UNCLASSIFIED

ORNL-101

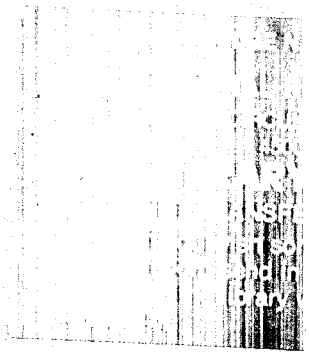
HEALTH & BIOLOGY-GENERAL

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES

3 4456 0360282 1

BIOLOGY DIVISION

THE CYTOGENETIC EFFECT OF SONIC ENERGY APPLIED SIMULTANEOUSLY WITH X-RAYS



OAK RIDGE NATIONAL LABORATORY

DIVISION OF

CARBIDE AND CARBON CHEMICALS CORPORATION

POST OFFICE BOX P

OAK RIDGE, TENNESSEE

UNCLASSIFIED

This documents of 10pages.
Copy 2 of 112 Series. A
ISSUED: 706-A- Library

UNCLASSIFIED

ORNL - 101
Health and Biology, General

THE CYTOGENETIC EFFECT OF SONIC ENERGY APPLIED SIMULTANEOUSLY WITH X-RAYS

by

Alan D. Conger

Biology Division

OAK RIDGE NATIONAL LABORATORY
division of
CARBIDE AND CARBON CHEMICALS CORPORATION

This document is based on work performed under Contract Number W-7405-eng-26 for the Atomic Energy Project at Oak Ridge National Laboratory.

July 19, 1948

Date Received: 7/21/48 Date Issued: 7/22/48

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES

3 4456 0360282 1

ORNL-101
Health & Biology-General

DISTRIBUTION:

1. G. T. Felbeck (C&CCC-N.Y.-N.Y.)
2-4. 706-A Library
5. 706-B Library
6. Training School
7. Biology Library
8. Health Physics Library
9-13. Central Files
14. E. J. Murphy
15. D. C. Bardwell
16. A. D. Conger
17. M. D. Peterson
18. A. M. Weinberg
19. A. Hollaender
20. H. Etherington
21. J. A. Cox
22. W. A. Johnson
23. C. D. Cagle
24. S. R. Sapirie (AEC-Townsite)
25. H. M. Roth (AEC-Townsite)
26. C. N. Rucker
27-36. Argonne National Laboratory
37. Armed Forces Special Weapons Project
38-39. Atomic Energy Commission, Washington
40. Battelle Memorial Institute
41-48. Brookhaven National Laboratory
49-52. Carbide & Carbon Chemicals Corporation (K-25 Area)
53-56. Carbide & Carbon Chemicals Corporation (Y-12 Area)
57. Columbia University (Failla)
58-61. General Electric Company
62-68. Hanford Directed Operations
69. Iowa State College
70-71. Kellex Corporation
72-74. Los Alamos
75. Massachusetts Institute of Technology
76-77. Monsanto Chemical Company, Dayton
78. National Bureau of Standards
79. Naval Radiological Defense Labs.
80. NEPA
81-83. New York Directed Operations
84. Patent Advisor, Washington
85-99. Technical Information Division, ORDO
100. UCLA Medical Research Laboratory (Warren)
101-105. University of California Radiation Laboratory
106-107. University of Rochester
108-111. Western Reserve University (Friedell)
112. AEC, Oak Ridge National Laboratory

ABSTRACT

Conger, Alan D. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.:

The Cytogenetic Effect of Sonic Energy Applied Simultaneously with X-rays.

An experiment has been performed to test the influence of sonic energy on the yield of X-ray induced chromosomal aberrations in a clone of Tradescantia paludosa. Chromosomal aberration frequency was determined at the microspore mitosis from aceto-carmine smears made 4 days after treatment.

Inflorescences were immersed in water contained in a steel cup used for the sonic treatment. The bottom of the cup was a vibrating diaphragm which transmitted the sonic energy to the plant buds via the water. The instrument used was a Raytheon Mfg. Co. magnetostriction oscillator, run at 9,100 cycles/sec. and at a power of 30 watts. Two treatments were given to inflorescences inside the sonic cup: a) 250 r X-rays (78 r/min.) as measured in air outside the cup, and B) 250 r X-rays (as above) plus sonic treatment begun with and continued for 5 minutes after the X-radiation ceased.

The frequency of all types of chromosome aberrations was higher when the sonic treatment was given in addition to the X-rays. The ratio of X-ray plus sonic energy/X-ray aberrations was 1.3/1 for both exchanges and simple deletions. The sonic treatment alone, as given here, did not cause aberrations.

This increased yield of aberrations is probably due to an increased amount of chromosome movement caused by the transmission of sonic energy through the cells during and after the X-radiation; movement would separate the broken ends from a single chromosome break (reducing restitution) and bring together broken ends from different breaks (increasing new reunions)—both would increase aberration frequency.

