STUDIES ON A MUTANT OF *ESCHERICHIA COLI* WITH UNBALANCED RIBONUCLEIC ACID SYNTHESIS[1]

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In studies on radiation resistance of starved *Escherichia coli* strain K_{12} we encountered a mutant, W-6, which on methionine starvation accumulated large amounts of ribonucleic acid, without increasing its desoxyribonucleic acid or protein content (Borek *et al.*, 1955). Since this mutant seems to be the first example in which such unbalanced accumulation of only one of the three large components of the bacterial cell can be induced by withholding an essential nutrilite, it seemed of interest to study it in detail for it might serve as a useful tool in exploring some metabolic interrelationships.

Since the accumulation of ribonucleic acid has been postulated as an obligatory antecedent to protein synthesis, the metabolism of the ribonucleic acid-engorged cells was studied after the restoration of methionine, to determine when synthesis of protein is resumed. It was found that the ribonucleic acid-enriched organisms have a diminished ability to synthesize protein. They are unable to form adaptive enzymes, for at least 2 hr after the restoration of the amino acid, and, even in the synthesis of their usual proteins, these organisms are the slowest of those tested. This retarded protein synthesis is not caused by any delay in the resumption of respiration, for the oxygen consumption during the first two hours after the restoration of the lacking nutrients is no different in the glucose and methionine starved organisms.

That the inability to produce adaptive enzymes is not due merely to the temporary deprivation of the amino acid but rather to some metabolic condition in these starved cells was demonstrated by analogous studies of two other mutants. *E. coli* strain W122-33 is a methionine requiring auxotroph with a genetic block apparently similar to that of *E. coli* strain K_{12} W-6. We found that this mutant does not accumulate ribonucleic acid upon starvation of methionine, nor does it lose its ability to form adaptive enzyme, after the amino acid is restored to it. *E. coli* strain K_{12} J-2-1 is a histidine-requiring auxotroph which on starvation of the amino acid also does not accumulate ribonucleic acid, and it was found that, as the mutant above, it does not lose its ability to form adaptive enzyme at its usual rate. While these studies were in progress very interesting findings were reported by Cohen and Barner (1955) on unbalanced cytoplasmic synthesis in a thymine deficient mutant, *E. coli* strain 15T- , which on starvation of thymine accumulates both ribonucleic acid and protein. This mutant loses the ability to multiply after such unbalanced synthesis has taken place extensively even though the lacking thymine is restored to it. This finding has led these authors to postulate that "cytoplasmic growth in the absence of nuclear synthesis soon establishes a condition in which the cytoplasmic environment becomes unfavorable for cell division." We therefore tested whether unbalanced ribonucleic acid synthesis without protein synthesis also dooms the organism. We could find no evidence, significantly beyond the error of viable cell counting, that accumulation of ribonucleic acid is also lethal. Thus, the lethality of unbalanced cytoplasmic synthesis may be restricted to organisms which accumulate both protein and ribonucleic acid.

We also report here findings on the uniqueness of ribonucleic acid accumulation in *E. coli* strain K_{12}W-6 and studies on the irradiation sensitivity of such unbalanced ribonucleic acid synthesis.

MATERIALS AND METHODS

Bacterial strains. *E. coli* strain K_{12}W-6 is derived from the mutant *E. coli* strain K_{12}58-161

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which when originally isolated (Tatum, 1945) required both methionine and biotin. It has been redesignated by Dr. J. Lederberg since it has lost its biotin requirement. The genetic block in this organism is before cystathionine (Simmonds, 1948). The requirement for methionine is absolute. Neither vitamin B_{12} nor thymine, thymidine nor thymidylic acid supports the growth of the organism.

Dr. Bernard Davis kindly provided a mutant of the W strain, *E. coli* strain W122-33; which has a similar genetic block to *E. coli* strain K_{12}W-6 as far as this can be determined by the analysis of the requisite nutrilites and accumulating metabolites. But this strain of *E. coli*, while lysogenic, is not inducible by ultraviolet irradiation (Jacob, 1953, personal communication). *E. coli* strain K_{12}W677 is threonine, leucine and thiamin requiring. *E. coli* strain K_{12}J-2-6 is tryptophan requiring. *E. coli* strain K_{12}J-2-1 is histidine requiring. All of the organisms, except the first, were kindly provided by Dr. B. Davis. To eliminate back mutants, every auxotroph was reisolated from colonies originating from single cells, half of the colony being placed in unsupplemented chemically defined medium, the other half into medium enriched with the requisite nutrilite. No such precautions were necessary with *E. coli* strain K_{12}W-6 which, 10 yr after its isolation, shows no detectable tendency toward back mutation.

