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EXPOSURE OF Tradescantia MICROSPORES TO PERIODIC VIBRATIONS OF 40-100 HERTZ

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JOINT REPORT

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EXPOSURE OF *Tradescantia* MICROSPORES TO PERIODIC VIBRATIONS OF 40-100 HERTZ*

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SUMMARY PAGE

THE PROBLEM

Russian scientists have found that space flight factors such as vibration and acceleration cause chromosomal disturbances. Of interest to us is whether or not these factors can exert an effect during earth-based experiments. We subjected in our laboratory one of their biosystems, microspores of Tradescantia anthers, to low frequencies (40-100 Hz) of vibrations at various levels of acceleration (5-40 g).

FINDINGS

Slight differences ($P<0.05$) were found in the number of spherical chromosomal fragments observed in the experimental and control groups of microspores, the greater number being among the experimentals. It was also observed that specimens vibrated for a short period (3 minutes) exhibited larger effects than those vibrated for longer periods of time (15-60 minutes). No significant impairments of the mitotic mechanism or growth disturbances were observed. Further experimentation is indicated in which Tradescantia microspores would be subjected to periodic and random vibrations of higher frequencies (100-2000 Hz) and accelerations (up to 110 g).

ACKNOWLEDGMENT

Dr. Dietrich E. Beischer has given much encouragement, helpful guidance, and assistance to this project for which we are very grateful.

INTRODUCTION

Russian space scientists have placed a great emphasis upon their findings (1-4,6) from exposure of living cells placed aboard Vostok and Voskhod spacecraft. They believe that certain space flight factors--weightlessness, radiation, vibration, and acceleration--may cause an increase in the occurrence of chromosomal aberrations. The influence of weightlessness and radiation as the inducing agents is minimized, and vibration and acceleration of missile ascent and descent are stressed as primary causes of chromosomal fragmentation without subsequent translocation or inversion. The deleted and rounded chromosome parts referred to by the Russians as "Spherical Fragments" are considered as such in our work.

In a previous study (5) in which a restricted vibration schedule and several biosystems were used only a mutagenic effect upon the life history stages of Drosophila was found. It was proposed then to investigate the effects of a wider range of periodic and nonperiodic frequencies of vibrations. The present report describes a series of ground experiments in which buds of the blue spiderwort plant, Tradescantia paludosa (Clone 3 of Sax), were exposed to frequencies of vibration between 40 and 100 Hertz (Hz). Microspores taken from these buds were studied for abnormalities. Slight differences in the number of spherical fragments were found in experimental and control groups, the greater being among the experimentals. It was also observed that specimens vibrated for a short period (three minutes) exhibited larger effects than those vibrated for greater lengths of time (15-60 minutes). No significant impairments of the mitotic mechanism or growth disturbances were observed.

PROCEDURE

VIBRATION APPARATUS

An MB Electronics Company exciter (EA 1500) with dual power amplifiers (2120MB) was used to vibrate the specimens. The amplifiers are driven by a Hewlett-Packard Company Model 200 CD oscillator. An MB Model N499 vibration meter monitored the output of a Columbia Research Laboratories accelerometer (Model 676-1), which is mounted in the moving element of the exciter. The meter and accelerometer are properly matched by an MB Model N504 integrator/amplifier. The meter allows direct readings of displacement, velocity, or acceleration of periodic or random vibration. Monitoring the frequency of vibration were a Computer Measurements Company Model 200C counter and a Model 410A printer. Figures 1-3 illustrate the vibration equipment.

METHOD

Inflorescences were collected from greenhouse plants and placed in water (pH 7). Buds were removed in the laboratory and injected with 2 per cent agar solution (pH 7). The prepared buds were embedded with additional 2 per cent agar solution in depressions made into 35 mm x 35 mm x 13 mm plastic blocks (Figure 2). The

Figure 1

Vibration system installed at the Naval Aerospace Medical Institute, Pensacola, Florida, for investigation of cellular reaction to low frequency periodic vibrations. The exciter and integrator/amplifier are on the laboratory bench. The cabinet contains (from top to bottom) the vibration meter, printer, counter, oscillator, and two power amplifiers. Note the specimen holder for buds of Tradescantia on top of the exciter.