THE CYTOGENETIC EFFECT OF SONIC ENERGY APPLIED SIMULTANEOUSLY WITH X-RAYS*

by

Alan D. Conger

It is generally agreed that the number of chromosome breaks initially produced by a given dose of X-rays is independent of conditions at the time of radiation. But the subsequent yield of observable chromosomal aberrations from a given number of initial breaks depends upon the ratio of those breaks which restitute (and are not detectable) to those which do not rejoin or which rejoin in detectable new associations. The majority of the breaks produced by X-radiation, under normal conditions, seem to rejoin in their original position and do not form observable aberrations[3].

Any factors which affect this process of rejoining will influence the yield of chromosomal aberrations obtained from a given X-ray dose. Several treatments used in conjunction with X-rays have been shown to increase (or decrease) the yield as compared with that obtained from the same dose of X-rays alone. The results of some of these treatments are summarized in Table I:

Table I

	Effect on X-Ray Aberration Frequency				
Treatment	Effect Alone	Before	During	After	Reference
Centrifugation	0	x	(2/1)*	0	(5)
Temperature (3°/36°)	0	0	(4/1)	0	(4)
Colchicine	0	(1/3)	x	x	(2)
Ultraviolet		(1/2.5)	x	(1/2)	(8)
Infrared	0	(2/1)	x	(2/1)	(9)

* ratio, X-ray + treatment/X-ray alone
x = not studied

In addition to the treatments above, the effect of the stage of division at the time of radiation, genetic differences, and differences between different tissues of the same individual have been studied[6].

Most of these artificial or natural influences (except ultraviolet and infrared) seem to produce their effect on the yield of chromosomal aberrations by altering somehow the amount or freedom of chromosome movement. An increase in the amount of movement after chromosomes have been broken by X-rays would enable the broken ends from one break to be separated from one another (reducing restitution) and would bring broken ends from different breaks together (increasing new reunions); either would increase the yield of aberrations. These considerations, and the work which had been done with centrifugation, suggested that sonic or supersonic energy would be a means of applying mechanical agitation to cellular structures during radiation, thereby increasing the yield of chromosomal aberrations.

Experimental Methods:

Inflorescences of a clone of Tradescantia paludosa Anderson & Woodson were subjected to simultaneous X-ray and sonic treatment. Aceto-carmine smears of the anthers were made 4 days after treatment, and the frequency of chromosomal aberrations determined at the microspore mitosis. X-rays were delivered at 250 KV peak, 15 ma, filtered through 1/2 mm. Cu and 1 mm. Al. The average intensity, measured with a Victoreen dosimeter in air at the same position as the buds were when exposed to radiation, was 78 r/minute. A dose of 250 r was given in all experiments.

Sonic treatment was begun with the X-radiation and continued for 5 minutes after radiation ceased. The sonic treatment was administered by a Raytheon Manufacturing Company Model R-22-3 magnetostriction transducer connected to an electronic oscillator operating at about 9,100 cycles/second.[7] The material was treated in a stainless steel cup, the bottom of which was

a diaphragm connected directly to a nickel magnetostriction rod which alternately contracted and expanded in an alternating electromagnetic field. The sound energy generated by the cup diaphragm was transmitted to the Tradescantia buds by water 3.4 cm deep (30 cc) in which the inflorescences were immersed, upside down, the center of the buds being 3 mm. beneath the surface of the water through which the sonic vibrations were transmitted. Temperature was kept constant by a continuous flow of tap water (18° C.) through the water jacket of the double walled sonic cup. Inflorescences were held in place during treatment by inserting the stem base of the inflorescence through a hole in a thin lucite cap fitted over the top of the cup. The machine was always operated at the same voltage level across the transducer, 30 volts, and at the same frequency, about 9,100 cycles/second. The sonic cup, with the flower buds held in position in the water, was centered under the X-ray tube; in both the control series (X-rays only) and the treated series (X-ray plus sonic energy), buds were held in the same position in the water inside the sonic cup during treatment. The relative X-ray dose received by the buds in the sonic and control series was the same; however, the absolute dose, due to scattering by the lucite cap, water and steel cup, may not have been the same as the 250 r measured by a dosimeter in the same position in air.

Results:

A series of six experiments was made. Since the results of all are essentially the same the data have been combined, and are presented in Table 2.

