNA.

The derepressed synthesis of β-galactosidase did not begin for 45 min (Fig. 2), although bulk protein synthesis was initiated much earlier (Fig. 4, 5). The length of the β-galactosidase lag did not depend on the time of addition of the inducer, on the broth concentration, or on the addition of the required amino acids to the medium. The rate of formation, but not the length of the lag, was dependent on the prior doubling of DNA. This is clearly demonstrated in Fig. 4, which shows the rate of β-galactosidase synthesis in a temperature-sensitive DNA synthesis mutant of *E. coli* (1). The rate of formation of β-galactosidase was considerably increased by the addition of the required amino acids to the medium.

Protein synthesis. Protein synthesis lagged for approximately 5 min, increased rapidly for the next 40 to 50 min, and then progressed at a slower rate. Results were the same whether protein was measured by ^{14}C-leucine incorporation or by the Lowry assay (Fig. 5). Protein synthesis

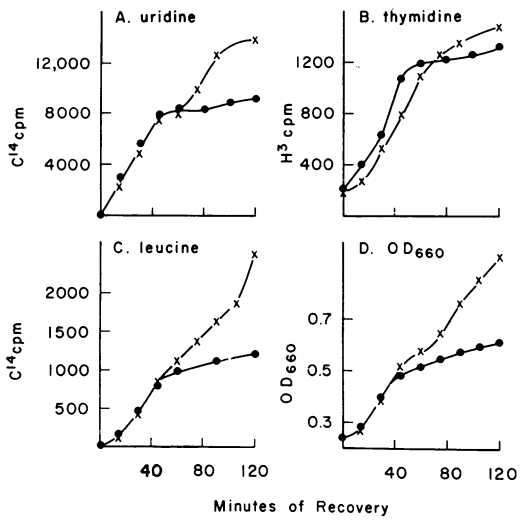

FIG. 3. *Effect of required amino acids on the events during recovery. Symbols: ●, normal recovery medium; ×, recovery medium supplemented with PATHB$_1$ (PATHB$_1$ = proline, arginine, tryptophan, and histidine at final concentrations of 20 μg/ml and vitamin B$_1$ at 1 μg/ml). Figures A, B, and C represent crude radioactivity.*

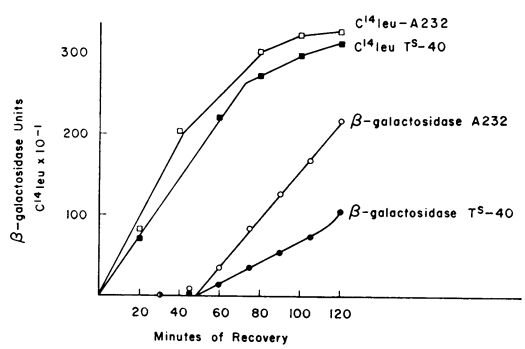

FIG. 4. *^{14}C-leucine incorporation and β-galactosidase synthesis in a temperature-sensitive DNA synthesis mutant of Escherichia coli (T^s-40). Results are expressed as the amount of crude ^{14}C-leucine incorporation in counts per minute or β-galactosidase activity per unit of optical density (660 mμ) of an induced culture at any given time (see ref. 1). The inducer isopropylthiogalactoside was added at zero-time. Note that recovery was carried out at the nonpermissive temperature (42 C).*

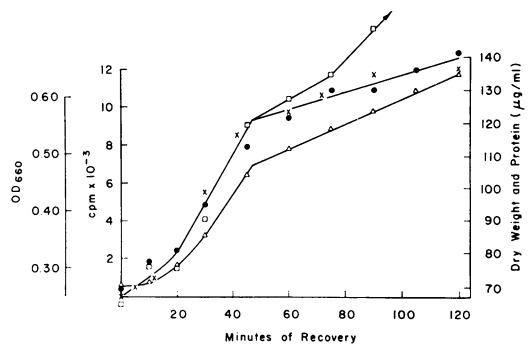

FIG. 5. *Protein and dry weight increase and ^{14}C-leucine incorporation during recovery. Symbols: ●, dry weight; ×, ^{14}C-leucine radioactivity; □, protein by the Lowry assay; △, optical density at 660 mμ.*

