cell type which is sufficiently modified to be considered smooth muscle.

With respect to sperm transport, it is generally assumed that the mature, nonmotile spermatozoa are carried along the seminiferous tubules simply by the flow of fluid which is constantly being absorbed into the lumen (*10*). The presence of smooth muscle cells suggests that the movement of the tubule contents may not be simply a passive phenomenon, but may also be, in part, an active process involving the peritubular cells.

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**References and Notes**

1. H. Stieve, in *Von Mölendorf Handbuch der Microskopischen Anatomie des Menchen* (Julius Springer, Berlin, 1930).
2. Y. Clermont, *Exp. Cell Res.* **15**, 438 (1958); D. Lacy and J. Rotblat, *ibid.* **21**, 49 (1960).
3. M. H. Ross, *Anat. Rec.* **148**, 32 (1964).
4. E. C. Roosen-Runge, *ibid.* **109**, suppl. 153, 413 (1951); B. A. Cross, "Hypothalamic Influences on Sperm Transport in the Male and Female Genital Tract," in *Recent Progress in the Endocrinology of Reproduction*, C. W. Lloyd, Ed. (Academic Press, New York, 1958), pp. 167–176.
5. M. H. Burgos, *Compt. Rend. Soc. Biol.* **154**, 2373 (1959); C. R. Leeson, *Invest. Urol.* **3**, 498 (1966).
6. E. S. Reynolds, *J. Cell Biol.* **17**, 208 (1963).
7. R. Caesar, G. A. Edwards, H. Ruska, *J. Biophys. Biochem. Cytol.* **3**, 867 (1957).
8. D. C. Pease and S. Molinari, *J. Ultrastruct. Res.* **3**, 447 (1960).
9. C. L. Prosser, G. Burnstock, J. Kahn, *Amer. J. Physiol.* **199**, 545 (1960).
10. K. E. Mason and S. L. Shaver, *Ann. N.Y. Acad. Sci.* **55**, 585 (1952).
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## Protein and Nucleic Acid Synthesis In Escherichia Coli: Pressure and Temperature Effects

Abstract. *The incorporation of glycine-$C^{14}$, leucine-$C^{14}$, and adenine-$C^{14}$ into the respective protein and nucleic acid fractions of* Escherichia coli *K-12 is markedly affected by application of moderately high hydrostatic pressure. Pressure application may result in either stimulation or inhibition of incorporation depending on the temperature.*

The disruptive but reversible effect of high hydrostatic pressure on the integrity of intracellular gel structures has been well documented (*1*). In bacteria, pressure effects on enzyme activity (*2*), growth (*3, 4*), cell-wall synthesis (*5*), and virus replication (*6, 7*) have been investigated. Little is known, however, about the effects of pressure on protein and nucleic acid synthesis. Zimmerman (*8*) reported that DNA sythesis in sea urchin eggs is not affected at pressures up to 5000 pounds per square inch (psi; 1 psi is equal to 0.068 atm), and Pollard and Weller (*9*) have reported pressure effects on induced enzyme synthesis in *Escherichia coli*. I now report the effect of pressure and temperature variations on the synthesis of protein and nucleic acid in *E. coli*.

Cultures of *E. coli* K-12 were grown to a cell density of $2.6 \times 10^8$ per milliliter. A portion (approximately 12 ml) of the suspension was transferred to the upper compartment of a mixing-type pressure chamber (*10*). The lower compartment contained an equal volume of nutrient broth containing $C^{14}$-labeled leucine, glycine, or adenine. The appropriate pressure was applied, and the contents of the two compartments were mixed. The pressure was released after a stipulated period of time and 20 ml of the cell suspension was placed in 2 ml of cold 50 percent trichloroacetic acid. This procedure took about 40 seconds. The protein and nucleic acid fractions were then isolated (*11*). The radioactivity is recorded in counts per minute per identical sample.

Temperature control was maintained by immersing the chambers in a water bath controlled by a direct-reading thermistor. Each pressure sample had its individual atmospheric control.

The radioactive amino acids used were leucine-1-$C^{14}$ and glycine-1-$C^{14}$ (specific activity 100 $\mu$c/mg). These were diluted for use in the chamber by addition of sufficient nutrient broth to yield a final radioactivity of 0.4 $\mu$c/ml. The adenine-$C^{14}$ (specific activity 0.01 mc/mg) was similarly diluted to 0.2 $\mu$c/ml.

Counts were made on quadruplicate plates to test the possible effect of pressure on the viability of the cells. Only application of 10,000 psi (the maximum pressure used) for 25 minutes (the maximum time period) resulted in a change in viable count, and this amounted to a decrease of 5 to 8 percent. Utilizing media other than nutrient broth, other workers (*6*) have reported a greater loss in viability under these conditions of pressure, temperature, and time. The nutrient broth seemed to have a protective influence and was therefore routinely used in these experiments.