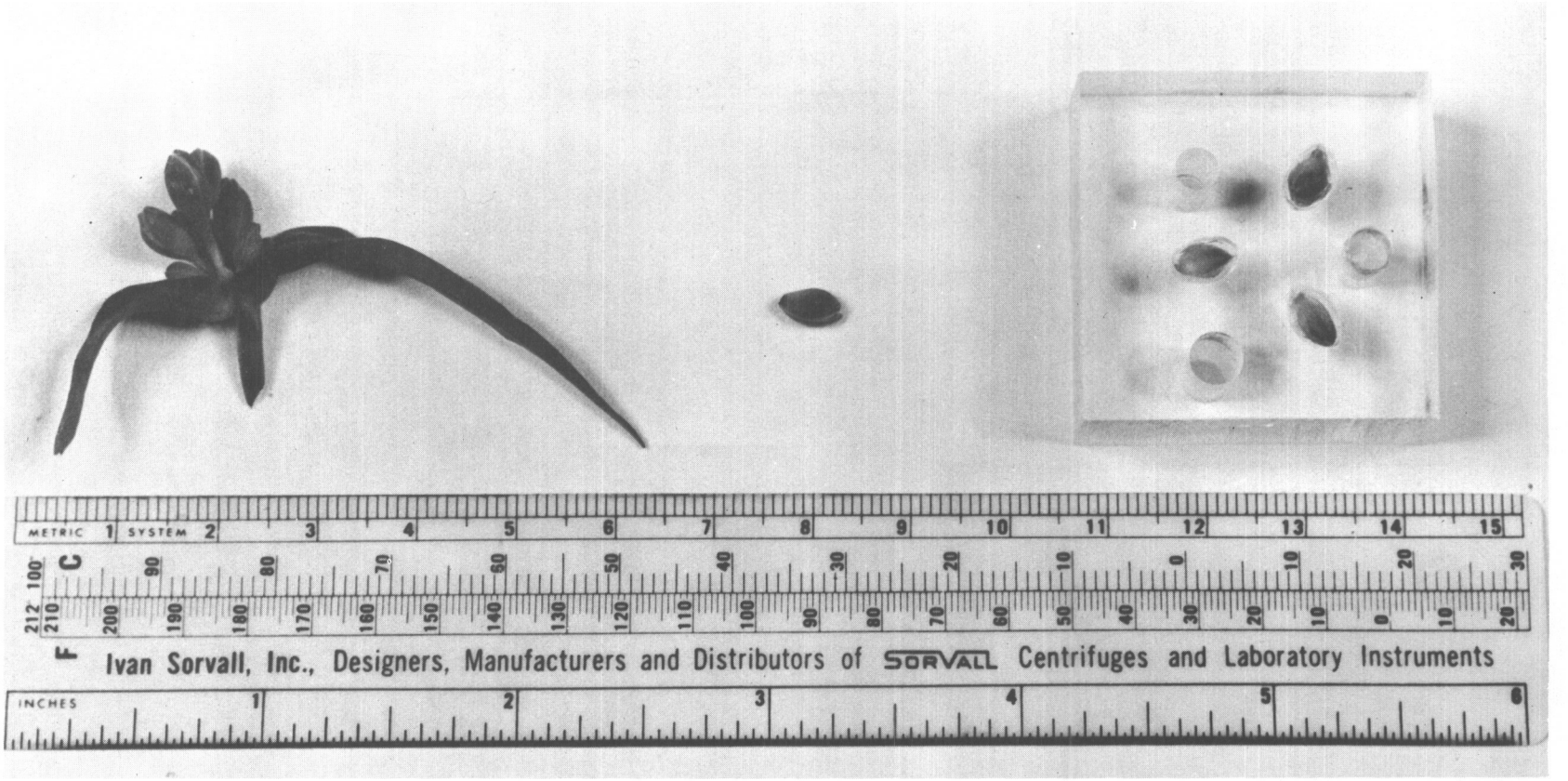


Figure 2

Tradescantia specimen holder with buds in depressions of the plastic block.
Left: inflorescence of buds; center: a single bud.