closely paralleled the increase in cell mass, but at no time during recovery did protein synthesis occur preferentially (Fig. 6). Because of the agreement between growth as measured by optical density and by dry weight (Fig. 5), we used optical density as a measure of cell mass. The incorporation of radioactive proline, glycine, phenylalanine, alanine, or a mixture of all amino acids followed the same pattern as the incorporation of leucine, so any one of the above could be used as a measure of protein synthesis in the recovering system.

Bulk protein synthesis did not depend on DNA synthesis (Fig. 4); however, the inability to make new RNA naturally depressed new protein synthesis. This conclusion is based on experiments

with a temperature-sensitive RNA synthesis mutant (1).

DNA synthesis. Of the three macromolecular syntheses studied, that of DNA was initiated last. Crude incorporation of ^{3}H-thymidine began within 5 min, but this radioactivity did not appear in DNA for 20 min (Fig. 7). The diphenylamine assay gave results comparable to the purified radioactivity determinations. From 20 to 45 min, DNA increased about 50%; this timing is in agreement with the time of "nuclear" division observed cytologically. Figure 6 shows that there was a preferential time during recovery for DNA synthesis and that after this time DNA synthesis was balanced with respect to cell mass increase. About 60% of the ^{3}H-thymidine incorporated by recovering cells was recoverable as purified radioactivity from 60 to 120 min of recovery.

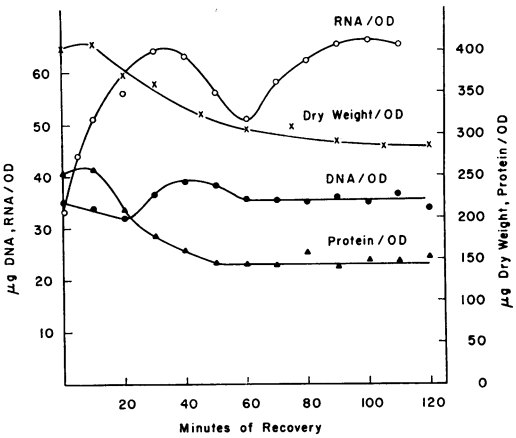

FIG. 6. *Differential dry weight, DNA, RNA, and protein changes in Escherichia coli cells recovering from glucose starvation. DNA, RNA, and protein levels were measured chemically, and the results are plotted as micrograms of material per unit of optical density at 660 mμ of the culture at each time of sampling.*

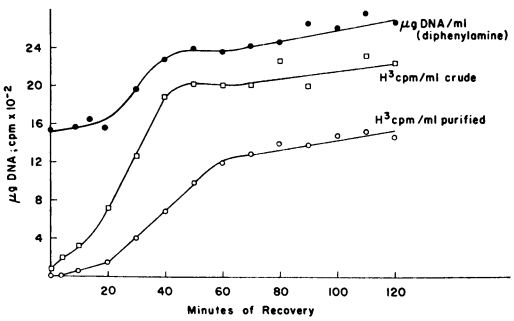

FIG. 7. *DNA synthesis and the incorporation of crude and purified thymidine radioactivity in recovering cells.*