The effect of pressure on the incorporation of glycine into protein is shown in Fig. 1. At 6,000 psi and 37°C, incorporation is identical to that of controls at atmospheric pressure; at higher pressures there is an inhibition, and at lower pressure a marked stimulation occurs. The stimulatory effect of 4000 psi, and similarly the inhibitory effect of 8000 psi, occurs within the first 5 to 10 minutes of the application of pressure. After this period, the rates are identical with that of the control. In several experiments, pressure was released after 10 minutes at 4000 psi, and samples were taken at successive 5-minute intervals. The resultant incorporation curve was identical to the one shown where 4000 psi was maintained over the full period of the experiment. At 10,000 psi, after a small initial uptake, almost complete inhibition occurs.

It has been shown that a pressure of 10,000 psi effectively prevents division of *E. coli* and that release of pressure is followed by an extended lag period prior to resumption of the division process (*4*). At this pressure, protein synthesis is almost completely inhibited, and the question arose whether release

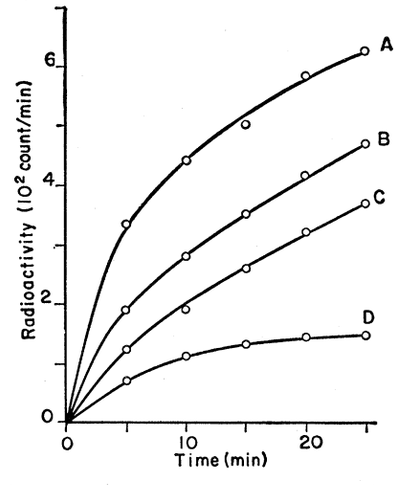


Fig. 1. Pressure effects on glycine-$C^{14}$ incorporation into protein at 37°C. A, 4000 psi; B, 6000 psi and 15 psi; C, 8000 psi; D, 10,000 psi.

of pressure would result in a similar lag. After protein synthesis is almost completely inhibited at 10 and 15 minutes subsequent to application of 10,000 psi at 37°C, release of pressure results in rapid resumption of amino acid incorporation. In fact, the incorporation rate may be somewhat greater than that found in nonpressurized specimens. Clearly then, the inhibitory effect of high pressure on protein synthesis is rapidly reversible, and it is possible that any subsequent lag in division may be due to an interference with cell-wall synthesis (*5*).

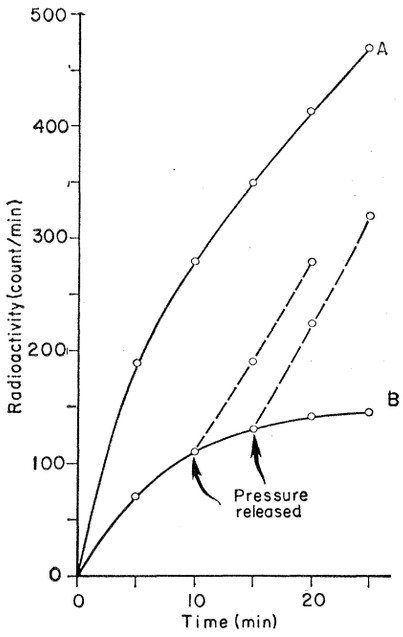


Fig. 2. The effect of release of inhibiting pressure (10,000 psi, 37°C) on glycine-$C^{14}$ incorporation. Incorporation into protein resumes rapidly. *A*, 15 psi; *B*, 10,000 psi.

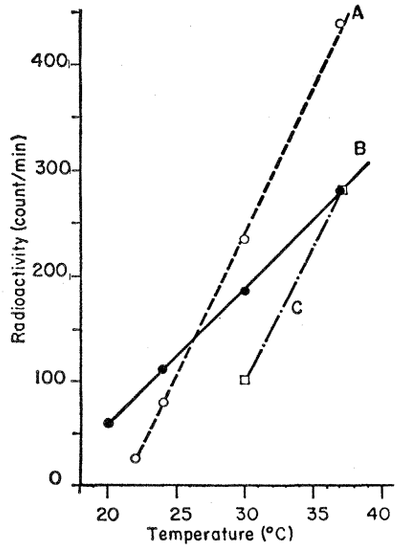


Fig. 3. Leucine-$C^{14}$ incorporation after 10-minute incubation as effected by pressure and temperature variation. A, 4000 psi; B, 15 psi; C, 6000 psi.

