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SUMMARY PAGE

THE PROBLEM

The assumption has been made that low-frequency vibration as experienced in air and space vehicles may increase the genetic load. This hypothesis was tested by studying the effects of vibration on the formation of biochemical mutants in Escherichia coli.

FINDINGS

No significant differences from unvibrated controls were observed either in growth or in number and kind of nutritionally dependent cells arising in E. coli vibrated at frequencies from 20 to 100 Hz. Exposure times varied from 5 to 60 minutes, and accelerations from 5 to 20 G. The experiments demonstrate that the single linkage chromosome structure of the protokaryotic E. coli is mechanically stable enough to withstand the mechanical forces of vibration within the limits used.

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INTRODUCTION

The genetic balance of a biological system in spaceflight may be disturbed not only by ionizing radiation but also by dynamic factors (acceleration, vibration, weightlessness) and by extreme physical factors (temperature, and others). It is often difficult to judge the relative importance of a single factor since synergistic action must be considered. Recently, one of the dynamic factors, vibration, has aroused special interest. Vibration occurs during periods of acceleration and deceleration. In addition to the possibility of direct vibration effects, vibration may also sensitize the biological system to cosmic radiation and possibly weightlessness in orbital flight.

Zhukov-Verezhnikov et al. (6) noticed in Vostok flights 3 to 6 a sensitizing effect of vibration during experiments with lysogenic E. coli K-12 (λ). Rybakov and Kozlov (5) showed in the laboratory that vibration by itself had no inducing effect on the prophage but caused sensitization of the lysogenic cultures to small doses of radiation. Even though the mechanism of this sensitization by vibration has not yet been elucidated, it may be speculated that the repressor of lysogeny is partially inactivated or its synthesis inhibited by vibration or that vibration actually causes structural changes in the prophage region of the genome of the bacterium. If one part of genome is assumed to become labile under the influence of vibration, the structure of other parts may also become unstable. This consideration led to the present search for vibration-induced nutritionally dependent mutants (auxotrophs). Experiments with E. coli vibrated at a number of frequencies between 20 and 100 Hz and at accelerations from 5 to 20 G for 5 to 60 minutes had negative results. The number of auxotrophs formed was found statistically insignificant; thus, it appears doubtful that vibration of the indicated characteristics causes nutritional genetic blocks.

MATERIALS AND METHODS

The vibration apparatus has been described previously by Knepton (4). Plexiglas holders were used to firmly couple the petri dishes and vials containing the specimens to the moving element of the vibrator.

The organisms were Escherichia coli strains ATCC 12435 and ATCC 10798 K-12 (λ).

Details of the media and growth factor solutions used in characterizing mutants are given in Appendix A.

In the search for mutants formed under the influence of vibration, two different methods were used:

1. Method A was the delayed enrichment agar layer technique described by Lederberg (4). Two experiments were performed by different experimenters at different times.

2. Method B was based on the penicillin agar layer method of Adelberg and Myers (1).

Nutritional mutants were characterized according to the methods of Lederberg (4) and the Beijerinck auxanographic method. Since the results depended on details in the procedure, all methods are fully described in Appendix B.

RESULTS

With the delayed enrichment agar layer technique (Method A) no significant difference between the experimental and the control groups was seen. Two different experimenters observed nine mutants in the vibrated specimen out of a total of 18,283 counts (Table I), while five mutants were found in 18,004 control counts. With the exception of one, the mutants in the vibrated samples were observed in samples vibrated at low frequency (≤ 70 Hz), at low G force (< 10 G), and during short vibration times (≤ 15 minutes). However, the limited experimental material does not allow the conclusion to be drawn that these vibration conditions favor the formation of auxotrophs.

No significant effect of vibration on the formation of auxotrophs was shown by the penicillin agar layer method (Method B). A summary of the results for all vibration conditions under Method B is included in Table I; detailed data are presented in Table II. Compared with the control values, no special vibration conditions seemed to favor the formation of auxotrophs.

Fifty auxotrophic mutants from vibration and control experiments were characterized concerning their specific growth factor needs (Table III). Forty-seven of these required addition of one or more amino acid for normal growth. Three isolates grew on complete medium, but would not respond to any of the minimal media supplemented by growth factors listed in Appendix A. No significant difference between the distribution of mutants in the vibrated and in the control groups was observed.

DISCUSSION

The fact that, in E. coli, no nutritionally dependent cells were formed in the force environment of the selected vibration ranges requires some discussion as to: 1) the transfer of force to the cells, 2) the methods of characterizing nutritional mutants, and 3) the meaning of the experimental results concerning the mechanical stability of the chromosome of E. coli.