Crude ^{3}H-thymidine incorporation by cells inhibited at zero-time with 50 μg of chloramphenicol per ml was about half normal, but the incorporation of this thymidine into DNA was virtually absent (Fig. 8). Chloramphenicol added at 12 or 26 min resulted in the cessation of thymidine incorporation after 30 min of inhibition, whereas the addition at 40 or 60 min had no detectable effect. It is evident in recovering cells that: (i) no DNA synthesis is possible when protein synthesis is inhibited by chloramphenicol at zero-time (purified counts per minute), (ii) radioactive thymidine enters molecules other than DNA (chloramphenicol at zero-time, purified versus crude counts per minute), and (iii) addition of chloramphenicol does not cause the overincorporation of ^{3}H-thymidine. The first two observations can be arrived at by additional means: (i) a temperature-sensitive protein synthesis mutant of *E. coli* incorporates crude thymidine equally well in the presence or absence of chloramphenicol, but exhibits no purified radioactivity in either case (Fig. 9A), and (ii) a temperature-sensitive DNA synthesis mutant incorporates 20% of the normal crude thymidine, but again incorporates virtually no purified radioactivity (Fig. 9B). The third observation is important because some temperature-sensitive *E. coli* mutants do overincorporate ^{3}H-thymidine during recovery (1).

RNA synthesis. During recovery, the first detectable synthesis was that of RNA. The pattern, but not the apparent timing (see Discussion), of RNA synthesis was the same whether measured by the orcinol reaction, by crude ^{3}H- or ^{14}C-uridine incorporation, or by purified radioactive uridine incorporation (Fig. 10).

Of the crude uridine incorporation, 80% was recoverable as purified radioactivity. This is the maximal recovery of RNA for our extraction conditions, as reported by Munro and Fleck (12).

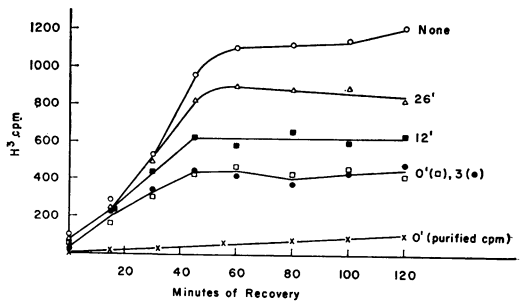

FIG. 8. *Effect of chloramphenicol inhibition on ^{3}H-thymidine incorporation in recovering cells. Chloramphenicol at 50 μg/ml final concentration was added to recovering cells at the times indicated in the figure. All curves, except the lowest, represent crude radioactivity.*

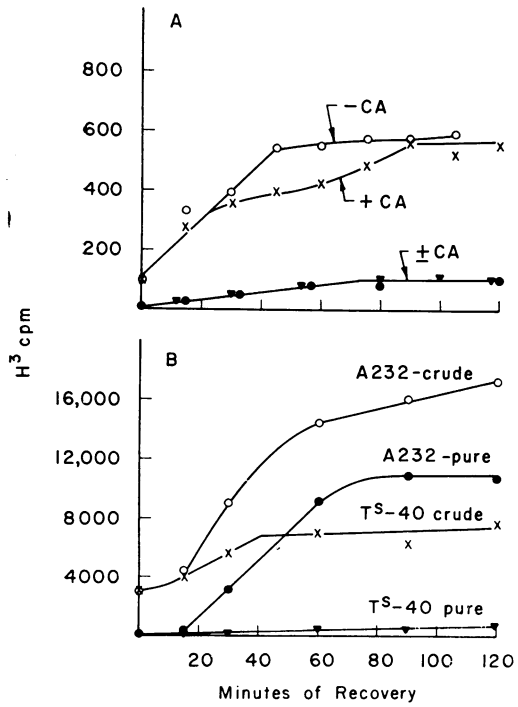

FIG. 9. *(A) Effect of chloramphenicol inhibition on ³H-thymidine incorporation in a temperature-sensitive protein synthesis mutant of Escherichia coli (Ts-68). Chloramphenicol at 50 μg/ml and ³H-thymidine at 1 μc/ml were added at zero-time. Symbols: ○, ×, crude ³H-thymidine incorporation; ●, ▲, purified ³H-thymidine incorporation. (B) ³H-thymidine incorporation in a temperature-sensitive DNA synthesis mutant of Escherichia coli (Ts-40). ³H-thymidine at 10 μc/ml was added to a recovering culture at zero-time. Recovery in experiments 9A and 9B was carried out at the nonpermissive temperature.*

