NSAM-924

THE INFLUENCE OF VIBRATIONS ON CHROMOSOMES

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JOINT REPORT

UNITED STATES NAVAL SCHOOL OF AVIATION MEDICINE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

April 1965

THE INFLUENCE OF VIBRATIONS ON CHROMOSOMES*

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Bureau of Medicine and Surgery
Project MR005.13-9010
Subtask 7 Report No. 1

NASA Order No. R-39

Approved by

Captain Ashton Graybiel, MC USN
Director of Research

Released by

Captain H. C. Hunley, MC USN
Commanding Officer

12 April 1965

*This research was conducted under the sponsorship of the Office of Biotechnology and Human Research, National Aeronautics and Space Administration.

U. S. NAVAL SCHOOL OF AVIATION MEDICINE
U. S. NAVAL AVIATION MEDICAL CENTER
PENSACOLA, FLORIDA

SUMMARY PAGE

THE PROBLEM

In man's travel through the atmosphere of earth and into outer space, he is subjected to "flight factors" of the vehicles in which he travels. Among the principle factors of air and space travel are the mechanical vibrations generated by motors and air buffeting. It has been observed by a few earlier workers that vibrations, such as are found in air and space ships, are deleterious to chromosomes. Russian workers have discovered chromosome rearrangements in microspores of Tradescantia paludosa which were subjected to the flight factors of their Vostok series of earth-satellite experiments. They stress the importance of vibration as a contributory factor to disturbances of chromatin material during microsporogenesis in Tradescantia. A study of the response of chromosomes to the predominant frequencies and accelerations of vibrations found aboard flight ships is of special interest.

FINDINGS

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This report gives the results of an initial series of experiments in which cells from various organisms were vibrated at frequencies of 40 and 70 cps with 10 and 20 G's. Microspores of Tradescantia paludosa (Clone 3 of Sax), conidia of Neurospora crassa (L-prolineless clock-mutant, FGSC No. 491a), Neurospora crassa (N.R.C. No. 865A), and ova, larvae, and pupae of Drosophila melanogaster (wild type) were studied for chromosomal rearrangements. No influence was found of these vibration frequencies and accelerations on N. crassa, T. paludosa, and the P_1 generation of D. melanogaster, but there was observed the presence of body color and wing shape mutants among the F_1 generation of D. melanogaster. In future work these organisms will be subjected to other frequencies and displacements of vibration.

Author

ACKNOWLEDGMENTS

Dr. Dietrich E. Beischer of this laboratory has given much encouragement and constructive advice during this entire study. I gratefully acknowledge all of his time and effort. In addition, Mrs. Nelda Moss and Mr. Clayton Ezell helped with various preparations and procedures that were used. To them I am very grateful.

Mr. Harold M. Forkois, U. S. Naval Research Laboratory, made available his facilities without which this series of experiments could not have been conducted. Among those in his laboratory who contributed directly to the technical aspects of the vibration equipment, and to whom I am appreciative, are Mr. J. P. Layher and Mr. B. P. Czarnaski.

Finally, there are those to whom I am indebted and appreciative who willingly furnished the living specimens that were used in this study. They are: Mr. William Ogata, Dartmouth College; Miss Mary Clement, National Research Council of Canada; Dr. Sheldon Wolff, Oak Ridge National Laboratory; and Mr. Walter Tarnok, U. S. Naval Aviation Medical Center.

INTRODUCTION

Sisakyan and co-workers (7) recently reported the effect of "space flight factors" upon the chromosomes of Tradescantia paludosa (Clone 3 of Sax) microspores. With cooperation of Cosmonauts Popovich and Bykovsky, aboard Vostoks 4 and 5, the Russian researchers by cytologic analysis found chromosomal rearrangements in the microspores which they believed were due to the action of factors connected with launch and re-entry of a space ship. The particular flight factors to which they refer are vibration and acceleration. Of interest to us are environmental conditions aboard air and space vehicles; therefore, we believed it worthwhile to conduct an investigation of the effects of vibration on chromosomes. In an exhaustive search of the literature dealing with the effects of vibrations upon living organs, only two other reports, besides that of the Russian workers, were found which touched upon the response of chromosomes to vibrations. One of these describes the observation of Sueda (8) in 1938 on wound healing; and the other one reports the findings of Nickerson and Paradijeff (4) in dogs of hyperlobulation in the polynuclear neutrophils and of inhomogeneities and the presence of intranuclear vacuoles in the lymphocytes. Consequently, we planned a pilot series of experiments that would reveal a feasible approach to discover whether or not the vibrations present aboard flight ships are harmful to living organisms, and in particular, to man's chromosomes.