Figure 3

Close-up of exciter showing Tradescantia specimen holder with aluminum plate covering the buds and bolted to the exciter's moving element.

bud-filled depressions were then covered with an aluminum plate and the entire preparation securely bolted to the top of the exciter's moving element (Figure 3). A control preparation was kept on a laboratory table during the vibration time period of each run. Times of the vibration runs varied between three and sixty minutes, and acceleration levels were between 5 g and 40 g.

Following each vibration run a smear from one anther of each bud was made. The cells were killed in an acetic acid (30 per cent)-ethyl alcohol (70 per cent) solution and fixed in 80 per cent ethyl alcohol. The material was stained with aceto-carmine, and semipermanent mounting was accomplished with cover slips held in place by clear fingernail polish. The prepared cells were scored for abnormalities, and appropriate photomicrographs were made using an American Optical Series 10 phase microscope, Model 10 Ortho-Illuminator, and a No. 682 camera with 4 inch x 5 inch Graflok back. Panatomic-X film was exposed for empirically derived time periods.

OBSERVATIONS

Microsporogenesis

Each T. paludosa bud was found to possess usually six anthers, each of which generally contains cells all in the same stage of microspore generation. There was an average of from 7,000 to 10,000 cells per anther. The length of the bud was correlated with the microsporogenetic stage of a single bud's cells. Since it was desired to vibrate buds that possessed anther cells in the mitotic division, buds of 5.0 mm - 5.5 mm were selected. Buds less than 5.0 mm contain cells that are in the meiotic phases while those greater than 5.5 mm have immature and mature microspores. Figure 4 illustrates the entire microsporogenetic cycle.

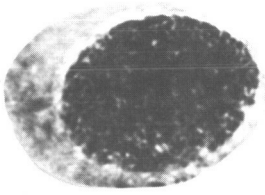
Chromosomal Aberrations

Aberrations were found most frequently among interphase-3, prophase-3, and immature microspores which contained spherical fragments, presumably broken off bits of chromosomes (Figure 5 b-d and g). Spherical fragments were also observed in tetrad, metaphase-3, anaphase-3, and telophase-3 cells but were less common, 5 per cent of the total fragments (see Figure 5 a, e-f). Axial rearrangement of chromosomes (Figure 5 h) was occasionally seen.

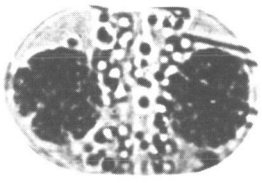
Bilobed generative nuclei were seen in one control and in twelve experimental immature microspores taken from buds that had been vibrated at 80 or 100 Hz and 14, 6, or 20 g (Figure 5 i-l).

Table I gives all the experimental data.

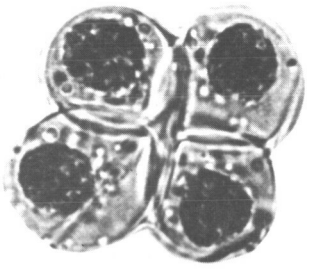
From these data it was calculated that, on the average, 0.95 per cent of the scored experimental cells and 0.70 per cent of the control cells possessed one or more spherical fragments ($P<0.05$ level of significance).