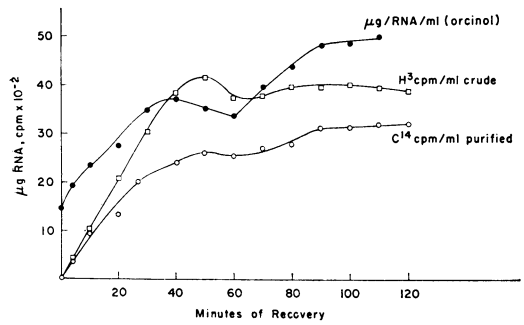

FIG. 10. *RNA synthesis and the incorporation of crude and purified uridine radioactivity in recovering cells. The ³H data were obtained from a different culture than the ¹⁴C and orcinol data. No difference was ever found between the patterns of ³H- and ¹⁴C-uridine incorporation.*

This extent of recovery supports the notion that, in recovering cells, most, if not all, of the radioactive uridine enters RNA.

Addition of chloramphenicol at the onset of recovery altered the initial rate of RNA synthesis and the final amount of RNA produced in the first round of synthesis (Fig. 11). The same result was obtained from a temperature-sensitive protein synthesis mutant (1). Addition of chloramphenicol to recovering cells at later times resulted in the overproduction of RNA.

The synthesis of RNA in *E. coli* A232 is normally under stringent control. However, after glucose starvation, this control mechanism was temporarily discarded, and during recovery the control of RNA synthesis was relaxed (Fig. 12).

Ribosome synthesis. Figure 13 demonstrates a typical polyribosome profile obtained from logarithmically growing cells lysed by the procedure described in Materials and Methods. An analysis of the amount of ribosomes present in a bacterial cell broken under our conditions is complicated by the fact that about half of the cellular ribosomes are not recovered in sucrose gradients, but remain tightly associated with the membrane fraction. Most of these ribosomes could be released from the membrane by ribonuclease (Fig. 14); low concentrations of ribonuclease released these ribosomes as small polyribosomes. A quantitative analysis of ribosomes in starved cells is further complicated by the fact that some of the ribosomes can no longer be released with ribonuclease. The following experiments, then, measure only ribosomes that could be released.

After glucose starvation, no ribosomes could be detected. They reappeared as soon as recovery started (Fig. 15, 16), and one of the first entities to appear was the 70S unit. These 70S units quickly disappeared, and then polyribosomes appeared (Fig. 15). The pool of polyribosomes reached a stable level by 30 min of recovery,

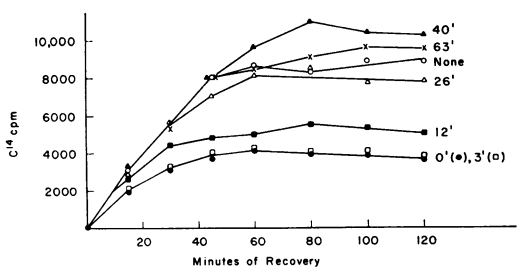

FIG. 11. *Effects of chloramphenicol inhibition on ³H-uridine incorporation during starvation. Chloramphenicol was added to recovering cells at a final concentration of 50 μg/ml at the times indicated in the figure. All curves represent crude radioactivity.*