From the vibration spectra of missiles and various reports concerning the effect of vibrations on organs and tissues it was determined that, in the first experiments, the vibration frequencies should be 40 and 70 cycles per second (cps) and for each frequency of vibration accelerations of 10 and 20 G should be present for periods of fifteen minutes. In future experiments other prevailing vibrations and accelerations of flight vehicles will be used. When the organisms to be vibrated were selected, five criteria were taken into consideration: 1) the medium in which to embed the specimens, 2) the ease of manipulating the cells of the particular organism, 3) their viability in the embedding medium, 4) their established use in genetic and chromosomal studies, and 5) their low chromosome number. With these criteria in mind, the following organisms were studied: conidia of Neurospora crassa (FGSC No. 491a and N.R.C. No. 865A); microspores of Tradescantia paludosa (Clone 3 of Sax); and ova, larvae, and pupae of Drosophila melanogaster (wild type). The findings were negative in the case of Neurospora and Tradescantia, but positive for Drosophila. Further studies should answer many questions that have been opened by this initial investigation.

VIBRATION APPARATUS AND METHOD

A vibration facility located at the U. S. Naval Research Laboratory, Washington, D. C., was used for this series of experiments. The exciter (shaker) is of the electromagnetic type and was manufactured by Calidyne Company, Winchester, Massachusetts. The driving amplifier is a Westinghouse Model NR25 with a McIntosh Model 2200 preamplifier. An MB Electronics (New Haven, Connecticut) oscillator is

used to sweep frequency. The test level (G force) is sensed by an Endevco accelerometer (Model 22210) and is read out from an MB Electronics Model N550 vibrator meter. Shown in Figure 1 are the exciter and accelerometer.

The specimen holder (Figure 2) was constructed from a pine wood block, 84 mm x 84 mm x 102 mm, and a piece of flat steel metal, 2.5 mm thick x 75 mm wide, that fitted around three sides of the block. Flanges of the steel clamp were bolted with four metal bolts to the horizontal platform of the exciter. The wooden block had a 23 mm diameter hole drilled 38 mm deep. The hole was widened to a 27-mm diameter, 11 mm from the top, and then the block was sawed in half. A glass specimen vial (also shown in Figure 2) could be fitted very snugly into the hole, a 0.5-mm thick fiber gasket placed between the wooden block halves, and the two pieces tightened together with four bolts, washers, and nuts. The whole assembly was clamped securely to the exciter's platform with the metal clamp.

The loaded capped specimen vial was vibrated in a vertical direction for fifteen minutes. Each kind of biological material used was vibrated at 40 and 70 cps and for each frequency they were exposed to two displacements (double amplitudes) which gave G forces of 10 and 20. The displacement at 40 cps and 10 G was 3.05 mm; for 40 cps and 20 G, 6.10 mm; for 70 cps and 10 G, 1.02 mm; for 70 cps and 20 G, 2.03 mm.

The laboratory temperature ranged between 24 and 25°C during the time of the entire experiment.

Neurospora crassa

Method

N. crassa, FGSC No. 491a, L-prolineless mutant, was obtained from the Fungal Genetics Stock Center, Dartmouth College, Hanover, New Hampshire. Conidia of this strain were harvested from mycelia growing on Gray's complete medium (see Brandt, ref. 2) and suspended in distilled water. Eight drops of the concentrated conidial suspension were mixed in each specimen vial with 4 ml of almost gelled 2% agar. This mixture was allowed to solidify in a cold water bath for five minutes. The specimen vial then was placed in the specimen holder, which was clamped to the exciter's platform and vibrated for fifteen minutes. The specimen vial was removed from its holder and from it one drop of suspension was taken and placed in each of eight six-inch long race tubes (see Ryan, ref. 6, for a description of the tubes). Also, single drops of suspension were placed in six petri dishes containing Gray's medium and on seven plates which held Vogel's (9) minimal medium with L-proline added (0.12 gm per liter of Vogel's medium). A nonvibrated set of control specimens was similarly treated. Two specimen vials were vibrated for each combination of frequency and displacement.