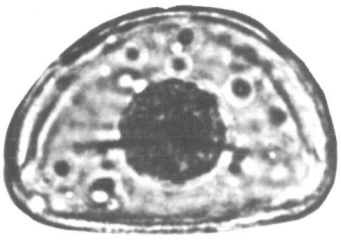
MOTHER CELL



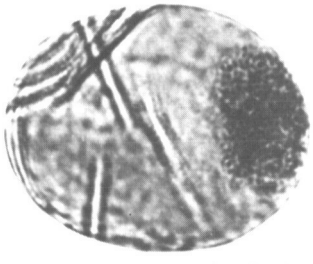
DIAD



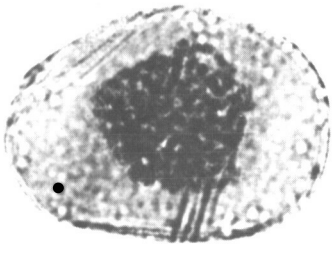
TETRAD



INTERPHASE-3



EARLY PROPHASE



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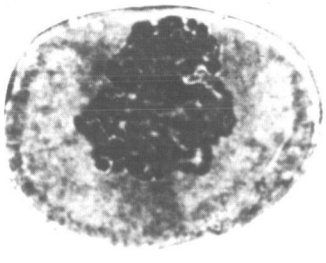

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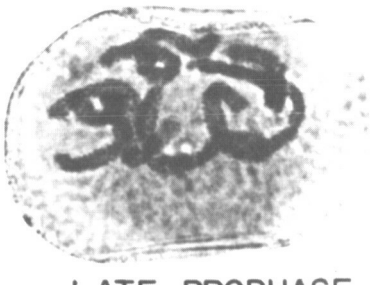
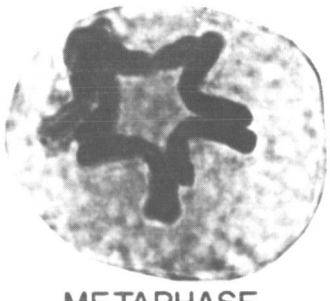
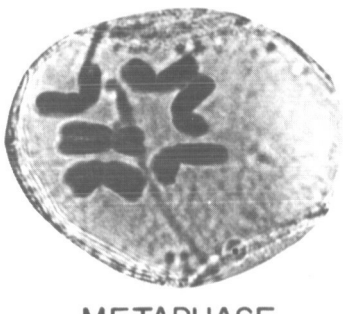


LATE PROPHASE



METAPHASE



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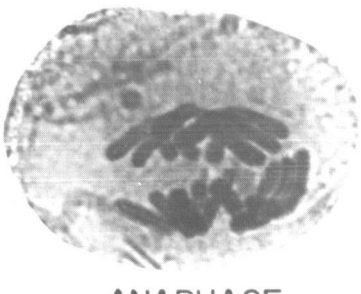
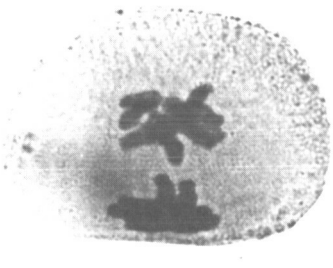
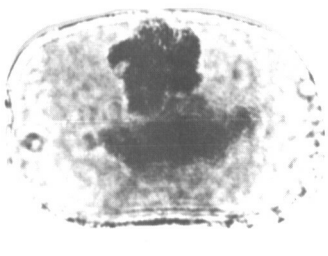


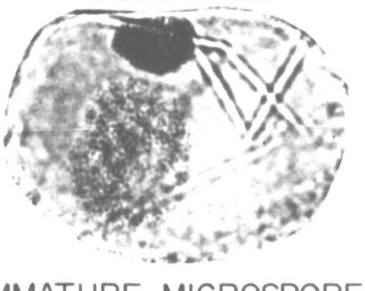
ANAPHASE



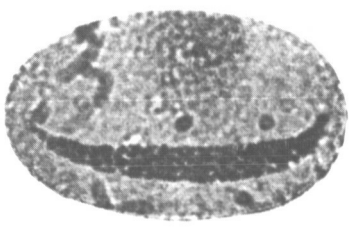
TELOPHASE



POST -TELOPHASE



IMMATURE MICROSPORE



MATURE MICROSPORE

Figure 4

Legend for Figure 4

Successive phases in the development of Tradescantia microspores.