Table 2

Yield of 4-day chromosomal aberrations per 100 cells.
250 r X-rays (av. 78 r/min.): av. 7 1/2 minutes sonic treatment

Treatment	No. of Buds	No. of Cells	Exchanges[1]		Deletions[2]	
			no.	per 100 cells	no.	per 100 cells
X-rays + sonic	21	1481	547	36.9 ± 4.6	751	50.7 ± 6.3
Control (X-ray alone)	8	650	189	29.1 ± 2.0	261	40.2 ± 2.5
Diff. (Sonic) - (X-rays) ± S.E. Diff.				7.8 ± 2.5		10.5 ± 1.0
Ratio. Sonic/X-ray				1.27/1		1.26/1

1 Dicentrics + tricentrics + centric rings
2 Terminal + small isodiametric fragments (acentric rings excluded)

Previous experiments had shown that the sonic treatment alone, as given here, did not cause detectable chromosomal aberrations*. The yield of X-ray induced aberrations has been increased by the application of sonic energy; the differences between the sonic treated and X-ray controls are statistically significant for both the one- and two-hit aberrations (difference > 3 times S.E. diff.).

Discussion:

The increase in aberration yield is somewhat less than that obtained by other treatments, where the ratio of treated/control has been about 2/1 for centrifugation, and infrared, about 4/1 for cold treatment. It is

* A recent experiment[10] reports the production of chromosomal aberrations, mutations, and other effects in Allium root tips, Helianthus stem apices, and Drosophila by treatment with ultrasonics alone. However, the treatment was quite different in frequency (400,000 cycles/sec.) and in other factors from the sonic exposures reported here.

believed that the smaller effect found with sonic energy, as administered in these experiments, is due to the fact that it is difficult to deliver a sonic dose intense enough to produce the effect desired without causing a disruption of the cells or entire tissue, making further observation impossible. An intensity of 30 volts across the transducer was empirically determined as the maximum intensity which could be used, and still have the buds and anthers survive intact. In these experiments, cells have been observed as much as 5 days after treatment in which the entire nucleus had been disrupted into numberous small droplets scattered throughout the cell, in other cases the nucleus had been condensed into a solid, amorphous pycnotic globule. It is interesting that some cells, 5 days after treatment, though still intact and at the same stage of cellular development as normal cells, had no vestige of the nucleus or nuclear fragments remaining, though the cytoplasm was uniformly darker stained than nearby normal cells. Apparently the nucleus was almost completely dispersed throughout the cells, yet 5 days of development and growth of the microspore ensued in such cells with a dispersion rather than an organization of genic material. There can be no doubt, however, that the transmission of sonic energy does cause movement of cellular constituents without disrupting the cell; such effects have been described in early biological studies made with sonics and supersonics.[1] Further, an X-ray induced chromosome fragment, because of its smaller mass and surface area, should have a smaller viscous drag and therefore be displaced by sonic vibration more than the heavier and larger chromosome from which it came. This may be observed qualitatively for isodiametric deletions ("minutes", or "dots") which following X-ray treatment alone are generally associated quite closely with the chromosomes; after combined X-ray and sonic treatment, the deletions are often completely dissociated from the chromosomes.

It is thought that by refinements in the method of treatment, and perhaps by using ultrasonic energy of much shorter wavelength than used here (9,100 cycles/sec. = about 16 cm. wavelength in water) greatly enhanced yields of aberrations may be obtained; if the problem of inducing considerable vibrational movement of cellular constituents without causing disruption of the cell wall can be solved, this should be possible. Ultrasonics with wavelengths of the order of magnitude of cell dimensions may be the way of doing it.

Summary:

The cytogenetic effects of simultaneous treatment of Tradescantia inflorescences with X-rays (250 r) and sonic energy (9,100 cycles/sec.) are described. Simultaneous treatment with sonic energy and X-rays increases the yield of X-ray induced chromosomal aberrations about 1.3 times the yield obtained with the same amount of X-rays alone. The sonic treatment alone did not cause aberrations. The increased yield is probably due to an increased movement of chromosomes and chromosome fragments caused by the sonic treatment, resulting in a decrease in the amount of restitution and an increase in the amount of detectable new reunions between the broken ends of chromosomes.

1. Bergmann, L., Ultrasonics, Their Scientific and Technical Applications, Chapter V, New York, John Wiley & Sons (1938)

2. Brumfield, R. T., P.N.A.S., 29: 190-193 (1943)

3. Lea, D. E., Actions of Radiation on Living Cells, New York, MacMillan Co. (1947)

4. Sax, K., Genetics, 32: 75-78 (1947)

5. Sax, K., P.N.A.S., 29: 18-21 (1943)

6. Sax, K., and C. P. Swanson, Amer. J. Bot. 29: 52-59 (1941)

7. Shropshire, R. F., J. Bact., 53: 685-693 (1947)

8. Swanson, C. P., Genetics, 29: 61-68 (1944)

9. Swanson, C. P., and A. Hollaender, P.N.A.S., 32: 295-302 (1946)

10. Wallace, R. H., R. J. Bushwell, and E. H. Newcomer, Science, 107: 577-8 (1948)