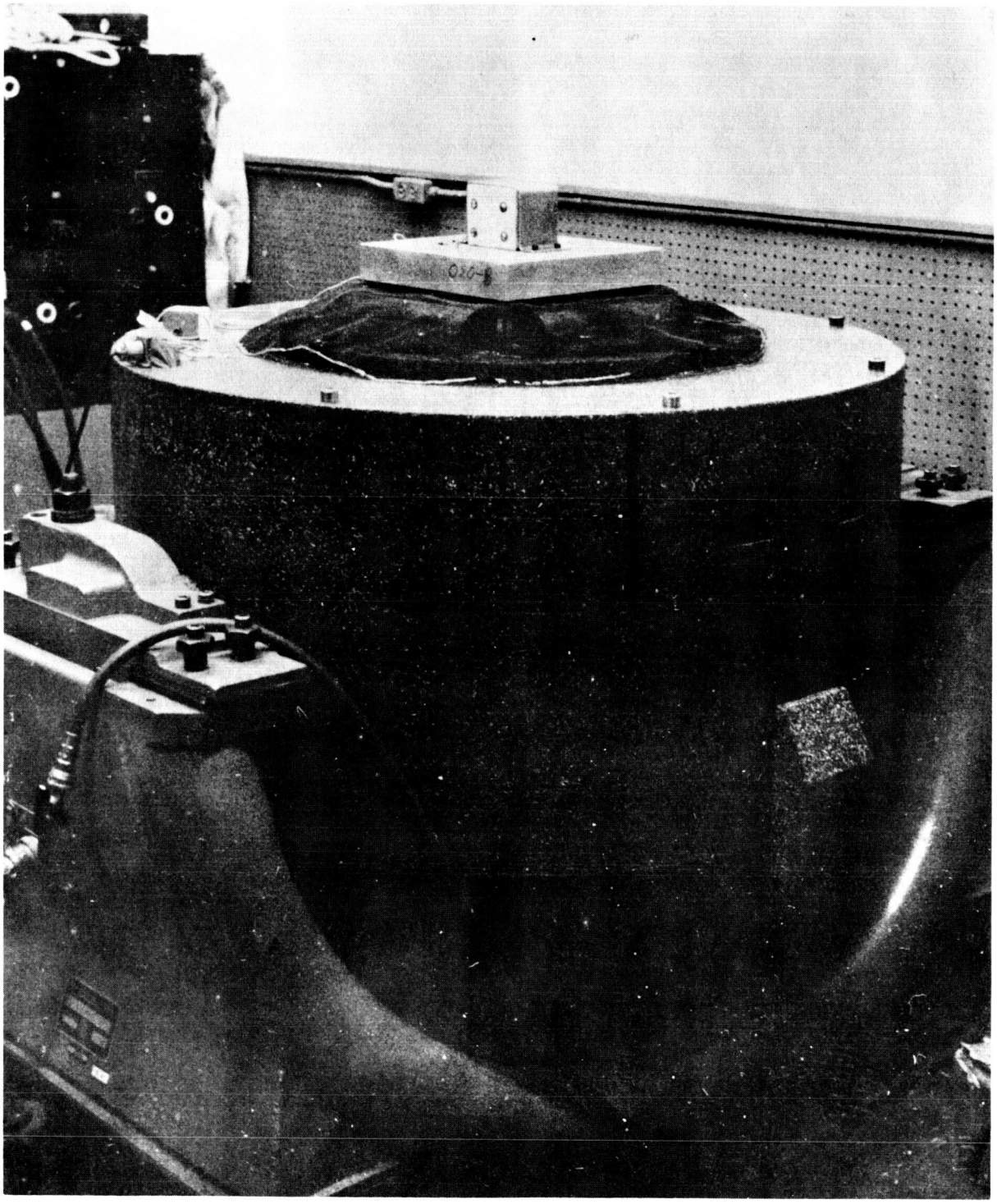

Figure 1

Exciter installation at the U. S. Naval Research Laboratory, Washington, D. C. The specimen holder and the small Endevco accelerometer to the left are shown mounted on top of the exciter's platform.