The medium, the method of culture of the microorganisms, the method of starvation and the analytical procedure for the assay of nucleic acids were previously described (Borek *et al.*, 1955). The accumulation of adaptive enzymes was assayed by following respiration on L-arabinose as carbon source and also by the determination of induced β-D-galactosidase by the method of Cohn and Monod (1951) using o-nitrophenyl β-D-galactoside as substrate. We are indebted for a gift of this substrate to Dr. M. Cohn of Washington University.

For the studies on the effect of irradiation on the ribonucleic acid synthesis in the starving organisms, 1-L cultures in logarithmic growth phase of about 2×10^8 cells per ml were harvested by centrifugation, were washed with synthetic medium containing glucose but no methionine and were resuspended in such medium to a concentration of about 2×10^8 cells per ml. They were immediately irradiated for various periods in 50-ml lots in an open petri dish at 1 meter distance from a 15-watt GE germicidal lamp. The irradiated organisms were then incubated aerobically in the dark without methionine and were assayed for nucleic acid content at various intervals.

For comparison, organisms in logarithmic growth phase at a concentration of 2×10^8 cells per ml were irradiated in their original medium in 50-ml lots for 150 sec. (Under these conditions over 95 per cent of the organisms were induced to form phage.) The irradiated organisms were pooled, incubated in the dark, and aliquots were removed at intervals for ribonucleic acid determination.

Total protein was assayed by the method of Lowry (1951) on washed pellets of counted, centrifuged bacteria.

RESULTS AND DISCUSSION

None of the mutants listed except *E. coli* strain K_{12}W-6 accumulated ribonucleic acid upon starvation of its requisite amino acid. Since these findings are negative, no data are presented. The disparity in ribonucleic acid accumulation between *E. coli* strain K_{12}W-6 and *E. coli* strain W122-33 upon methionine starvation was striking. This may possibly stem from differences in the two genetic blocks. For, although they accumulate the same intermediates, the mechanism of the genetic failure may not be the same. Of course, there are other differences between the two mutants, for example, while both strains are lysogenic only the K_{12} strain is inducible.

In table 1, the viability, determined as colony formers of *E. coli* strain K_{12}W-6 during 6 hr of methionine starvation and 100 min after the restoration of the amino acid, is presented. Un-

TABLE 1

The viability of Escherichia coli during 6 hr of methionine starvation and after the restoration of the amino acid

Colony formers per ml $\times 10^{-8}$

Methionine Starvation			Methionine Added to Starved Culture					
Hours			Minutes					
0	3	6	0	20	40	60	80	100
1.8	1.8	1.8	1.8	1.9	1.9	2.3	2.2	2.2

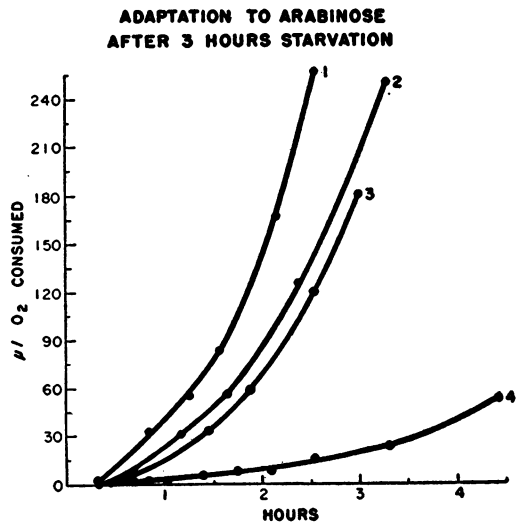

Figure 1. Respiration on L-arabinose as substrate after 3 hr of starvation of various metabolites. Curve 1, *Escherichia coli* strain K_{12} W-6 prestarved of glucose. Curve 2, *E. coli* strain W122-33 prestarved of methionine. Curve 3, *E. coli* strain K_{12}J-2-1 prestarved of histidine. Curve 4, *E. coli* strain K_{12}W-6 prestarved of methionine. All the deprived metabolites, except glucose, were restored. The cell counts at the end of the starvation were, 1.2, 1.0, 1.1, and 1.4 × 10^8 per ml respectively. The standard Warburg techniques were followed on 2-ml aliquots of bacterial culture.