The effect of temperature on the incorporation of leucine-$C^{14}$ is shown in Fig. 3. A pressure of 4000 psi stimulates incorporation above 27°C and inhibits below this temperature, while 6000 psi has no effect at 37°C and inhibits at lower temperatures. This type of pressure-temperature relationship has been shown in bacterial luminescence (*12*) and cardiac-rate studies (*13*), and is probably indicative of effects on a series of different, but interrelated, biochemical events.

The incorporation of adenine-$C^{14}$ into the nucleic acid fraction of the cell (Fig. 4) is basically the same as that for amino acid incorporation into protein with, however, two important differences. Although at 37°C, 4000 psi stimulates, 6000 psi has no effect, and 10000 psi inhibits incorporation, the stimulated rate continues for at least 20 minutes; and the inhibition, while marked, is never complete. Therefore, while protein synthesis is completely inhibited after 20 minutes at 10,000 psi, nucleic acid synthesis is maintained, although at a greatly reduced rate. Pollard and Weller (*9*) report a similar effect and show that the uptake is chiefly in the synthesis of RNA.

Possibly the effect of pressure is primarily on the active transport of amino acids, but some experiments on a cell-free system indicate that direct effects on protein synthesis mechanisms occur. There is little doubt that the application of pressure can play a major role in the various enzyme-substrate relationships of the living organism. A qualitative, though perhaps over-simplified, analysis of the opposite effects of the same pressure at different temperatures would follow that given for cardiac rate (*13*).

In essence, the primary rate-controlling reaction is postulated to involve an activated enzyme. A reversible thermal inactivation of this enzyme would begin at about 25° to 27°C. Both the primary reaction and the enzyme inactivation would be inhibited by pressure application, with the latter displaying a greater sensitivity. Therefore, the application of 4000 psi at 37°C would have little repressive effect on the primary reaction and result in a stimulation by affording a greater amount of activated enzyme. On the other hand, 6000 psi might also result in a greater enzyme supply, but it may, in turn, inhibit the primary reaction to a greater extent, thus yielding no net change in the rate. Although Pollard and Weller (*9*) attribute their results on an induced enzyme system to the effect of pressure on transcription, my data is not sufficient to allow for a specific interpretation of the site of the primary pressure effect. The system is, however, amenable to further analysis.

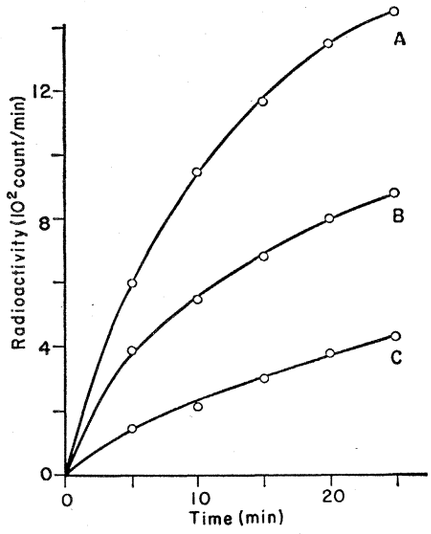


Fig. 4. Adenine-$C^{14}$ incorporation into nucleic acids at various pressures and 37°C. A, 4000 psi; B, 15 psi; C, 10,000 psi.

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**References and Notes**

1. D. A. Marsland, *Int. Rev. Cytol.* **5**, 199 (1956).
2. R. D. Haight and R. Y. Morita, *J. Bacteriol.* **83**, 112 (1962).
3. F. H. Johnson and I. Lewin, *J. Cell. Comp. Physiol.* **28**, 77 (1946).
4. C. E. Zobell and A. B. Cobet, *J. Bacteriol.* **84**, 1228 (1962).
5. L. R. Berger, *Bacteriol. Proc.* **(1959)**, 129 (1959).
6. L. Rutberg, *Acta Pathol. Microbiol. Scand.* **61**, 81 (1964).
7. L. Rutberg and C. G. Hedén, *Biochem. Biophys. Res. Commun.* **2**, 114 (1960).
8. A. M. Zimmerman, *Exp. Cell Res.* **31**, 39 (1963).
9. E. C. Pollard and P. K. Weller, *Biochim. Biophys. Acta* **112**, 573 (1966).
10. J. V. Landau and L. Thibodeau, *Exp. Cell Res.* **27**, 591 (1962).
11. G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.* **161**, 83 (1945).
12. D. E. S. Brown, F. H. Johnson, D. A. Marsland, *J. Cell. Comp. Physiol.* **20**, 151 (1942).
13. J. V. Landau and D. A. Marsland, *J. Cell. Comp. Physiol.* **40**, 367 (1952).
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