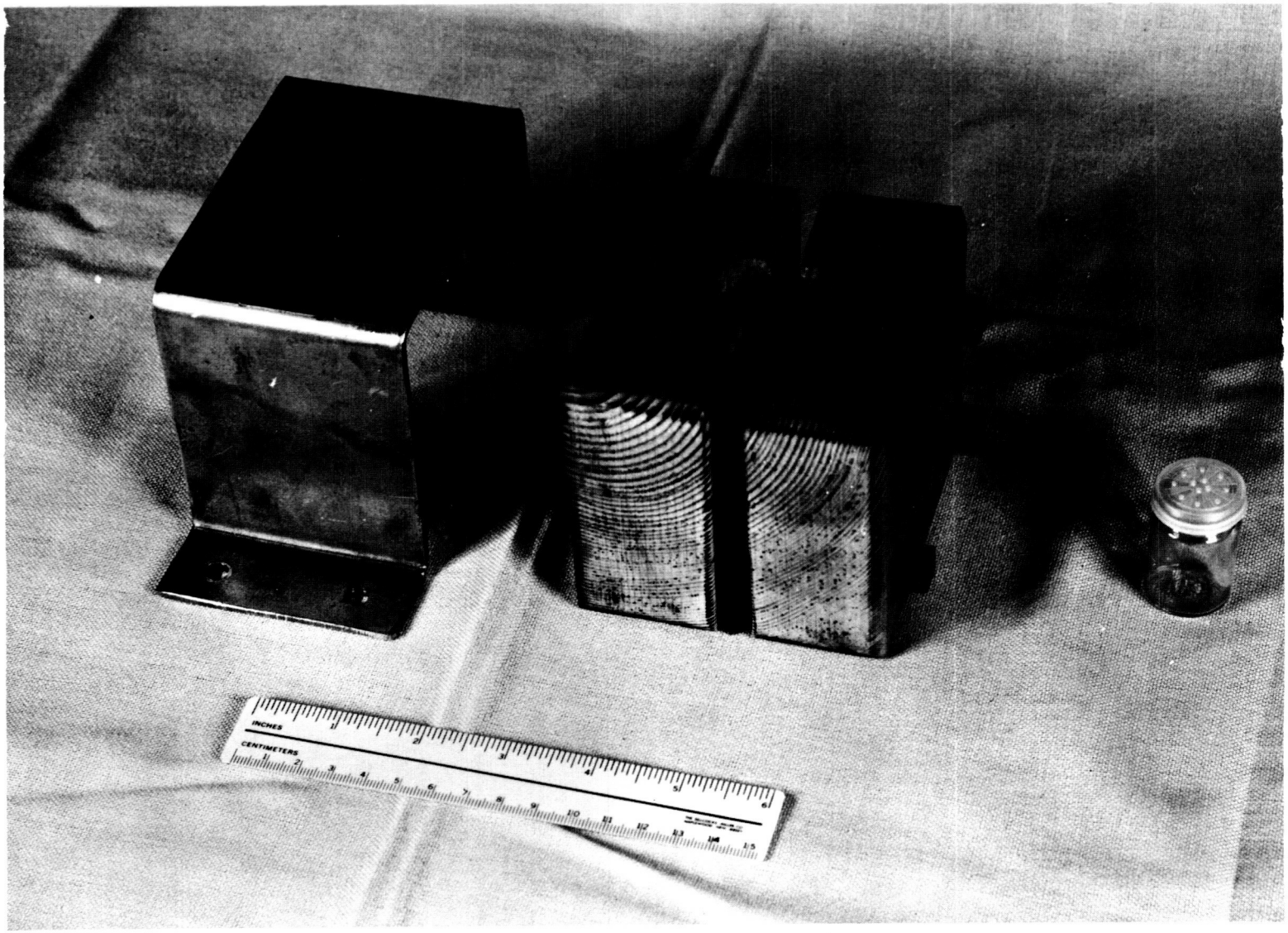

Figure 2

Specimen holder and vial used for vibration experiments.

Results

The typical "patch" or "clock" growth of this strain was not altered in any way by the exposure of the conidia to the specified frequencies and displacements. This is demonstrated in Figure 3. Growth rates in the race tubes of the experimentals were not significantly different from those of the controls. There was no change in the mycelial growth of those on the plates with Gray's medium and with Vogel's plus L-proline. Consequently, it is believed that the vibratory frequencies and G forces used in this experiment did not alter the genetic material (chromosomes) of this strain's conidia.

Method

N. crassa, N.R.C. No. 865A, was obtained through the generosity of the National Research Council, Ottawa, Canada. Conidia were harvested from mycelia of this strain, and specimen vials were prepared and vibrated in the same manner as described for N. crassa, FGSC No. 491a. Single drops from the experimentals and controls were placed on petri dishes containing either Gray's or Westergaard's (10) minimal medium. Also, single drops of conidial suspensions were deposited upon opposite sex (N.R.C. No. 866a) mycelia-containing petri plates which held Westergaard's medium.

Results

This wild type strain's vibration-exposed conidia grew equally as well on the minimal as on the complete media. The experimental and control plates, inoculated to bring about sexual reproduction, for some undetermined reason did not form mature perithecia. Therefore, it is concluded that the vibrations used in this experiment did not produce nutrient mutants. However, still further investigation is needed to clarify the reasons why sexual reproduction was not consummated.

Tradescantia paludosa (Clone 3 of Sax)

Method

Each specimen vial was prepared by placing five buds taken from inflorescences of T. paludosa, which were supplied by the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, on top of an approximately 2 mm thick layer of 2% agar. Each bud was of a different length--approximately 3, 4, 5, 5.5, and 6 mm. It was believed that, by using such a wide variation in bud sizes, a consequent variety of microspores in the various meiotic and mitotic stages of sporogenesis would be present. This proved later not to be the case, but what was actually vibrated were microspores that were in the interphase stage prior to the last cell division. Due to difficulty in obtaining Tradescantia inflorescences, the condition of cell division stage could not be controlled; therefore, the types of microspores that were available were vibrated in this pilot series of experiments. The buds were gently pressed into the