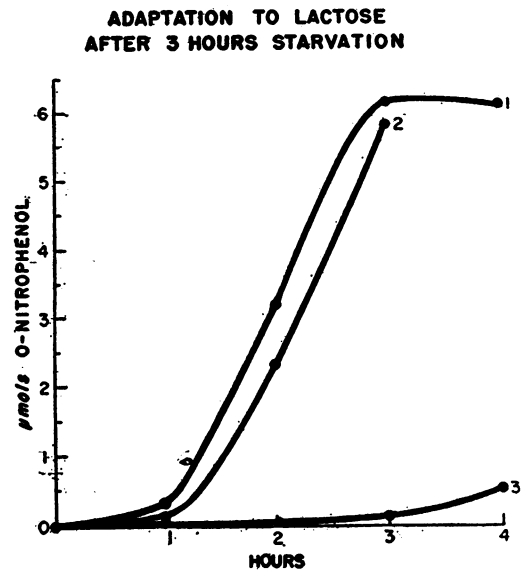

Figure 2. Induction of β-D-galactosidase after 3 hr of starvation. Curve 1, *Escherichia coli* strain K_{12}W-6 prestarved of glucose for 3 hr. Curve 2, *E. coli* strain W122-33 prestarved of methionine for 3 hr. Curve 3, *E. coli* strain K_{12}W-6 prestarved of methionine for 3 hr. After the starvation the organisms were washed free of glucose; lactose and in 2 and 3 methionine were then provided. The cell count at the start of the adaptation was 2 × 10^8 cells per ml in all cases. β-D-Galactosidase was assayed on 2-ml lysed aliquots by the addition of 0.5 ml of 0.013 M *o*-nitrophenyl-β-D-galactoside in 0.2 M phosphate buffer, pH 7 and incubation for 1 hr.

The findings with histidine starvation of *E. coli* strain K_{12}J-2-1 are not presented for it was found that this mutant, unlike its parent strain, contains β-D-galatosidase constitutively.

like the thymine deficient mutant, *E. coli* strain 15T- studied by Cohen and Barner, *E. coli* strain K_{12}W-6 remain completely viable during this period. This comparative stability probably stems from the more extensive metabolic crippling of the methionine deficient mutant. The thymine-requiring auxotroph, being able to synthesize both protein and ribonucleic acid, can begin some of the initial processes of cell division but then, blocked at some point, it is probably unable either to complete cell division or to reverse the initiated processes. That this is the condition of these cells is evidenced by the very rapid cell division that can ensue when thymine is restored to them after a propitious interval (Barner and Cohen, 1955). On the other hand, the methionine deficient auxotroph which can make no protein during the starvation probably does not initiate the processes toward division and thus retains the integrity of its structure. In contrast to *E. coli* strain 15T- no "synchronization of cell division"—as measured by an accelerated rate of cell division —could be detected by the addition of methionine at any time during the starvation of *E. coli* strain K_{12}W-6.

Figure 1 summarizes the studies on adaptation to L-arabinose after 3 hr of prestarvation by three auxotrophs. *E. coli* strain K_{12}W-6 was starved of glucose and methionine separately; *E. coli* strain W122-33 and *E. coli* strain K_{12}J-2-1 were starved of methionine and histidine, respectively. The only organism which was incapable of metabolizing L-arabinose 2 hr after the restoration of the lacking nutrient was *E. coli* strain K_{12}W-6 after methionine starvation.