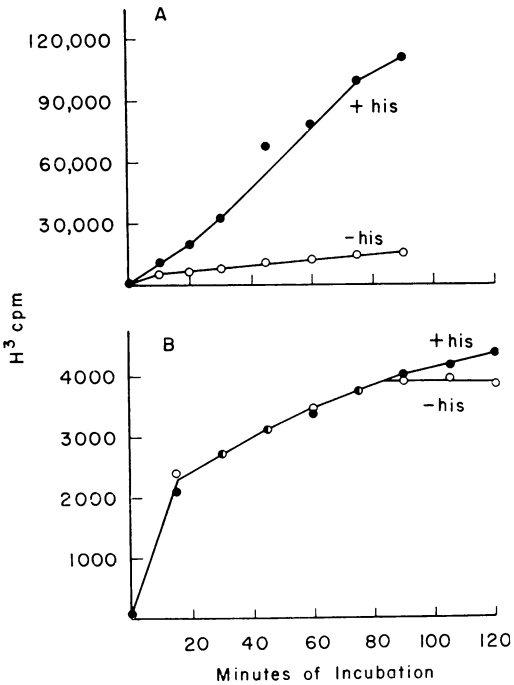

FIG. 12. *RNA control in logarithmically growing and recovering cells. (A) Cells growing logarithmically in SC basal medium were harvested, washed once with SC medium, and resuspended in the same medium containing 1 μc/ml of ³H-uridine with (●) or without (○) histidine. Crude incorporation of ³H-uridine was measured. (B) Cells were starved as described in Materials and Methods. After starvation, glucose at 2 mg/ml, ²H-uridine at 10 μg/ml, ³H-uridine at 1 μc/ml, vitamin B_1 at 1 μg/ml, and the required amino acids at 20 μg/ml, with (●) or without (○) histidine, were added, and crude uridine radioactivity was followed with time. No optical density increase was observed in either culture for the first 2 hr of recovery.*

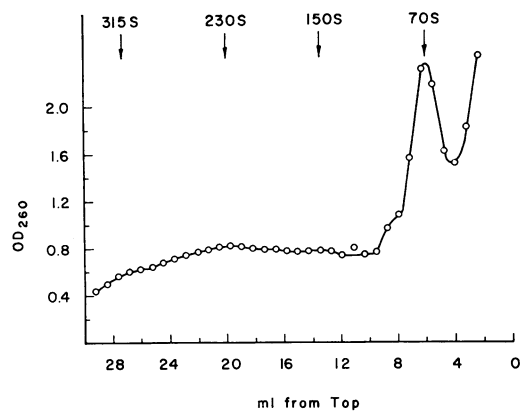

FIG. 13. *Escherichia coli polyribosomes. Polyribosomes from 22 optical density units (660 mμ) of E. coli cells growing in L broth at 37 C were isolated and then centrifuged for 120 min.*

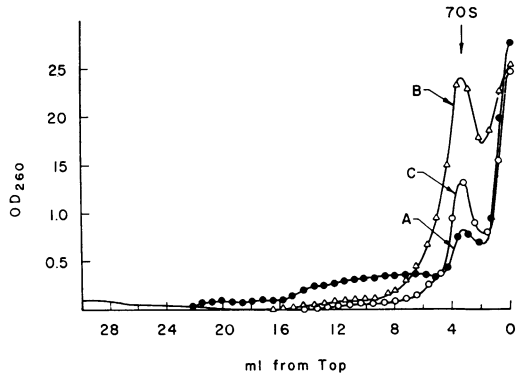

FIG. 14. *Effect of ribonuclease on the number of free ribosomes. Cells (optical density at 660 mμ of 10.5) grown in Nutrient Broth at 30 C were broken and were (A) clarified, then centrifuged; (B) treated with ribonuclease, clarified, then centrifuged; or (C) clarified, treated with ribonuclease, then centrifuged. Centrifugation was for 75 min.*

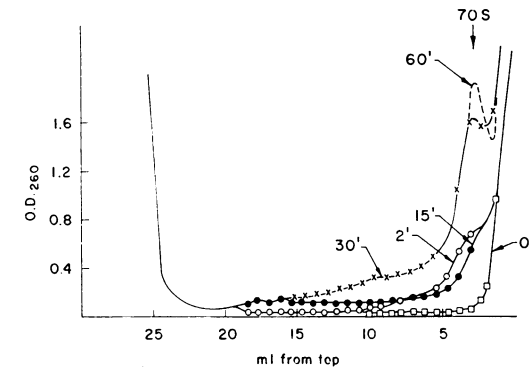

FIG. 15. *Polyribosomes and 70S ribosomes in recovering cells. Samples (27 ml) of a recovering culture were made 20 μg/ml in chloramphenicol, harvested, then processed as described in Materials and Methods. The unclarified lysate was centrifuged for 75 min.*

