CONSERVATION OF NUCLEIC ACIDS DURING BACTERIAL GROWTH*

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The work of Schoenheimer and his collaborators (1) established that most of the constituents of non-proliferating tissues undergo continual material replacement at rates measurable in days. The rate of desoxypentose nucleic acid (DNA) replacement is notably less, however, and probably cannot be distinguished from cellular replacement in adult tissues (2, 3).

Recently, Stevens *et al.* (4) have attempted to measure the rate of replacement of DNA in actively growing tissues. Their results seem to show that the replacement in dividing cells is very fast indeed, but this conclusion must be regarded as controversial for the present (5).

Unicellular microorganisms are well suited to experimentation along these lines. Populations consisting of a single cell type can be labelled with isotope in a variety of ways, and the retention of isotope during subsequent growth in isotope-free medium can be measured under environmental conditions subject to rather complete experimental control. The multiplication itself can be measured with precision, and with assurance that all or most of the cells are multiplying.

In spite of these favorable circumstances, replacement of phosphorus (6), nitrogen (7), RNA (8), DNA (9), adaptive enzyme activity (10), or radioactivity (11) during growth of microorganisms has not been observed. Some replacement of labelled protein has been reported (12), but the replacement depended in an unexplained way on shifts from one culture medium to another, and was not correlated with growth. More important in the present connection, these experiments showed clearly that multiplication of cells labelled in arginine could occur without replacement of the labelled arginine by arginine supplied in the culture medium.

The uniformity of these results leads one to ask whether unicellular organisms, owing to their peculiar ecology, may not have developed economical ways to minimize losses from cells to medium. If this were so, replacement of atoms in a specified substance could not be detected as a net loss of isotope

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from the cells. It could, however, be detected as an intracellular shift of isotope from one substance to another, as from RNA to DNA (9) or from adenine to guanine. Such shifts should, moreover, respond in a predictable way to competitive substrates supplied in the culture medium.

The experiments described below were designed with these considerations in mind, and also differ from most previous experiments in spanning severa

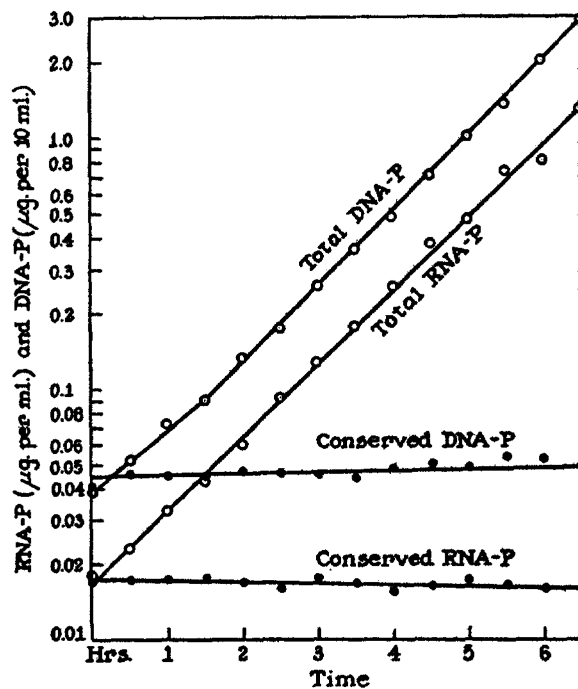

FIG. 1. Total DNA and RNA phosphorus, determined as continuously assimilated P^{32}, and conserved phosphorus, determined from a parallel culture containing only preassimilated P^{32}, observed during 6 generations of bacterial growth. The cultures contained 5×10^6 uniformly labelled cells per ml. at the start of the experiment.

generations of growth. Nevertheless, we have not detected any replacement of atoms in the nucleic acids of *Escherichia coli*.

P^{32}-labelled cells.—Fig. 1 shows that there is little or no unequal exchange of phosphorus between RNA and DNA, and no significant loss from either, during 6 generations of bacterial growth. If the phosphorus in either substance is subject to exchange with common precursors, the rates in both directions for both substances must be proportional to the net rates of synthesis, or very slow relative to these. The methods of analysis have been described earlier (13).

C^{14}-labelled cells.—Table I shows that when uniformly C^{14}-labelled cells multiply through 6 generations in the presence of any one of several nucleosides, there is no measurable change in the amounts or distribution of radioactivity among the purines or pyrimidines of the bacterial DNA. Most of these nucleosides compete effectively with glucose as a source of nucleic acid carbon, and the competition is specific as to purines and pyrimidines (14).