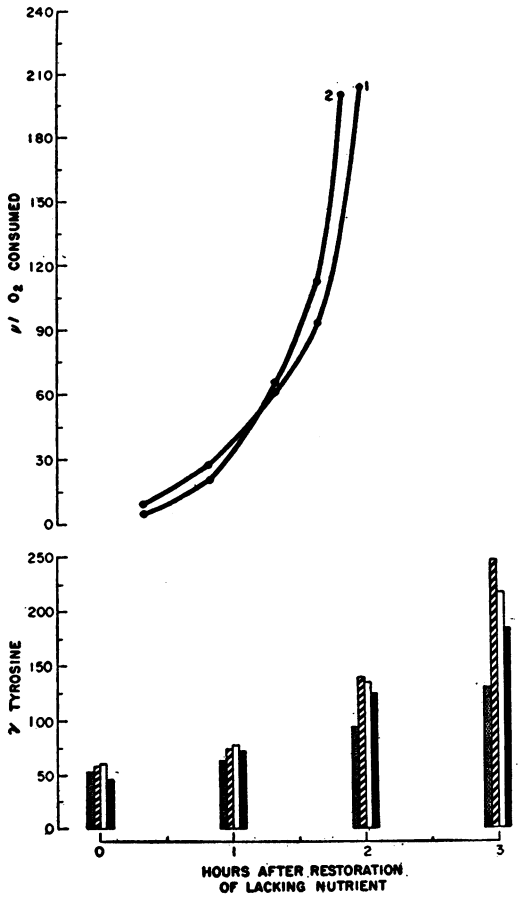

Figure 3. Post-starvation metabolism by various microorganisms. Curve 1, oxygen consumption by *Escherichia coli* strain K_{12}W-6 after 3 hr of methionine starvation. Curve 2, oxygen consumption by *E. coli* strain K_{12}W-6 after 3 hr of glucose starvation. The lower bar graphs represent total protein content, expressed as micrograms of tyrosine per 10-ml aliquots of culture. First bar, *E. coli* strain K_{12}W-6 after starvation of methionine. Second bar, *E. coli* strain W122-33 starved of methionine. Third bar, *E. coli* strain K_{12}J-2-1 starved of histidine. Fourth bar, *E. coli* strain K_{12}W-6 starved of glucose. The initial cellular populations before the deprived nutrilites were restored were 1.0, 1.6, 1.2, and 0.8 × 10^8 cells per ml respectively.

In figure 2, a similar experiment with lactose as a substrate and the assay of the intracellular β-D-galactosidase is represented. The only organism unable to synthesize adaptive enzyme to utilize lactose as the carbon source in 3 hr was the ribonucleic acid-enriched *E. coli* strain K_{12}W-6.

The metabolism of the ribonucleic acid-enriched organisms after the restoration of methionine is compared with that of organisms prestarved of other nutrilites in figure 3. Although there is no difference in the initial rate of respiration between glucose-starved, and methionine-starved *E. coli* strain K_{12}W-6, protein synthesis in the latter is slower. On the other hand, the rate of synthesis in the other two amino acid prestarved auxotrophs is faster than that of the glucose starved *E. coli* strain K_{12}W-6.

The loss in metabolic resiliency of the ribonucleic acid-enriched organism may be due, as has been suggested by Gale and Folkes (1953) for penicillin-treated bacteria, to the *kind* of ribonucleic acid which accumulates. It has been shown by Dunn and Smith (1955), for example, that the desoxyribonucleic acid in the thymine-deficient auxotroph, *E. coli* strain 15T- is ab-

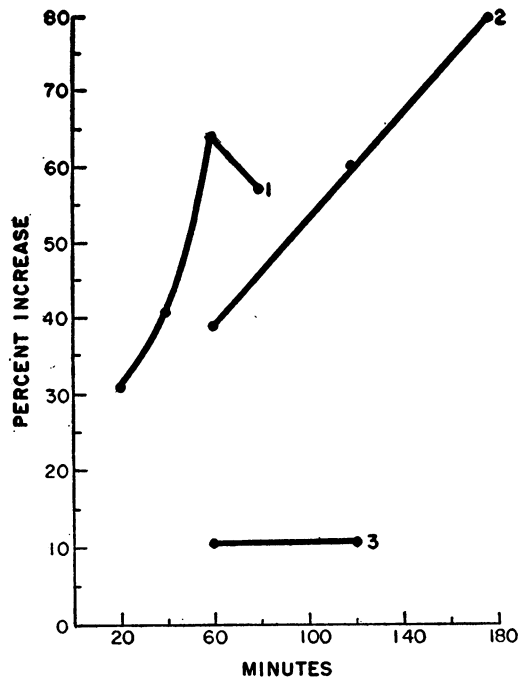

Figure 4. Ribonucleic acid accumulation in *Escherichia coli* strain K_{12}W-6. Curve 1, irradiated 150 sec and methionine provided. Curve 2, unirradiated, methionine deprived. Curve 3, irradiated 60 sec, methionine deprived. The level of ribonucleic acid phosphorus when the organisms were freshly harvested from logarithmic growth phase was 130 μg/10^{10} cells. The ordinate represents per cent increase in ribonucleic acid from those initial levels.

normal in both composition and physical properties.