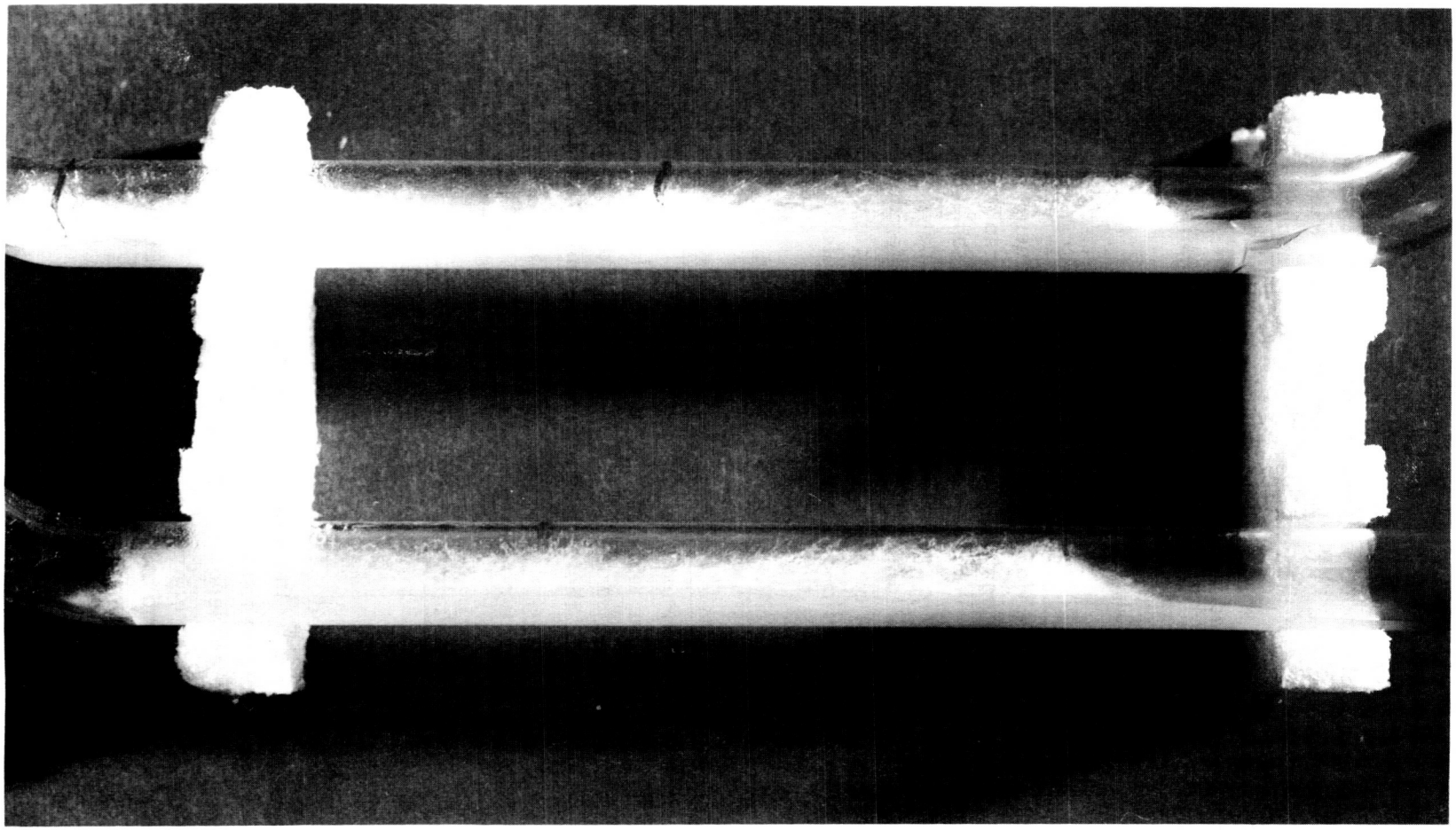

Figure 3

Side views of experimental (top) and control (bottom)
mycelia of Neurospora crassa (#491a) "clock" strain growing in six-inch race tubes.

Growth originated at left ends of race tubes. Middle and right end crayon marks indicate the terminations of two 24-hour periods of growth. During the daylight phase there is not so much conidiation as during the night phase, consequently the "patches" of conidia.

agar, then about a 1-mm thick layer of cool 2% agar was poured over them and allowed to gel in a cold water bath for five minutes. The specimen vial was then clamped to the exciter and vibrated. A control set of nonvibrated buds was also prepared in a similar manner.

After exposure to the vibrations and G forces just described, the buds were removed from the vial and from each there were made two slides of anther smears, according to the standard aceto-carmine squash technique.

Results

The slides were immediately scored and from the examination of a total of approximately 9,520 microspores were found that were in only the interphase stage which precedes the final cell division in the microsporogenetic process. In none of the examined microspores could there be detected any chromosomal rearrangements as described by Russian workers (1). The conditions for vibrations in this experiment do not cause aberrations of chromosomes in interphase microspores of T. paludosa.

Drosophila melanogaster, Wild Type

Method

Twenty each of ova, larvae, and pupae were collected from cultures, derived from an original culture purchased from the Genetics Research Unit, Carnegie Institute of Washington, Cold Spring Harbor, New York, 11724, and appropriately placed in the control and experimental specimen vials.

The ova had a maximum age of three hours when removed from a culture-medium-containing stendor dish (over which adult D. melanogaster had lived for three hours) and placed on a small piece of wet absorbent paper. (All culture medium used for this experiment was prepared according to Burdick, ref. 3.) The wet piece of paper then was laid, ova down, inside a specimen vial which had a 2-mm thick layer of 2% agar in its bottom. A 2-mm thick layer of cooled 2% agar was then poured over the ova and paper. The prepared specimen vials were vibrated, after which the ova were removed and placed in a regular culture bottle with medium. The ova were subsequently observed through the F_1 generation adult stage.

Third instar larvae were placed on 3 ml of solid 2% agar inside the specimen vials and allowed to move into the medium. A layer of aluminum was placed between the cap and vial top, the vial capped, and the larvae vibrated. After each vibration fifteen larvae were removed to regular culture bottles which contained medium and were observed through the F_1 generation. The salivary glands of the five remaining larvae were smeared and stained for chromosomes according to the method described by Painter (5). A cover slip was placed on top of the prepared chromosomes and sealed with vaseline. The slides were inspected the following day.