SPORE MOTHER CELL: Cell in Interphase-1; length 30μ; nuclear diameter 20μ. First meiotic division (division-1) ends in Interphase-2 represented by DIAD: each nucleus diameter is 11μ. Second meiotic division (division-2) results in TETRAD (nuclei are same size as those in the diad). After each cell of the tetrad separates, the cell is referred to as INTERPHASE-3: cell length 33μ; nuclear diameter 12μ. The onset of the mitotic division (division-3) is indicated by movement of the interphase-3 nucleus to the cell's edge (EARLY PROPHASE). The cell subsequently goes through PROPHASE-3, METAPHASE-3, ANAPHASE-3, TELOPHASE-3. There is no cytokinesis, only karyokinesis. In the POST-TELOPHASE-3 stage, the generative nucleus moves to the cell's edge and the tube nucleus remains as a less darkened structure near the center. This is more evident in the IMMATURE MICROSPORE and MATURE MICROSPORE (cell length, 50μ) that have a spindle or crescent-shaped generative nucleus, the tube nucleus remaining obscure as a round, lesser staining body.

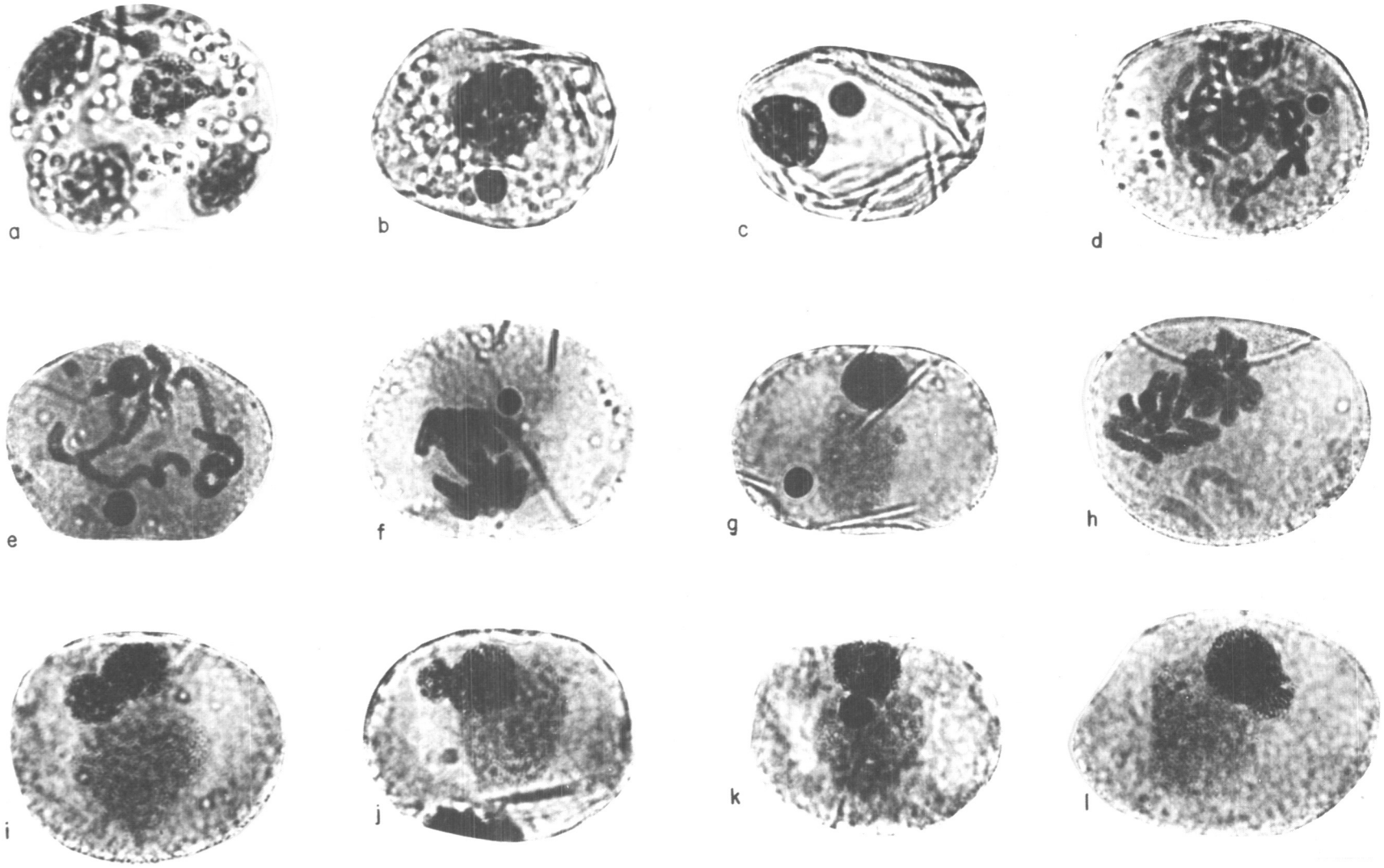


Figure 5

Legend for Figure 5

Cellular observations in Tradescantia anther smear preparations from vibrated as well as control specimens.

Spherical fragments found in tetrad (a), interphase-3 (b), prophase-3 (c-d), early metaphase-3 (e), anaphase-3 (f), and immature microspore (g); axial rearrangement at anaphase (h); bilobed generative nucleus of immature microspore (i-l).

Table I

Data From Vibrated Specimens of Tradescantia

Vibration Duration: Minutes	40 Hz				70 Hz				80 Hz						90 Hz		100 Hz			
	7.3g		20g		7.3g		20g		5g		10g‡		20g▲		30g		40g		20g		7.3g		14.6g		20g	
	T*	SF+	T	SF	T	SF	T	SF	T	SF	T	SF	T	SF	T	SF	T	SF	T	SF	T	SF	T	SF	T	SF
3	453	14	1043	15	1072	5	729	12	1781	26	3801	48	3868	53	957	5	5659	29	910	20	1870	7	1330	12	937	8