1. The mechanical coupling of the E. coli cells to the vibration generator was different in methods A and B. In method A the cells were suspended in a semisolid agar medium that transferred the energy from the vibrator head to the cell. The mode of the coupling of the cells by the semisolid agar on the open-faced plates is not well defined. It is realized that the coupling may distort the vibration pattern. A careful analysis of this mechanical problem was not possible in the present study.

In method B the cells were suspended in liquid broth that filled vials fastened to the vibration head, and, in contrast to method A, were free to move. Vibration causes a certain limited movement of the cells in the broth. It can be assumed that cells and broth are "stirred" and that more nourishment becomes available to the cells. However, no readily noticeable differences in growth rate were observed between the vibrated cultures and their controls.

2. The two methods used to characterize the nutritional mutants are different in effectiveness and sensitivity. In a given time method B allows the screening for mutants of a considerably larger number of cells than method A and is therefore more useful in the present task. The high mutation frequency found in method A indicates that a certain number of slow growing mutants are picked up by this procedure that do not get through in method B. A comparison of the experimental values of method B with the control values determined by the same method shows with great certainty that low-frequency mechanical vibration up to 20 G does not change the natural rate of occurrence of auxotrophic mutants.

3. The experiments demonstrated the mechanical stability of the threadform chromosome in E. coli under the conditions of the selected vibration-force environment. This stability can be expected if only the potential energy imparted to the chromosome by the vibrating medium is considered. Energy values far below the thermal agitation energy kT are found in calculations that make reasonable assumptions about the mass of the chromosome and the buoyant force of the surrounding medium. However, the tensile strength of the genetic thread in E. coli could be surpassed in a different way. It is well known that the E. coli chromosome is very sensitive to hydrodynamic shear (2); flow out of a narrow pipette can cause breakage of the molecule. The E. coli cell contains a number of inclusions in the form of smaller and larger granules up to 500-Å diameter. Movements of these inclusions in the cytoplasm could set up the conditions for shearing forces on neighboring DNA threads. The negative experimental results of this study exclude the possibility of hydrodynamic shear inside the cell under the chosen vibration conditions.

COMMENT

The chromosome structure of protokaryotic systems with their single linkage groups can be assumed to be more sensitive to mechanical disturbances than the chromosomes of eukaryotic organisms where the genetic material is separated from the cytoplasm by a membrane which encloses the nucleus. From negative findings in the protokaryotic E. coli, it appears most unlikely that the specific and well-protected structure of chromosomes in eukaryotic organisms such as man is mechanically injured by short-duration low-frequency vibration up to 20 G.

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Table I

Summary of Mutation Frequencies*

	Experimental	Control
Method A		
Experiment I	$4/6227 = 6.4 \times 10^{-4}$	$1/6230 = 1.6 \times 10^{-4}$
Experiment II	$5/12,056 = 4.2 \times 10^{-4}$	$4/11,774 = 3.4 \times 10^{-4}$
Total	$9/18,283 = 4.9 \times 10^{-4}$	$5/18,004 = 2.8 \times 10^{-4}$
Method B	$147/55.5 \times 10^{5} = 2.7 \times 10^{-5}$	$127/51.1 \times 10^{5} = 2.5 \times 10^{-5}$

*Frequency = Number mutants per number colony-forming units.

Table II

Mutation Frequencies* Observed at Three Different Vibration Times When Using Method B

Frequency In Hz	Force In G	5 min		15 min		30 min		Control	
		No. of Mutants	Freq. X 10^{-5}	No. of Mutants	Freq. X 10^{-5}	No. of Mutants	Freq. X 10^{-5}	No. of Mutants	Freq. X 10^{-5}
20	5	10	5.07	15	7.61	14	7.10	35	6.0
40	5	0	-	0	-	0	-	1	0.8
70	5	15	9.55	13	8.28	1	0.63	20	4.3
100	5	8	8.24	8	8.24	2	2.06	12	4.2
20	20	6	2.53	7	2.95	4	1.68	24	3.4
40	20	6	2.06	1	0.34	6	2.06	9	1.0
70	20	5	1.72	2	0.68	1	0.34	8	0.9
100	20	6	1.50	8	2.00	9	2.25	18	1.5

*Frequency = Number mutants per number colony-forming units.