Nine-day old pupae were put on bits of wet absorbent paper and transferred with the paper, pupae-side down, to a specimen vial containing a 2-mm thick layer of 2% gelled agar. They were then covered with a layer of the same thickness of cooled 2% agar and allowed to set in a cold water bath for five minutes, after which the specimen vial was removed and vibrated. Following each vibration the pupae were placed in culture-medium-containing bottles and observed through the F_1 generation.

Results

An examination of the salivary gland slides showed that there was no visual evidence of chromosomal aberrations. In a count of stock cultures it was found that there were no mutants among 1,756 adult D. melanogaster, wild type. As shown by the data in Table I, there was no mutagenic effect detected in the parent generations (P_1), but there was an induced mutant effect to the extent of 1.18 per cent among the offspring (F_1 generation) adults of the vibrated specimens. There were no mutants among the controls F_1 adults. The types of mutations found were deviations from the wild type body color and from wing size and shape. The body color in eleven females and one male was black instead of the wild type body color of gray. Wing size and shape varied from that of the normal in that it was vestigial, club, or dumpy in fifteen males and thirteen females. The sex ratio of the experimentals was not significantly different from that of the controls or from the ratio of 45.8 per cent males to 54.2 per cent females of the stock specimens.

DISCUSSION

In this pilot investigation, from the beginning there was the important matter of selecting the frequencies and accelerations of vibration. A thorough search of the vibration literature gave indications that the predominant vibration frequencies present aboard air- and spacecraft are between 1.2 and 1,000 cps. Accelerations of as much as 58 G have been recorded from missiles during launch, but most usually the launch-time G forces are between 10 to 20. Because the Russians report ground-based experiments on cells using 70 cps and 7.9 G, it was decided to start with 70 cps vibrations at 10 and 20 G. In the vibration spectra of some aircraft and space ships there is a prevailing vibration frequency, among others, of 40 cps; therefore, this frequency was also used in these experiments. In future work, it is planned to use other frequencies and accelerations of vibrations which are within the 1.2 - 1,000 cps range, with vibratory accelerations of 5 - 60 G.

Little is known of the mechanism by which vibration produces biological changes, but it is hypothesized that damage is related to the absorption of energy at resonant frequency. It has been empirically demonstrated that test human and animal organs have specific resonant frequencies and at these frequencies major damage occurs. The absorption of vibratory energy results in mechanical damage. It is thought that there is a resonant frequency of vibration and displacement for each type of chromosome which will cause mechanical damage to it. This damage will result in a chromosomal rearrangement or aberration. It is the ultimate purpose of a projected

TABLE I

DATA FROM VIBRATED SPECIMENS OF DROSOPHILA MELANOGASTER, WILD TYPE

STAGE IN LIFE CYCLE	P_1 MUTANTS (TOTAL NO. IMAGOES)						F_1 MUTANTS (TOTAL NO. IMAGOES)						
	CONTROL	40 cps 10 Gs	40 cps 20 Gs	70 cps 10 Gs	70 cps 20 Gs	TOTAL	CONTROL	40 cps 10 Gs	40 cps 20 Gs	70 cps 10 Gs	70 cps 20 Gs	TOTAL	% MUTANTS
OVA	0(4)	0(9)	0(8)	0(14)	0(14)	0(49)	0(153)	2(332)	0(350)	6(368)	3(396)	11(1599)	0.69
LARVAE	0(8)	0(7)	0(9)	0(3)	0(9)	0(36)	0(6[C])	0(61)	5(256)	0(19)	2(233)	7(575)	1.22
PUPAE	A	B	B	B	B	—	A	10(448)	5(167)	4(302)	3(297)	22(1214)	1.81
TOTAL	0(12)	0(16)	0(17)	0(17)	0(23)	0(85)	0(159)	12(841)	10(773)	10(689)	8(926)	40(3388)	1.18
% MUTANTS	0	0	0	0	0	0	0	1.43	1.29	1.45	0.86		

A = No development of P_1 pupae

B = P_1 adults accidentally lost

C = Portion of these cultures dried before counting of imagoes was possible.

series of experiments to determine the resonant frequencies and vibratory accelerations that are deleterious to chromosomes and, in particular, to those of man. Experiments will be conducted not only using living cells, but also colloids of elastic, viscous medium in which chromosome-like particles are dispersed. The latter type of experiment should give some indication as to which frequencies and accelerations should be tried using live cells.

Still of concern is the matter of absolute transference of the primary frequency and acceleration of vibration to the living cells. This is known only by attaching a G-level sensor to the cell which at the present stage of technical development is impossible. The only alternative is to make sure that the cells are coupled as best as possible to the vibrating platform. In the experiments of this report, it was decided that a 2% solution of agar would adequately do the coupling; an investigation of the correctness of this assumption is planned.