It has been shown by Siminovitch (1953) that after induction, *E. coli* strain K_{12} continues to synthesize both ribonucleic and desoxyribonucleic acid, differing profoundly in this respect from the metabolism of a bacterial cell invaded by virulent phage. Since irradiation and subsequent phage development do not interfere with ribonucleic acid synthesis in the well nourished organism, it seemed of interest to determine the effect of irradiation on ribonucleic acid synthesis in the organism deprived of methionine and which consequently is unable to develop phage. The results of such a study are presented in figure 4.

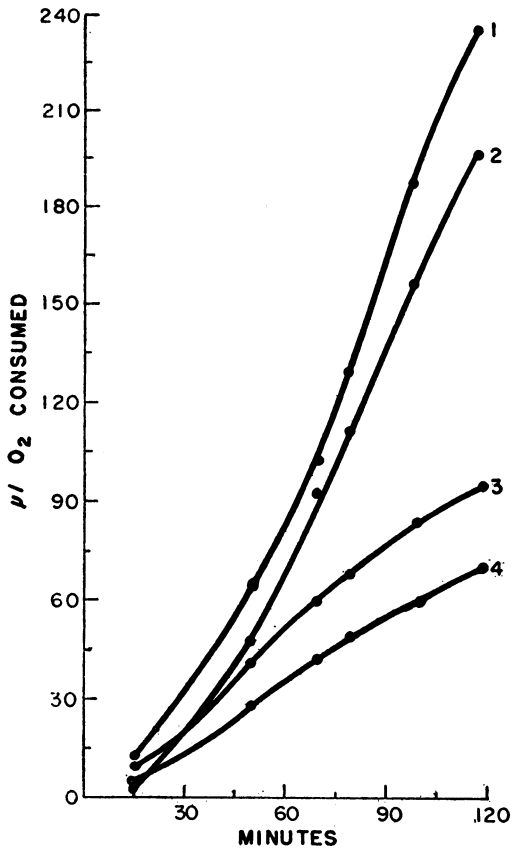

Figure 5. The effect of irradiation on the respiration of *Escherichia coli* strain K_{12}W-6 with and without methionine. Curve 1, respiration without irradiation, methionine provided. Curve 2, the same after 60 sec irradiation. Curve 3, respiration without irradiation, methionine deprived. Curve 4, respiration after 60 sec irradiation, methionine deprived.

Even 40 per cent of the normally inducing dose of irradiation—60 sec at 1-meter distance—is sufficient to inhibit the synthesis of ribonucleic acid in the methionine-deprived organisms. This finding is in striking contrast to the effect of ultraviolet irradiation not only on the lysogenic organisms but on nonlysogenic organisms as well since it was shown by Kelner (1953) that ultraviolet irradiation which prevents 90 per cent of the organisms from giving rise to visible colonies still permits marked increase in ribonucleic acid content in *E. coli* strain B/r.

That the failure to accumulate ribonucleic acid is not due to inhibition of respiration by the irradiation is shown in figure 5 in which the effect of irradiation on the respiration of *E. coli* strain K_{12}W-6 with and without methionine after the irradiation is presented.

The increased radiation sensitivity of ribonucleic acid synthesis in the absence of methionine can stem from too large a variety of causes to be discussed fruitfully at the present time, but the findings do serve as additional evidence to those offered by Heinmets and Kathan (1954) on the importance of considering the metabolic condition of the "target" before valid conclusions can be drawn from studies on radiation dosage and physiological *sequelae* in bacteria.

SUMMARY

Metabolic studies on a mutant of *Escherichia coli* strain K_{12} which accumulates ribonucleic acid on methionine starvation are presented. The ribonucleic acid-enriched organism is uniquely slow in synthesizing both adaptive enzymes and its usual proteins. Unbalanced cytoplasmic synthesis of ribonucleic acid, unlike unbalanced synthesis of ribonucleic acid and protein, was found to be not lethal. The synthesis of ribonucleic acid in the organism starved of methionine is uniquely sensitive to radiation.

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