Table III

Characterization of Auxotrophic Mutants

Requirement for Normal Growth	Source of Culture	
	Control	Experimental
Proline	7	9
Histidine	7	4
Cysteine	7	8
Proline - Histidine	1	0
Amino acid (unidentified)	2	2
Growth in complete medium only	2	1
	26	24

Appendix A

Media and Growth Factor Solutions

Method A: Agar layer technique of Lederberg:

Complete Broth (CB)

Casein digest	10 g
Yeast extract	5 g
Glucose	5 g
K_2HPO_4	3 g
KH_2PO_4	1 g
Distilled H_2O	1000 ml

Minimal Broth (MB)

Glucose	1.0 g
K_2HPO_4	7.0 g
KH_2PO_4	2.0 g
$Na_3Citrate \cdot 5H_2O$	0.5 g
$MgSO_4 \cdot 7\ H_2O$	0.1 g
$(NH_4)_2SO_4$	1.0 g
Distilled H_2O	1000.0 ml

Complete Agar (CA)
Add to Complete Broth 15 g agar

Minimal Agar (MA)
Add to Minimal Broth 15 g agar

Minimal Broth without glucose (MB-G)

Method B: Penicillin agar layer method of Adelberg and Myers:

Complete Broth and Agar (CB and CA)

Casein digest	10 g
Yeast extract	5 g
K_2HPO_4	3 g
KH_2PO_4	1 g
Glucose	5 g
H_2O	1000 ml
Purified agar	15 g

Minimal Broth and Agar (MB and MA)

K_2HPO_4	7.0 g
KH_2PO_4	3.0 g
$Na_3Citrate \cdot 3\ H_2O$	0.5 g
$MgSO_4 \cdot 7\ H_2O$	0.1 g
$(NH_4)_2SO_4$	1.0 g
Glucose	2.0 g
H_2O	1000.0 ml
Purified agar	15.0 g

Adjusted to pH 7.0

Minimal Broth without glucose (MB-G) was prepared as above except that no glucose was added.

Purified agar was thoroughly washed in distilled water and 95-per cent ethanol to remove any traces of growth factor.

Penicillin G (Chas. Pfizer, U.S.P.) from two different lots

Penicillinase (Difco-Penase, 2000 L.U./ml)

Growth factors - Stock solutions of growth factors for the identification of auxotrophic mutants were made as follows and used at a concentration of 1/10 of the stock solutions.

A. Water Soluble Vitamins

	mg/l
1. Thiamine	0.1
2. Riboflavin	50.0
3. p-amino benzoic acid	10.0
4. Nicotinic acid	10.0
5. Pantothenic acid	10.0
6. Pyridoxine	10.0
7. Pteroylglutamic acid	1.0
8. Choline	200.0
9. Inositol	100.0
10. Biotin	0.1
11. Vitamin K	100.0
12. Vitamin B_{12}	0.1

Three mixed vitamin solutions were prepared; the first contained vitamins 1, 2, 3, and 4; the second 5-8, and the third 9-12.

B. Amino Acids (l-isomers)

	mg/l
1. Lysine	100
2. Arginine	100
3. Methionine	100
4. Cystine	500
5. Leucine	100
6. Isoleucine	100
7. Valine	100
8. Phenylalanine	100
9. Tyrosine	100
10. Tryptophan	100
11. Histidine	100
12. Threonine	200
13. Glutamic Acid	100
14. Proline	100
15. Aspartic Acid	100
16. Alanine	100
17. Glycine	100
18. Serine	100
19. Hydroxyproline	100
20. Cysteine	100

Six mixed solutions of amino acids were prepared: A. amino acids 1-4; B. 5-7; C. 8-9; D. 11-15; E. 16-19; and F. 20.

C. Purines, Pyrimidines, Nucleosides, Nucleotides (PPNN) in aqueous solutions of 100 mg/l

Purines: Xanthine
Hypoxantine
adenine
guanine

Pyrimidines: Uracil
thymine
cytosine

Nucleosides: Ribose uridine
" cytidine
" xanthosine
" inosine
" adenosine
" guanosine
Deoxyribose thymidine

Nucleotides: Adenosine-3-phosphoric acid (yeast adenylic acid)
Adenosine-5-phosphoric acid (muscle adenylic acid)
Xanthylic acid
Guanylic acid
Uridylic acid
Cytidylic acid

Appendix B

Methods

Method A: Delayed enrichment agar layer technique.