The Russian researchers (1,7) have described vibratory effects in microspores of T. paludosa which were in the interphase (prior to the last mitotic division) and metaphase stages of microspore formation. The two genetic defects that they reported are spherical fragments and complex recombinations. The former appear as micronuclei and are most easily distinguished in the cytoplasm of interphase microspores. Figure 4 illustrates normal microspores in interphase, and Figure 5 depicts a normal microspore in metaphase. These researchers also have described several kinds of complex recombinations, most of which involve the mating of different chromosome types during microsporogenesis. The negative results of the experiments reported in this paper do not necessarily indicate an absence of vibration effect on Tradescantia chromosomes inasmuch as only two frequencies of vibration were used. There are many other frequencies prevailing in the vibration spectra of missiles and aircraft during take-off, flight, and landing which have not been used. This also could possibly be the reason for the negative results in the case of the Neurospora conidia.

The discovery of mutants in the F_1 generations of vibrated specimens of Drosophila and none in the P_1 generations is considered a significant finding. Future work will involve a repetition of this experiment and a more critical quantitative genetic analysis of the mutants.

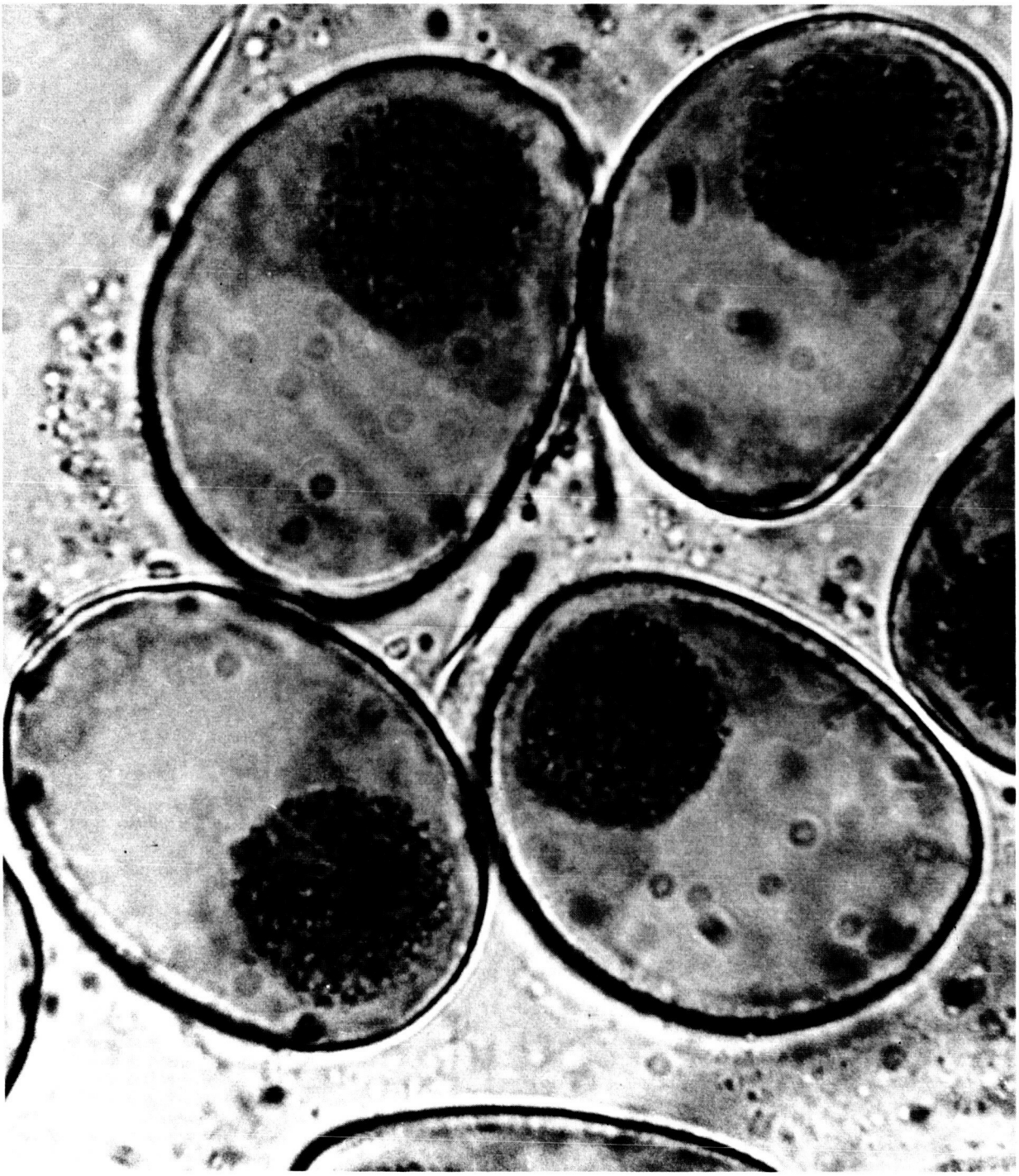

Figure 4

Immature microspores of Tradescantia paludosa (Clone 3 of Sax) in interphase prior to the last cell division of microsporogenesis.

Each microspore is about 33 microns long, and each nucleus is approximately 12 microns in diameter.

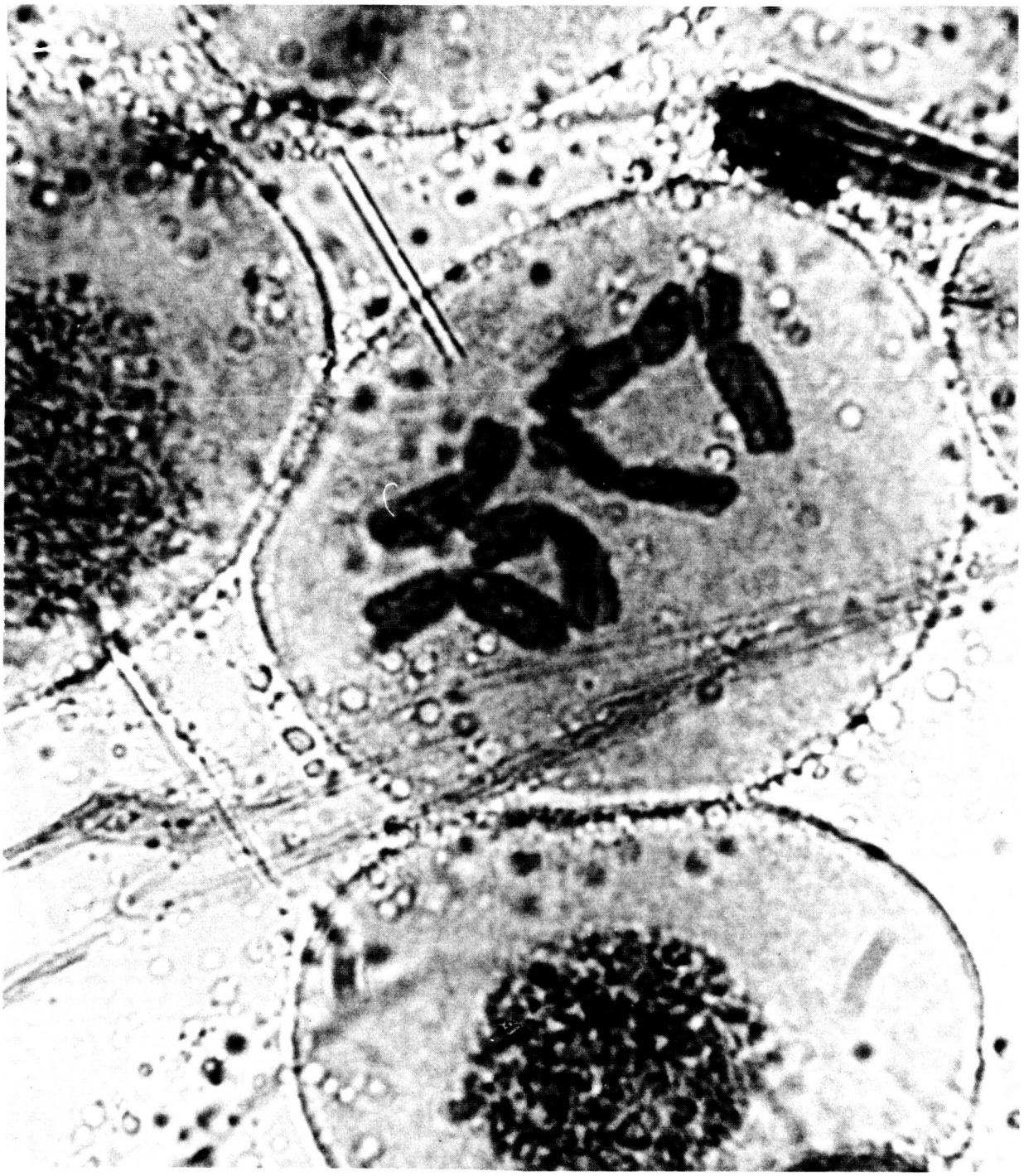

Figure 5

Entire cell in middle of the picture in metaphase of last cell division of microsporogenesis in Tradescantia paludosa (Clone 3 of Sax)

Chromatids are easily discerned. The haploid number is six. Each chromosome is approximately 1.06 microns in width.

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Security Classification

Unclassified **DOCUMENT CONTROL DATA - R&D**

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
U. S. Naval School of Aviation Medicine
Pensacola, Florida

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP
Not applicable

3. REPORT TITLE
The Influence of Vibrations on Chromosomes.

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(Last name, first name, initial)*
Knepton, James C., Jr.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
12 April 1965	14	10

8a. CONTRACT OR GRANT NO. NASA R-39

b. PROJECT NO. BuMed MR005.13-9010
Subtask 7

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
NSAM-924

9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
1

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DD FORM 1 JAN 64 1473

Unclassified
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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Chromosomes						
Conidia						
Drosophila melanogaster						
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Microspores						
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It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS), (S), (C), or (U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, roles, and weights is optional.