An. Inst. Biol. Univ. Nal. Autón. Méx. 49 )1978), Ser. Biol. Exp. (1): 33-39. 28-XII-1982

# THE MICRONUCLEUS TEST APPLIED TO *Vicia faba* ROOT MERISTEM CELLS EXPOSED TO X-RAYS OR ULTRASOUND

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## ABSTRACT

X-irradiation (200R) but not ultrasound (1.1 MHz, 8 W/cm² spatial peak intensity, 1 min continuous wave exposure) induced a statistically significant increase in the frequency of micronuclei in root meristem cells of *Vicia faba* 6 to 36 hours post exposure. The distribution of autoradiographic $^{3}H$-label among cells with nuclei and micronuclei for control and X-irradiated cells was comparable.

The data further confirm that (1) a clinically used level of ultrasound does not appear to induce breakage types of chromosomal anomalies and (2) micronuclei can perform DNA synthesis, often in concert with the parental nucleus.

## RESUMEN

Se comprobó en el material tratado con rayos x (200 R), pero no al que se aplicó ultrasonido (1 minuto de exposición a ondas continuas de 1.1 MHz con valor máximo de intensidad de 8 W/cm²), la inducción de un aumento estadísticamente significativo de micronúcleos en las células meristemáticas de la raíz de *Vicia faba* desde las 6 horas hasta las 36 horas después de la exposición. Fue similar la distribución autorradiográfica del $^{3}H$ en las células con núcleos y micronúcleos de los testigos y de los tratados con rayos X.

Los datos confirmaron que: (1) el nivel de ultrasonido utilizado en la clínica no parece inducir anomalías cromosómicas y (2) aunque generalmente los micronúcleos llevan al cabo su síntesis de DNA simultáneamente con los núcleos a los que están asociados, no siempre sucede eso.

## INTRODUCTION

Attempts to induce classical "breakage" type chromosome aberrations with clinical ultrasound have been largely negative. MacIntosh and Davey (1970, 1972) initially reported such aberrations which a subsequent experiment conducted "double blind" failed to confirm (MacIntosh *et al.*, 1975). Other reports have indicated an absence of ultrasonically induced chromosome aberrations (Buckton and Baker, 1972; Coakley *et al.*, 1972; Hill *et al.*, 1972; Watts *et al.*, 1972). Unfortunately, the number of mitotic cells scored in most experiments has been small, typically between 50 and 150 cells. A number of reports suggests, however, that at

certain levels ultrasound induces non-breakage types of chromosomal aberrations such as bridged agglomerated and laggard mitotic figures (Gregory *et al.*, 1974; Cataldo *et al.*, 1973; Kokhar and Oliver, 1975; Lehman *et al.*, 1954; Newcomer and Wallace, 1949; Selman, 1952; Slotova *et al.*, 1967; Woeber, 1951, and Woeber, 1952).

The loss of acentric chromosomal fragments from metaphase complements results in the formation of chromatin-containing micronuclei in post mitotic interphase cells. The presence of acentric chromosomal fragments at mitosis and their subsequent involvement in the formation of interphase micronuclei indicate that acentric chromosomal fragments are capable of performing at least those condensation functions related to mitosis, and subsequent chromosomal despiralization and nuclear envelopment processes requisite for nucleus formation. For example, a $G_1$ X-irradiated cell must pass through S and $G_2$ to reach mitosis; the presence of chromosome fragments at metaphase indicates survival of the chromosome fragments for at least one interphase to mitosis transit. That these acentric chromosomal fragments are capable of entering mitosis and reentering interphase in the form of a micronucleus is evidence of some functional ability.

A variety of physical and chemical stresses has been used to induce classical "breakage type" chromosomal aberrations and micronuclei. Boller and Schmid (1970), Matter and Schmid (1971), Heddle (1973), and von Ledebur and Schmid 1973) independently developed a "micronucleus test" which, in comparative studies, indicates a good correlation between incidence of micronuclei and chromosomal aberrations obtained under similar conditions (Boller and Schmid, 1970: Muller *et al.*, 1972; Heddle, 1973). The micronucleus test involves scoring the frequency of micronucleus occurrence in a population of cells. The advantages of the "micronucleus test" for screening of a suspected chromosomal mutagen are that "it is reliable, easy, and very much more rapide than the traditional method" (Heddle, 1973); also relatively large numbers of cells can be scored.