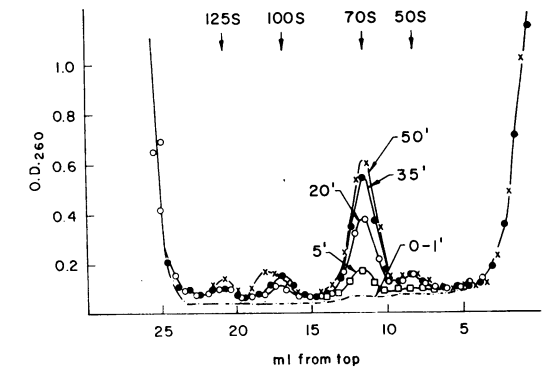

FIG. 16. *"Total" ribosomes in recovering cells. Same as in Fig. 15 except that the lysate was treated with ribonuclease and centrifuged for 3.5 hr.*

but by this time there was an abnormally large pool of 70*S* units (when compared to the log phase ratio of polyribosomes to 70*S* ribosomes). The fact that a polyribosome preparation essentially free from 70*S* units could be obtained (at T = 15 min) argues against degradation of polyribosomes during preparation and gives meaning to the appearance of 70*S* units at later times.

The profiles presented in Fig. 16 show very few 50*S* subunits and virtually no 30*S* subunits. This may not be a real indication of the state of the ribosomal population, however, since we have recently found that cells lysed by other procedures exhibit considerable quantities of both subunits.

DISCUSSION

Removal of glucose, as well as required amino acids, from the growth medium affects *E. coli* in a manner analogous to the withdrawal of magnesium (11), or phosphate (8) ions. DNA and RNA are degraded during starvation; however, the DNA complement *per viable cell* remains unchanged, whereas the RNA content per viable cell is drastically reduced. The total amount of protein appears to be conserved during starvation, resulting in a doubling of the protein content per viable cell.

Dresden and Hoagland (4) observed that polyribosomes are converted to 70*S* units during short-term glucose starvation. At first glance, it appears that the *entire* ribosomal population is destroyed during prolonged starvation, but the observations that about 15% of a prestarvation uridine label is recovered in RNA at the end of the starvation period, that this RNA has the usual properties of ribosomal RNA, and that the label is found tightly associated with the membrane indicate that some ribosomes survive the starvation procedure and that the surviving ribosomes are membrane-bound. In this connection, experiments not reported here reveal that these ribosomes exist as 30*S* and 50*S* subunits. Disappearance of the ribosomal population during magnesium or phosphate starvation has been reported (8, 11), but there is some indication that this effect may represent the depletion of glucose from the growth medium during starvation (9). The immediate tasks of the recovering cell, then, include the resynthesis of RNA, the reassembly of ribosomes, and probably the repair of the cell membrane.

The first detectable event to occur during recovery is the production of RNA. RNA synthesis, as measured by the orcinol reaction, shows two periods of synthesis, separated by what is probably a period of catabolism between 40 and 60 min. When RNA synthesis is measured by uridine incorporation, the initial burst of synthesis appears to last slightly longer—as if pre-existing RNA fragments significantly contribute to the newly made RNA and or the first RNA to be turned over is preferentially old RNA. Although the curves approach linearity near the end of the measured recovery period, it is apparent from the plot presented in Fig. 6 that RNA synthesis is unbalanced at all times. The RNA synthesized during the early period appears to be primarily ribosomal (70%) and 4*S* (30%) on the basis of sedimentation properties. That made during the second period exhibits a more heterogeneous sedimentation profile.

The initiation of RNA synthesis during recovery occurs without a detectable lag, and the chloramphenicol experiments indicate that it does not require the formation of a new protein. New protein synthesis is required, however, for the *full production* of RNA and for the *termination* of the first round of synthesis. The former protein(s) appears to be made uniformly throughout the recovery period, whereas the latter is probably not made during the first 30 min of recovery.