15			957	7			1020	12	2151	35	3325	32	3565	37	996	4	3890	34	1143	10	1229	6	688	3	609	5
30	668	2			1359	6																				
45			1016	6			875	1					810	3					725	9	1025	2	729	4		
60	533	2			762	2																				
Controls	1492	1	2135	10	4014	12	2346	16	3044	34	4495	33	5962	61	1267	6	4975	39	1805	17	2943	20	2000	10	1754	18

n of experimentals = 16. n of controls = 16.
Mean percentage of scored cells with spherical fragments in experimentals = 0.95%.
Mean percentage of scored cells with spherical fragments in controls = 0.70% .
Standard deviation of experimentals = 0 .39. Standard deviation of controls = 0.36.
"Students" value for t = 1.735. Degrees of freedom = 28. P = 0.05 level of significance.

*T = Total number of cells that were scored.

+SF = Number of scored cells with spherical fragments.

‡These figures represent a total of two series of vibrations.

▲These figures represent a total of three series of vibrations.

Figure 6 illustrates the relationship of time of vibration and the percentage of spherical fragments at different frequencies and accelerations.

Giant Cells

Larger than normal cells were seen in eight of the experimentals and in two of the controls. Their diameters were from 36 - 40μ, with most being in prophase-3. The nuclei were of normal size.

RESULTS AND DISCUSSION

The experimental conditions of Antipov, et al. (1) deviate in two main respects from the ones described in the present report. The Russian authors used specimen containers which allowed freedom of movement for the Tradescantia spores while our specimens were firmly coupled to the vibration inducing specimen holder. Only this last procedure allows judgment of the effect of selected vibration conditions. In a loosely coupled specimen the vibration of the specimen may grossly deviate from the vibrating source.