TABLE I

Conservation of DNA Purines and Pyrimidines

Experiment	Competitor	DNA-C^{14} per 10^{13} starting bacteria			
		Guanine	Adenine	Cytosine	Thymine
		μc.	*μc.*	*μc.*	*μc.*
1	Start	46	45	38	52
		53	48	43	52
1	Thymidine	51	44	45	51
		53	52	53	53
2	Start	54	51	45	55
		56	54	52	64
2	Desoxyadenosine	44	49	60	74
		80	56	59	77
2	Desoxyguanosine	51	55	57	63
		73	56	58	63
2	Desoxycytidine	48	54	61	65
		54	51	59	64
2	Uridine	48	54	56	73
		93	52	57	68

The cells were labelled by growth for 4 generations in C^{14}-invert sugar, 0.025 $\mu c./\mu g.$ C, washed, and allowed to grow for 30 minutes in non-radioactive invert sugar. At this time a sample containing 10^9 cells was precipitated with carrier DNA for analysis, and identical starting samples were transferred to 100 ml. of the non-radioactive medium containing 0.2 mg./ml. of competitor. Free growth continued in these for 6 generations at one generation per hour. Entire terminal cultures were fractionated, and 40 per cent of each hydrolysate was applied to a single chromatogram, which caused excessive streaking of guanine. Results are shown for duplicate chromatograms.

Thymidine is a specific precursor of DNA (15, 16). Evidently neither bacterial DNA nor RNA exchanges atoms with the simpler precursors of these substances at rates commensurate with rates of synthesis during growth.

Most of the analytical methods used here have been described previously (17). Purine and pyrimidine C^{14} was assayed after elution from the chromatograms in water, and evaporation in sample dishes. Sample thickness did not exceed 0.5 mg./cm.^{2} Contaminating radioactivity eluted from paper blanks cut from the rims of the spots varied from 17 per cent for thymine to 8 per cent for adenine. Adenosine in competition with C^{14}-sugar reduced the radio-

activity of the purines, and uridine that of the pyrimidines, to the level of the blanks. Radiographs showed that the high level contaminants were well separated from the purines and pyrimidines.

SUMMARY

In experiments of 6 hours duration, no replacement of phosphorus or purine and pyrimidine carbon in DNA, nor flow of these atoms from RNA to DNA, could be detected in rapidly growing cultures of *E. coli*. The slow replacement that has been demonstrated for many substances in non-proliferating tissues of other organisms, though it may occur also in bacteria, is not greatly accelerated under conditions of rapid cellular growth, and therefore cannot be a characteristic feature of synthetic processes.

REFERENCES

1. Schoenheimer, R., The Dynamic State of Body Constituents, Cambridge, Harvard University Press, 1942.
2. Davidson, J. N., The Biochemistry of the Nucleic Acids, New York, Wiley and Sons, 1950.
3. Hevesy, G., *Adv. Biol. and Med. Physics*, 1948, **1**, 409.
4. Stevens, C. E., Daoust, R., and Leblond, C. P., *J. Biol. Chem.*, 1953, **202**, 177.
5. Barton, A. D., *Fed. Proc.*, 1954, **13**, 422.
6. Kaudewitz, F., *Z. Naturforsch.*, 1953, **8b**, 500.
7. Abrams, R., Hammarsten, P., Reichard, P., and Sperber, E., *J. Gen. Physiol.*, 1949, **32**, 271.
8. Manson, L. A., *J. Bact.*, 1953, **66**, 703.
9. Fujisawa, Y., and Sibatani, A., *Experientia*, 1954, **10**, 178.
10. Rickenberg, H. V., Yanofsky, C., and Bonner, D. M., *J. Bact.*, 1953, **66**, 683.
11. Cohn, M., personal communication.
12. Podolsky, R. J., *Arch. Biochem. and Biophysic.*, 1953, **45**, 327.
13. Hershey, A. D., *J. Gen. Physiol.*, 1953, **37**, 1.
14. Graham, A. F., Siminovitch, L., and Lesley, S. M., data to be published.
15. Kozloff, L. M., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18**, 209.
16. Reichard, P., and Estborn, B., *J. Biol. Chem.*, 1951, **188**, 839.
17. Hershey, A. D., Dixon, J., and Chase, M., *J. Gen. Physiol.*, 1953, **36**, 777.