An overnight culture of E. coli ATCC 12435 was diluted to give approximately 50 cells per ml in MA. Two-milliliter amounts of this dispersion were dispensed into petri dishes on a previously formed layer of 5 ml of MA. The seeded plates were then refrigerated. Two plates were removed from the refrigerator for the experiment. One was vibrated at a specified frequency, acceleration, and time period, while the other remained on the desk top and served as a control. The plates were vibrated at 5, 10, and 20 G; 40, 70, 80, 90, and 100 Hz for 5, 10, 15, 30, and 60 minutes. After vibration, both plates received a 5-ml MA overlay and were incubated at 37° C. Twenty-four and 48 hours later the colonies were marked and counted. At 48 hours the plates were overlayed with 5 ml of CA, reincubated, and new colonies marked and counted at 24 hours. For verification, transfers were made from these new colonies to CB, and incubated for 24 to 72 hours, and the presence or absence of growth was recorded. Further transfers of mutant colonies were made from the complete broth tubes to MB as well as CB and incubated 24 to 48 hours. Finally, the culture was transferred from the second CB tube to fresh MB. Any isolates appearing to grow slowly in MB were considered partially blocked auxotrophs, and those growing only in CB were considered auxtrophic mutants. Both were transferred from the second CB tube to CA slants and retained for further studies.

Method B: Penicillin agar layer method of Adelberg and Myers.

Cultures of E. coli ATCC 10798 K-12 (λ) were maintained on minimal medium and transferred daily during the course of the experiment. One ml of a suspension of 24-hour growth in MB-G was added to 100 ml of sterile MB-G and incubated at 37° C for 16 hours. The resulting suspension was diluted with MB-G to give a reading of approximately 50 per cent transmission at 4200 angstroms in a Coleman Jr. Spectrophotometer. In filling the vials for vibration and control, about 3 ml of the suspension were slowly injected into a sterile rubber-stoppered vial until it was completely filled. All vials to be vibrated in a series and the controls were prepared at the same time and held at room temperature. A series of cell suspension was vibrated at 5 and 20 G and 20, 40, 70, and 100 Hz for 5, 15, and 30 minutes. The experimental and control cell suspensions were diluted 1:200 in MB-G for plate counts. A mixture of 1 ml of each resulting suspension with 7 ml of MA was overlayed on a previously prepared plate already containing a 7-ml MA layer. After the agar containing the cell suspension hardened, a protective layer of MA was overlayered. The plates were incubated at 37° C for 6 hours, then 7 ml of MA containing 250 units/ml of penicillin were added. The plates were refrigerated overnight, then incubated at 37° C for 24 hours and colonies marked. A layer of 7 ml of MA containing 260 units of penicillinase was added and the plates incubated at 37° C for 48 hours. Colonies appearing at 24 and 48 hours were marked. Finally, a 7-ml layer of CA was added and the plates incubated for a final 24 hours. New colonies were marked, picked to CA slants, and incubated for 24 hours. Those showing growth were transferred to MA. Isolates showing growth on MA slants at 24 hours were considered prototrophs and discarded; those giving growth at

48 hours were considered partially blocked auxotrophs and retained for further studies. Those giving growth only on CA slants were considered auxotrophic mutants and also retained for further studies.

Characterization of Nutritional Mutants

Characterization of nutritional mutants was carried out according to the method of Lederberg using solid media and the Beijerinck auxanographic method as mentioned by Lederberg. A small amount of growth from the complete agar stock culture of the isolates was suspended in sterile physiological saline. One loopful of this suspension was streaked over the surface of each of the following types of agar slants: MA + amino acids, MA + vitamins, and MA + PPNN. The slants were incubated for 24 hours at 37° C and growth recorded. Those isolates showing growth on none of the four slants were incubated for an additional 24 hours. Those still showing no growth were reinoculated. When growth occurred on one of the slants, plates of MA containing appropriate subgroups of the growth factors were streaked with the isolate. When growth occurred on such a plate, the single growth factor was identified by making a pour plate of MA containing the organism. Small drops of solutions of each of the single growth factors were dropped on sectors of the plate. After overnight incubation at 37° C, the plates were examined for growth.

Viability Counts - One-tenth ml of the appropriate dilutions of the cell suspension made in MB-G was combined with 7 ml of MA in sterile plastic petri dishes. After the agar hardened, the plates were inverted and incubated for 48 hours, the colonies were counted, and the average of four plates made for each dilution was used to calculate the number of colony-forming units in the suspensions.

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13. ABSTRACT

No significant differences from unvibrated controls were observed either in growth or in number and kind of nutritionally dependent cells arising in E. coli vibrated at frequencies between 20 and 100 Hz. Exposure times varied between 5 and 60 minutes, and accelerations between 5 and 20 G. The experiments demonstrate that the single linkage chromosome structure of the protokaryotic E. coli is mechanically stable enough to withstand the mechanical forces of vibration within the limits used.

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