The hypothesis tested in the present research was that if ultrasound at certain clinically used levels does not induce breakage type chromosomal anomalies, then there should be no difference in frequencies of micronuclei between control and sonicated cells. The presence of micronuclei in somatic cells is normally an infrequent event. The purpose of this study was to increase information on the potential mutagenicity of clinical ultrasound. A set of experiments was also conducted with a mutagenic level of X-irradiation to check that a known mutagenic agent could induce micronuclei formation in the cell system being used (the cells of the root meristem of *Vicia faba*). As a corollary, the distribution of $^3$H-label over cells with nuclei and micronuclei from control and X-irradiated roots was determined.

## MATERIALS AND METHODS

Seeds of *Vicia faba* (obtained from Suttons Seeds, Reading, England) were used for all experiments. Seeds were soaked overnight in running tap water and then planted in trays containing moist vermiculite (19°C ± 1°). Five days later, a

population of seedlings with roots measuring 10-13 cm was selected. After seed coats and plumules were removed, the remains of the seedlings were transferred to a 20-liter tank containing continuously aerated Voth and Hamner's (1943) No. 5 inorganic nutrient solution. Roots were grown for 2 days in the nutrient solution before experimentation.

Roots were then exposed to either X-rays or ultrasound (or sham exposed) and then returned to the growth tanks. At 0, 6, 12, 18, 24 and 36 hours post-exposure root meristems were fixed overnight in 3:1 ethyl alcohol: glacial acetic acid, Feulgen stained, squashed and adhered to the slides by the dry ice method of Conger and Fairchild (1963). Slides were scored "blind" for the frequency of micronuclei per 1,000 cells; each meristem was squashed onto one slide. There were 3 slides per fixation interval per treatment.

X-irradiations were from a GE Maxitron operated at 300 kVp at 20 mA; the h.v.l. was 1.5 mm Cu, the intensity was 140 R/min, and the total dose was 200R. Ultrasound was delivered by means of a ceramic transducer powered by an oscillator and amplifier (Miller *et al.*, 1975). The frequency employed was 2 MHz, the spatial peak intensity was 8 W/cm$^2$ continuous wave, as determined by the deflection of a steel ball radiometer (Dunn and Fry, 1972). The duration of exposure was 1 min. in a clear Lucite chamber filled with nutrient solution. The transducer was at the bottom of the chamber, pointed upward, and each root was placed 12 cm above the transducer and the meristem centered in the sound field. A rubber absorber was placed above the root to reduce acoustic reflections.

For determination of nuclear DNA synthesis some roots were exposed for 2 h to 2 $\mu$Ci/ml $^3$H-thymidine ($^3$H-TdR) (6.7 Ci/mM specific activity) before fixation; autoradiographs were prepared by dipping the slides in Kodak NTB-3 liquid emul-

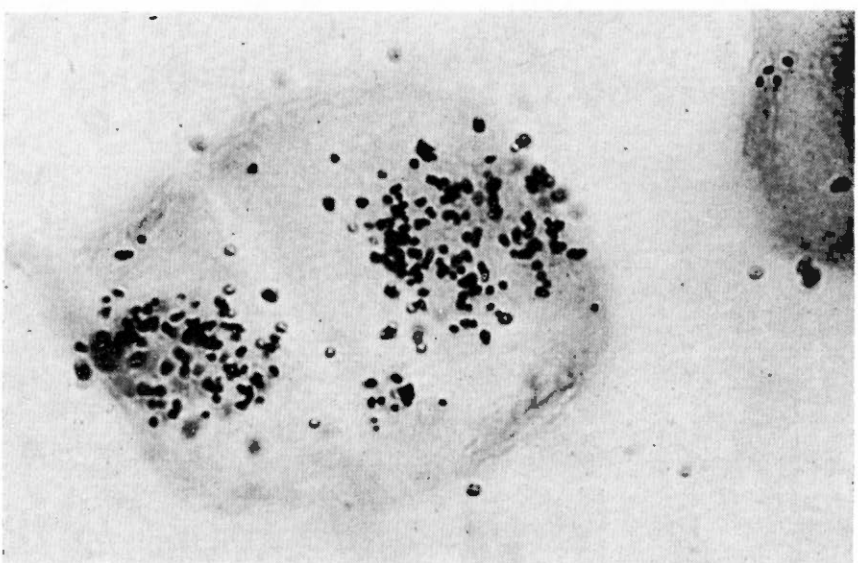

Figure 1. A $^3$H-labeled interphase cell in which the micronucleus (arrow) is also $^3$H-labeled (2300X).

sion, dried, placed in light tight slide boxes, stored at 4°C for 12 days in the dark, and then developed using Kodak D-19 developer and fixer. Slides were scored "blind" for the location of $^3$H-label in $^3$H-labeled interphase cells with micronuclei. Nuclei with $\geq$ 20 $^3$H-grains were scored as "$^3$H-labeled"; micronuclei had to have at least 3 $^3$H-grains to be scored as "$^3$H-labeled" (Fig. 1); nuclear grain counts fewer in number led to ambiguity of micronuclear $^3$H-labeling characteristics.

## RESULTS

There were no apparent differences between control and sonicated root meristem cells concerning the frequency of micronuclei/1000 cells ($p > 0.05$) sampled at 12, 18, 24, 30 and 36 h post-sonication (Fig. 2). On average there are about 4 micronuclei/1000 control cells for each of the six treatments fixation intervals. A previous study (Miller *et al.*, 1975) had demonstrated that cell progression was initially slightly perturbed (3.2% mitotic index at 6 h post-sonication) with exposure to 2.2 MHz 8 W/cm$^2$ ultrasound, but that recovery to near control mitotic index occurred within 17 h.

For X-irradiated root meristems the yield of micronuclei rises from control levels immediately after radiation exposure to about 85 micronuclei at 12 h post-X-

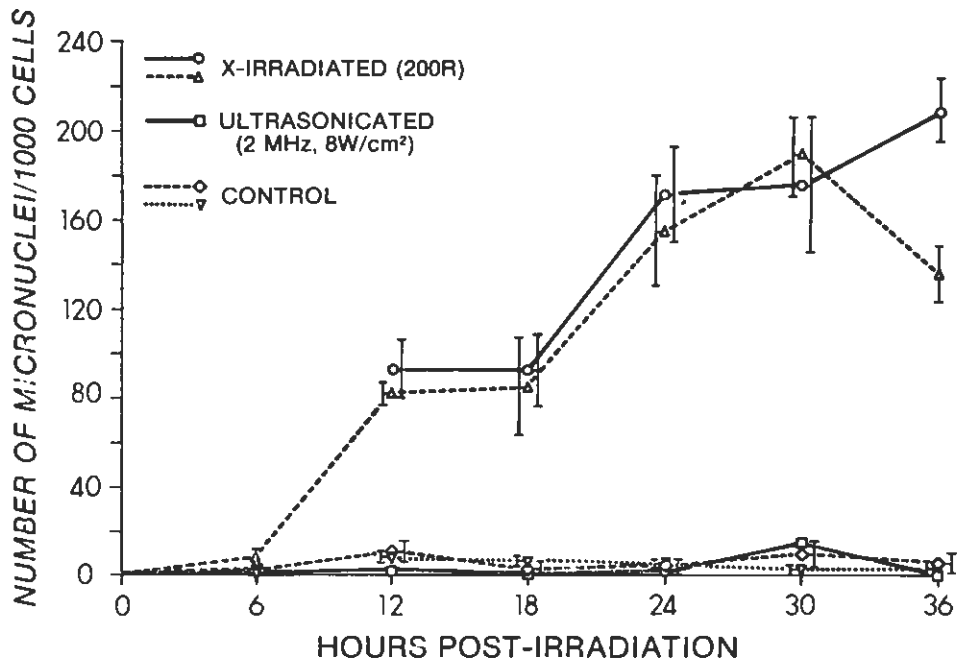


Figure 2. The relation between number of micronuclei per 1000 meristematic cells and post-irradiation fixation interval (h) for control, sonicated, and X-irradiated roots. The points represent mean $\pm$ S.E.M.

irradiation to about 170 micronuclei at the 24-36 intervals. In comparison to controls, these values are highly significantly different ($p < 0.001$). Similar results were reported by Evans *et al.* (1959) for *Vicia faba* root meristem cells exposed to 188 rad.

The relation between the nucleus and its micronucleus for DNA synthesis is shown in Table 1; the numbers represent the total for all the fixation intervals (3 slides/fixation interval/regimen). Por control cells with micronuclei 68.6% had both the nucleus and micronucleus $^3$H-labeled, 21.5% had only the nucleus $^3$H-labeled, and 9.9% had only the micronucleus $^3$H-labeled. For X-irradiated cells, while the frequency of micronuclei dramatically increased (Fig. 1) above control values, the relative distribution of $^3$H-label among the nucleus-micronucleus categories was similar to that of control values; approximately two-thirds of the $^3$H-labeled nuclei wtih micronuclei also had the $^3$-label associated with the micronucleus. A small percentage of cells had the micronucleus $^3$H-labeled but not the nucleus.

TABLE I

TOTAL NUMBER (%) OF AUTORADIOGRAPHICALLY $^3$H-LABELED* CELLS *WITH* MICRONUCLEI FROM CONTROL AND X-IRRADIATED (200 R) ROOT MERISTEMS.

| *Regimen (Fixation Intervals)* | *N* + Mn** | *N* + Mn* | *N + Mn** |
|---|---|---|---|
| Control (12, 18, 24, 30, 36) | 118 (68.6) | 37 (21.5) | 17 (9.9) |
| X-irradiated (12, 18, 24, 30, 36) | 489 (62.9) | 247 (31.8) | 41 (5.3) |

Cellular $^3$H-labeling* pattern of cells with nuclei (N) and micronuclei (Mn):

N* + Mn* = nucleus *and* micronucleus $^3$H-labeled; N* + Mn = only nucleus $^3$H-labeled; N + Mn* = only micronucleus $^3$H-labeled.

The clinical use of ultrasound involves ultrasonic frequencies of 1-20 MHz and spatial average intensities from a few milliWatts per square centimeter (mW/cm$^2$) (diagnostic applications) to a few Watts per square centimeter (W/cm$^2$) (therapeutic applications). The ultrasonic exposure used in the present study was about 1000X greater than that used in diagnosis and about 8X greater than that used in therapy. The absence of a statistically significant increase in micronuclei from sonicated proliferating meristem cells is further evidence for the absence of ultrasonically-induced breakage type chromosomal aberrations previously reported (Buckton and Baker, 1972; Coakley *et al.*, 1972; Hill *et al.*, 1972; MacIntosh *et al.*, 1975; Watts *et al.*, 1972; Miller *et al.*, 1975). That the *Vicia faba* system is, however, capable of forming micronuclei after treatment with a know chromosomal mutagen (X-rays) was confirmed.

Thus, the one main point that emerges from the present data is a confirmation that ultrasound at a clinically used intensity and frequency does not appear to induce breakage type of chromosomal anomalies in plant root meristem cells.

The appearance of $^3$H-label over micronuclei after $^3$H-thymidine application suggests that chromosomal fragments, in addition to being able to form chromosomes at mitosis and "nuclei" at interphase, are also capable of performing post-mitotic macromolecular synthesis. The presence or absence of $^3$H-label during the nuclear DNA synthesis indicates that the timing of micronuclear DNA synthesis does not always correspond with parental nuclear DNA synthesis. Such occurrences may represent an additional illustration that not all nuclear chromosomal material replicates simultaneously (e.g., *vide* Church, 1965; Miller, 1976; McLaren, 1972).

## ACKNOWLEDGEMENT

This paper is based on work performed under contract with the U.S. Department of Energy at The University of Rochester Department of Radiation Biology and Biophysics and has been assigned Report No. UR-3490-1525. This work was also partially supported by USPHS FD00694.

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