Newsletter of Environmental Mutagen Soc. No. 3, 25-26 (1970)

Brain cells and embryonic cells showed fine intracytoplasmic vacuoles and granules, changing cell outlines, rounding up, and detachment from the surface and destruction of the monolayer. These cytopathogenic effects could be observed within 1 hr, reached a peak at 2 hr, and maintained the same conditions at 18 hr. The cell-killing effect of nicotine increased abruptly above 250 μg/ml in 2 hr both in brain cells and embryonic cells (Table 1). In the case of tissue culture cells, the cytopathogenic effects were regarded as more reliable, more accurate, and more significant than the cell-killing effects; thus the cytotoxic dose 50 (CTD_{50}) was obtained by plotting the dose-effect curve on log-probit paper according to the method of J. T. Litchfield, Jr. and F. Wilcoxon [4] (Table 2). The CTD_{50} of nicotine on brain cells was 140 μg/ml for 2-hr treatment and 155 μg/ml for 18-hr treatment. The CTD_{50} of nicotine on mouse embryonic cells was 135 μg/ml for 2-hr treatment and 100 μg/ml for 18-hr treatment. Statistical analyses indicate no significant difference in cell-damaging activity of nicotine between brain cells and embryonic cells or between 2-hr and 18-hr treatments. Exposure of these cells to 1-2 μg/ml of nicotine, which could be comparable to a smoking dose of 1-2 mg/kg, did not bring any significant cytologic change ($p<0.05$ for all cases).

Compared with the LD_{50} of nicotine on newborn rats, which was reported [2] as 14.55 mg/kg, our CTD_{50} is just 10 times the LD_{50}. It is well-known that nicotine has a tendency toward high accumulation in the brain, and calculation shows that nicotine uptake in 5 min after intravenous injection of 2 mg of ^{14}C-nicotine [3] is 13.8 μg/g tissue of mouse brain, and that is just one-tenth of our CTD_{50}. Even though the dosage level was much greater than would hold for the most inveterate nicotine inhaler and though culture cells cannot be compared reasonably and directly with chronic smokers, it could be assumed that the culture cells could not pick up all the nicotine added to the medium, and that the living embryonic cells, in fact, could show such cytotoxic effects even with a smoking dose of nicotine. This is in view of the fact that our nicotine dose was calculated per ml of medium, cells were exposed just once, and treatment was short in duration.

YOUNG JOE KWON
GEORGE H. MICKEY

New England Institute
Ridgefield, Connecticut 06877

References

1. Public Health Service Review, The Health Consequences of Smoking. Dept. of Health, Education and Welfare, USPHS, 1967, p. 185.
2. King, J. E. and Becker, R. F., *Amer. J. Obstet. Gynecol.* 95, 508 (1966).
3. Schmiterlöw, C. G., Hansson, E., Andersson, G., and Appelgren, L. E., *Ann. N. Y. Acad. Sci.* 142, 2 (1967).
4. Litchfield, J. T., Jr. and Wilcoxon, F., *J. Pharmacol. Exp. Ther.* 96 99 (1949).

Chromosome breakage in cultured Chinese hamster cells induced by radio-frequency treatment

George Mickey Mickey

The chromosome-breaking ability of radio-frequency radiation has been demonstrated in garlic root tips [1,2,3]. Mammalian chromosomes are also susceptible to breakage by radio waves, as shown in human lymphocytes and Chinese hamster lung cultures [3]. This report describes techniques used and the degree of damage induced in these cultured dividing cells.

The established cultures used were from the "Don" strain of male Chinese hamster lung cells. They were grown in modified McCoy's medium containing 20% fetal calf serum. Stock cultures were cultivated in a Bellco cell production roller apparatus in bottles (225 cm^2) with the aid of Bellco "Rotadapters." Log phase growth was maintained by subculturing every 24 hr and incubating at 37°C. The division cycle occupies 12 hr under these conditions.

Trypsinized cells were concentrated by centrifugation and transferred to Falcon plastic Petri dishes (35 X 10 mm). Approximately 4 X 10^4 cells were planted on a 22-mm square cover glass in the Petri dish. Subsequently about 3 ml of growth medium was added very carefully so as not to disturb the cells. The cultures were incubated at 37°C for 24 hr in 5% CO_2 atmosphere at saturated humidity.

Just before treatment the growth medium was removed; the cells were then washed with warm Hank's balanced salt solution and barely covered with Hank's BSS to prevent drying out during treatment.

All cultures were treated in the same fashion, the only variables being the frequency of radio waves and times of recovery after treatment. Every experimental culture was matched with a control culture that received identical treatment except for exposure to radio frequency.

After exposure the Hank's BSS was replaced by 3 ml of the McCoy's growth medium and the cultures were returned to the CO_2 incubator for the length of the recovery periods: 0, 6, 18, 24, and 30 hr.

Most cultures were subjected to treatment with Colcemid, final concentration of 10^{-6} *M* for 2 hr, were placed in hypotonic solution for 20 minutes, and then fixed in 3 parts methanol to 1 part glacial acetic acid. The fixed cells were air dried on the cover glass, stained in 1% aceto-orcein, and mounted in euparal. Thus all the cells, both dividing and nondividing, were preserved for examination and scoring under phase-contrast microscopy.