The second difference of the two experimental series might also influence the results considerably. The Russian investigators derived their results from actual space flight experiments (Table II) in which vibration was only one component, but it was implicated as a main factor since the greatest effects on Tradescantia were observed during powered flight. In the present report vibration was investigated as the only physical force on the biological specimen.

In our vibration experiments we found spherical fragments in both experimental and control groups; the Russian investigators did not find such abnormalities in their controls (see Table II). The frequencies with which such aberrations were found in our experiments were 0.70 per cent in the controls and 0.95 per cent in the experimentals. Figure 6 shows that, after 15 minutes' vibration, this effect is no greater in the vibrated than in the unvibrated specimens, which indicates that this aberration is caused very soon after the cells are subjected to vibration and also suggests a repair process.

Up to the present we have not discovered a critical frequency of resonance which would probably enhance spherical fragment formation, but an attempt is underway to search for this condition in the frequency range of 100-3500 Hz.

From studies of our photomicrographs, it appears that the spherical fragment material is derived from the nucleus. This is also the opinion of the Russian workers who have hypothesized that portions of the chromatin material are sheared off under certain conditions in space flight. It may be conjectured that these fragments are formed in the way pictured in Figure 5 i-k by lobulation and subsequent breaking away from the main body of the nucleus. This accounts for their formation only in the interphase and early prophase. We did not find chromatid or chromosomal fragments

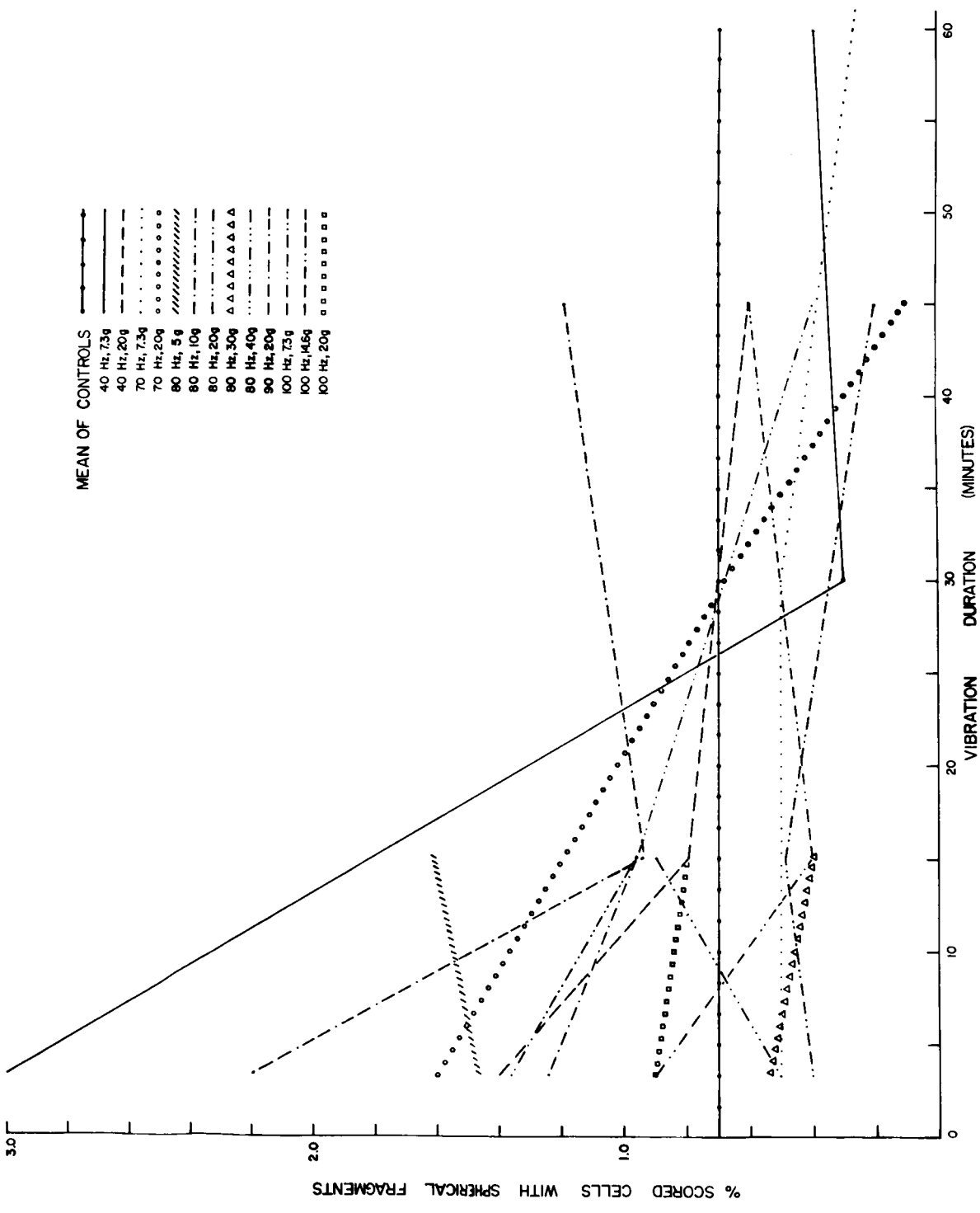

MEAN OF CONTROLS
40 Hz, 7.3g
40 Hz, 20g
70 Hz, 7.3g
70 Hz, 20g
80 Hz, 5g
80 Hz, 10g
80 Hz, 20g
80 Hz, 30g
80 Hz, 40g
90 Hz, 20g
100 Hz, 7.3g
100 Hz, 14.6g
100 Hz, 20g
% SCORED CELLS WITH SPHERICAL FRAGMENTS
3.0
2.0
1.0
0
10
20
30
40
50
60
VIBRATION DURATION (MINUTES)

Figure 6

Legend for Figure 6

Percentage of scored vibrated Tradescantia bud cells with spherical fragments in relation to duration of treatment. The line for the controls represents the mean.

Table II

Data of Russian Experiments with Tradescantia Exposed to Space Flight Factors Aboard Vostok Spaceships

Type of Aberration	Percentage* of Cells on Vostok Flight								
	1	3	4^+	4^+	Control	5^+	5^+	6	Control
Impairment of Mitotic Mechanism									
Displaced nucleus	0.25	0.25	0.42		0	0.38	0.76	0.70	0.20
Rosettes	0.40	0.50	0.84		0	0.56	0.94	0.90	0.10
Axial rearrangement	0.28	0.25	0.56	1.20	0	0.80	1.00	1.10	
Separation delay	0.05		0.28	1.20	0	0.08	0.20	0.09	
3- and 4- pole mitoses	0.01	0.80	0.20	0.40	0	0.07	0.10	0.20	
Immature microspore axial rearrangement		2.60	2.20	1.40	0	0.20	2.90	2.50	
Spherical Fragments	2.40	4.80	4.00	1.10	0	9.60	4.50	4.20	
Growth Impairment									
Giant cell				(4 cells)					
Appearance of germinating pollen			(2 cells)						

*Data derived from Delone, N. L., et al., Kosmicheskiye Issledovaniye, 1:200-224, 1963; and ibid, 2:233-251, 1964.

+Different times of fixing.

probably because our technique did not allow easy observation of such aberrations. Electronmicroscopic studies will be conducted in which the origin of the fragment material may be more readily recognized.

The smaller number of spherical fragments found by us may be due to the different coupling or to the absence of other factors of space flight like linear acceleration, radiation, and others.

CONCLUSIONS

Our data show an influence of short term, low frequency, sinusoidal vibrations (40 - 100 Hz and 5 - 20 g) on T. paludosa microspores. The effect is seen mostly in the form of spherical fragments, probably of chromosomal origin, appearing in the experimentals to a slightly greater degree than in the controls.

This implicates vibration as a contributing factor in the formation of nuclear abnormalities in space flight.

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13. ABSTRACT

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