Though this strain of *E. coli* controls RNA synthesis stringently during logarithmic growth, control is relaxed for at least the first 40 to 50 min of recovery. This phenomenon was noted by Nakada and Marquisee (13) in cells recovering from magnesium starvation. At about the time of restitution of stringent control, the ribosomal population is approaching a balanced growth level, cell division begins, and the cells become capable of producing the derepressible enzyme, β-galactosidase.

Protein synthesis in recovering cells begins after a lag of some 5 min. Most, if not all, of this initial synthesis is dependent on the prior or concomitant production of RNA, but not DNA. The observation that the synthesis of β-galactosidase cannot commence for 45 min is analogous to observations made on magnesium-starved cells (D. Giacomoni and S. Spiegelman, *unpublished data*).

Ribosomes appear immediately during recovery and reach a near maximal level by 30 to 40 min. Estimates on the number of ribosomes present in recovering cells are complicated by the technical difficulties described in the Results section, and solutions to these difficulties are presently being sought.

Julien, Rosset, and Monier (8) reported that, in cells recovering from phosphate starvation, ribosomes (at least 30*S* and 50*S* subunits) can be assembled without concomitant protein synthesis. Assembly of ribosome precursors in the absence of protein synthesis has also been observed in recovering magnesium-starved cells (13). It is pos-

sible that the same situation occurs in cells recovering from glucose starvation, especially since total cellular protein is conserved throughout starvation.

The synthesis of DNA in recovering cells appears to be synchronous. Little synthesis is detectable by the diphenylamine assay for the first 20 min of recovery; then DNA increases by about 50%. Inhibiting cells with chloramphenicol at the onset of recovery prevents the commencement of DNA synthesis and suggests that a new protein must be synthesized to carry out this function. The involvement of an unstable protein in DNA duplication has been proposed (7).

Crude ^{3}H-thymidine incorporation into recovering cells does not solely measure the production of DNA. This effect is most serious during the first 20 min of recovery, when little or no DNA production is taking place. At times later than 60 min, 60% of the crude thymidine incorporation is recoverable as purified DNA radioactivity. If all the thymidine were entering completed DNA, we would expect 80% recovery (12). About 20% of the thymidine, then, is not recoverable as completed DNA.

Nevertheless, crude thymidine incorporation is valuable as a measure of DNA synthesis on a gross scale. The assay has been used in the recovery system to measure the relative DNA synthetic capacities of temperature-sensitive mutants of *E. coli* (1), and crude incorporation results are consistent with purified radioactivity and diphenylamine measurements (A. Jacobson and D. Gillespie, *unpublished data*).

Finally, at a point 25 min after the onset of DNA synthesis, cell division begins. All of the macromolecules studied were produced at a slower rate during the division period than before or after, and the act of division may preclude these functions. By the first division, DNA and protein have reached balanced growth levels, while the amount of RNA and the number of ribosomes is below this level. At this time, β-galactosidase (induced at the beginning of the recovery period) is made, and the predominant type of RNA being synthesized appears to be message RNA.

From this study, it is apparent that the syntheses of DNA, RNA, and protein are not as interdependent during recovery from glucose starvation as during balanced growth. This fact alone makes the study of mutants blocked in macromolecular synthesis more amenable in this system than in balanced growth. There are other obvious advantages, too. The protein-synthesizing machinery must be rebuilt after starvation, and under this condition protein synthesis mutants can be treated as auxotrophs, enabling the study of accumulated intermediates while minimizing complicating degradation reactions. Moreover, the starved cell is a bag of ribosomal proteins and it should be possible to reconstruct ribonucleoprotein particles in a cell-free system and facilitate the study of ribosome mutants.

Once the phenotype of any given mutant is determined in the starvation system and in vitro, it will be possible to assay the effect of the lesion in the same mutants undergoing balanced growth.

The following communication (1) reports the selection of temperature-sensitive *E. coli* mutants and their screening by use of the glucose-starvation system.

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