During treatment, the Petri dishes containing the cell cultures were placed in a capacitor gap 12 mm wide. The capacitor plates were made of copper and were 55 mm in diameter. Cultures were exposed to radio-frequency energy at the following frequencies: 15, 19, 21, and 25 MHz. All were exposed for 30 min, with 50 microseconds pulse width, 100 repetitions per second, and 3000 volts peak to peak per centimeter. Field strength varied from 1.5 to 5.5 and the dose was calculated to be approximately 50 milliwatts per cm^2.

Fourteen experiments (including matched controls) were scored; this involved analysis of 12,463 cells. Observations recorded for each slide included: the number of dividing cells vs. the number of nondividing cells; the number of diploid, tetraploid, octoploid, or aneuploid mitoses; chromatid breaks; isochromatid breaks; translocations; ring chromosomes; dicentric chromosomes; gaps; and anaphase bridges. For calculating the total number of breaks, a chromatid or chromosome break was scored as one break; each translocation, ring chromosome, or dicentric chromosome was scored as two breaks. Chromatid gaps and anaphase bridges were omitted from calculations.

Under conditions of these experiments, radio-frequency treatment at 19 and 21 MHz produced significant numbers of chromosome breaks in Chinese hamster cells grown in tissue culture. Few breaks appeared immediately after treatment, but many occurred at 3 and 6 hr of recovery, which was during the first division after treatment; fewer breaks appeared during the second division; and the incidence of breaks after 24 or 30 hr of recovery (during subsequent divisions) dropped to the control level.

Incidence of the different types of chromosome aberrations, from most prevalent to least, was as follows: chromatid breaks, chromosome breaks, ring chromosomes, translocations, acentric fragments, and dicentric chromosomes.

The only valid comparisons must be between experimental and control groups within each experiment. Thus, in one experiment (19 MHz and 3-hr recovery period) the experimental value was 1.55% vs. 0.081% in the controls; in another (21 MHz and 6-hr recovery period) there were 1.2% breaks in the treated cells and none in the control cells; and in another (21 MHz and 18 hr recovery) there were 0.96% breaks in the treated cells but only 0.062% in the controls. The lowest number appeared after treatment with 15 MHz radio frequency, which produced no discernible breaks.

There was no correlation between the percentage of tetraploid or octoploid cells and either the radio frequency or the recovery period. Matched pairs usually showed similar values.

This work was supported by ONR Project No. NR101-756, and by the John A. Hartford Foundation, Inc.

GEORGE H. MICKEY
LOLA KOERTING

The New England Institute
Ridgefield, Connecticut 06877

References

1. Mickey, G. H., *N.Y. J. Med.* 63 1935 (1963).
2. Heller, J. H. and Mickey, G. H., in *Digest, International Conference on Medical Electronics*. New York, 1961, p. 152.
3. Heller, J. H. in *Cellular Effect of Microwave Radiation*, Symposium on the Biological Effects and Health Implications of Microwave Radiation, in press.

Flavin-sensitized reversal of UV photoproduct of DNA

Riboflavin and flavin mononucleotide were found to be capable of mediating the reversal of a photoproduct formed in cellular or purified DNA by far-UV radiations. The reversed photoproduct is thought to be pyrimidine dimer on the basis of spectrophotometric studies, correlation with acetophenone-sensitized thymine dimerization, and use of acriflavine under conditions that allow the dye to associate selectively with sequential nucleotides containing thymine.

Specific activity was displayed by flavin in the dark, optimal temperature 35°C, pH 7.0. The activity was markedly enhanced either by the presence of nicotinamide-adenine dinucleotide (NAD) or by illumination between 380 and 480 nm. The illumination effect was time- and temperature-dependent, and a maximal effect was obtained when flavin was illuminated completely prior to its introduction to the irradiated DNA. This effect disappeared in 3 hr at room temperature, and could not be restored. Flavin activity was rapidly destroyed by far-UV irradiation, but was relatively resistant to irradiation by polychromatic light from a xenon source. These results, together with changes in the absorbance spectra of the flavins, suggest that an active intermediary is formed from flavin, possibly lumichrome. Through its semiquinone free radical, lumichrome might interact with triplet thymine dimer, possibly with molecular oxygen as an intermediate.

It is proposed that, in evolving organisms, flavins may have functioned as photosensitizing agents to protect genetic material from the harmful wavelengths of unfiltered sunlight, utilizing the visible spectrum of solar radiations for a primary source of energy. Ultimately, endogenous electron carriers such as NAD may have coupled with flavins to provide a dark bioenergetic pathway of higher forms of repair. Studies now in progress in our laboratory have demonstrated that flavins and NAD protect mammalian cells from lethal effects of a far-UV irradiation, aromatic hydrocarbons, and diepoxides, thus suggesting a protective biological role for these metabolic agents.

D. ROTH

Department of Pathology
New York University Medical Center
New York City 10016

Improved lymphocyte culture

Improvements in the handling of lymphocyte cultures have provided us significant increases in both viable cultures and the number of metaphases generated in successful cultures (Table 1).

Basically, the changes in the standard methodology which have given us higher mitotic indices comprise: