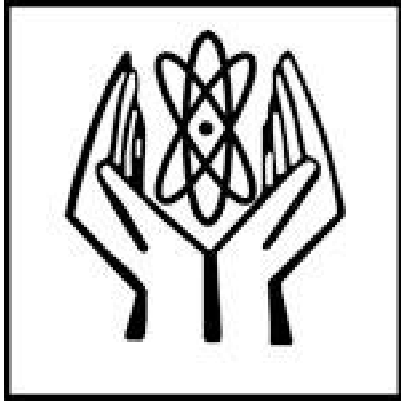

Glioma Proliferation Modulated in Vitro by Isothermal Radiofrequency Radiation Exposure

Author(s): Stephen F. Cleary, Li-Ming Liu and Randall E. Merchant

Source: *Radiation Research*, Jan., 1990, Vol. 121, No. 1 (Jan., 1990), pp. 38-45

Published by: Radiation Research Society

Stable URL: https://www.jstor.org/stable/3577561

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at https://about.jstor.org/terms

*Radiation Research Society* is collaborating with JSTOR to digitize, preserve and extend access to *Radiation Research*

JSTOR

This content downloaded from
158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms

RADIATION RESEARCH 121, 38–45 (1990)

# Glioma Proliferation Modulated *in Vitro* by Isothermal Radiofrequency Radiation Exposure

STEPHEN F. CLEARY,* LI-MING LIU,* AND RANDALL E. MERCHANT†

*Department of Physiology and Biophysics and †Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298-0051

CLEARY, S. F., LIU, L.-M., AND MERCHANT, R. E. Glioma Proliferation Modulated *in Vitro* by Isothermal Radiofrequency Radiation Exposure. *Radiat. Res.* 121, 38–45 (1990).

Isothermal (37 ± 0.2°C) exposure of glioma cells (LN71) for 2 h to 27 or 2450 MHz continuous-wave radiofrequency (RF) radiation *in vitro* modulated the rates of DNA and RNA synthesis 1, 3, and 5 days after exposure. The alterations indicate effects on cell proliferation and were not caused by RF-induced cell heating. The dose response for either frequency of the radiation was biphasic. Exposure to specific absorption rates (SARs) of 50 W/kg or less stimulated incorporation rates of tritiated thymidine ($^3$H-TdR) and tritiated uridine ($^3$H-UdR), whereas higher SARs suppressed DNA and RNA synthesis. Statistically significant time-dependent alterations were detected for up to 5 days postexposure, suggesting a kinetic cellular response to RF radiation and the possibility of cumulative effects on cell proliferation. General mechanisms of effects are discussed. © 1990 Academic Press, Inc.

## INTRODUCTION

The purpose of this study was to characterize direct effects of exposure of a human glioma cell line (LN71) to isothermal radiofrequency (RF) radiation. The exposures were to 27 and 2450 MHz continuous-wave (CW) radiation over the specific absorption rate (SAR) range 0 to 200 W/kg, and the responses studied were viability, morphological change, and DNA and RNA synthesis assayed by cellular incorporation of [$^3$H]thymidine ($^3$H-TdR) or [$^3$H]uridine ($^3$H-UdR). Precise balance of the rate of RF energy input and the rate of energy dissipation by conductive and convective heat transfer permitted cells to be exposed at 37°C with a minimum temperature control precision of 0.5% and an accuracy of 0.03%. The effects of exposure to RF radiation could thus be interpreted as resulting from direct cellular interactions without the confounding element of heating. *In vitro* exposure of noninteracting cells in suspension precluded indirect effects caused by cell-to-cell or systemic interactions. The study design thus avoided inherent uncertainties encountered in *in vivo* studies that result from cellular interactions and imprecise control of temperature and/or temperature gradients.

This was the third of a series of studies of the effects of isothermal RF radiation on mammalian cells *in vitro*. Since these three studies on different cell types were conducted under identical conditions, comparison of the results made it possible to establish that the effects noted were a general cellular response. In this study, it was determined that isothermal RF exposure induced a biphasic dose- or dose-rate-dependent alteration in the proliferation rate of glioma cells in culture, without detectable effects on viability or morphology. This response was similar to effects detected in human peripheral lymphocytes exposed to the same RF fields (*1*). Suppression of glioma or lymphocyte proliferation occurred at SARs that had been shown to induce functional deficits in mouse spermatozoa exposed to RF radiation, as determined by an *in vitro* fertilization assay (*2*).

## PROCEDURES

Densitometry, dosimetry, and procedures for the isothermal exposure of glioma cells in suspension to continuous-wave 27 MHz RF radiation were identical to those used for exposure of erythrocytes (*3*), neutrophils (*4*), unilamellar liposomes (*5*), mouse spermatozoa (*2*), or lymphocytes (*1*). The temperature control precision of the coaxial RF cell exposure chamber for the SARs used in these studies was ±0.2°C; the accuracy was 0.01°C. The waveguide exposure procedure, dosimetry, and densitometry for 2450 MHz glioma exposure were the same as previously used for exposure of liposomes (*5*), mouse spermatozoa (*1*), or lymphocytes (*1*). This exposure system afforded a temperature control precision of ±0.1°C and an accuracy of ±0.01°C at 37°C, the cell exposure temperature used in this study.

Five-milliliter aliquots of glioma cells ($10^6$ cells/ml) suspended in Dulbecco's modified Eagle's medium (DMEM) were simultaneously exposed, or sham-exposed, to 27 or 2450 MHz for 2 h at 37°C. Temperature was continuously monitored during exposure, or sham exposure, with nonperturbing thermistor probes (Vitek Electrothermia). After exposure six aliquots of the glioma cell suspension were transferred to culture plates for culture at 37°C. For both radiofrequencies each dose rate (i.e., SAR) was independently replicated at least twice.

The radioactive disintegration rates (dpm) and logarithm (dpm) of $^3$H-TdR or $^3$H-UdR were statistically analyzed by unbalanced randomized block analysis of variance (ANOVA), type III sums of squares (*7*). Statistical significance of differences in mean dpm and log(dpm) were determined by two-tailed F-tests. For purposes of comparison of relative effects of the radiofrequencies used in this study (27 and 2450 MHz), and dose dependence, the ratio of the mean normalized (see below) dpm of RF-exposed



0033-7587/90 $3.00
Copyright © 1990 by Academic Press, Inc.
All rights of reproduction in any form reserved.

This content downloaded from 158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms

TABLE I
**Incorporation Rates of $^3$H-TdR in Glioma Cells (LN71) 3 Days after *in Vitro* Exposure to Continuous-Wave 2450 MHz RF Radiation**

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | *F* value | *P* value | Sample size |
|---|---|---|---|---|
| 74.2 | 1.02 | 1.58 | 0.2156 | 48 |
| 50 | 1.24 | 4.82 | 0.0308 | 104 |
| 25[a] | 1.39 | 17.06 | 0.001 | 12 |
| 25 | 2.61 | 213.05 | <0.0001 | 22 |
| 5 | 1.42 | 95.64 | <0.0001 | 36 |

*Note.* Exposure was for 2 h at 37 ± 0.1°C.
[a] Thymidine uptake 1 day postexposure.

TABLE II
**Incorporation Rates of $^3$H-UdR in Glioma Cells (LN71) 3 Days after *in Vitro* Exposure to Continuous-Wave 2450 MHz RF Radiation**

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | *F* value | *P* value | Sample size |
|---|---|---|---|---|
| 74.2 | 0.97 | 1.36 | 0.2492 | 48 |
| 50 | 1.21 | 0.00[a] | 0.9918[a] | 65 |
| 25[b] | 1.81 | 17.47 | 0.0019 | 12 |
| 25 | 2.51 | 98.01 | <0.001 | 8 |

*Note.* Exposure was for 2 h at 37 ± 0.1°C.
[a] Logarithmic transformation *F* value (3.94), *P* value (0.052).
[b] Uridine uptake 1 day after exposure.

to sham-exposed cells was calculated for each experiment and denoted as the uptake ratio. A mean uptake ratio was then calculated from the uptake ratios for each SAR-RF combination, weighting each ratio by sample size. The SAR ranges were limited by available RF exposure systems. In the case of 2450 MHz continuous-wave RF radiation, gliomas were exposed to SARs of 0, 5, 25, 50, and 74.2 W/kg; at 27 MHz SARs of 0, 5, 25, 50, 80, and 200 W/kg were used. Within one-half hour after RF exposure cell concentrations were determined by cell counting, and trypan blue dye exclusion was used to determine field-induced alterations in cell membrane permeability. Cell concentrations were adjusted to $10^5$ cells/ml and plated in 96-well plastic culture dishes.

*Glioma Cell Culture and Assay*

Glioma cells were incubated for 1, 3, or 5 days in DMEM supplemented with 5% heat-inactivated fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. At the time of pulse labeling, cell concentrations were determined for each treatment group by quadruplicate measurements of total mitochondrial activity using the "colormetric" microtiter assay described by Green *et al.* (*6*). Log-phase cells were pulse-labeled for 6 h with $^3$H-TdR (100 μCi/mg) or $^3$H-UdR (200 μCi/mg) and incorporation was determined by liquid scintillation counting after cell filtration and washing to remove unincorporated labeled precursors. The radioactivity (dpm) of the samples was normalized to equal cell concentrations, and mean uptake ratios were calculated as described. Cell samples were fixed and prepared for examination by light or electronmicroscopy.

## RESULTS

A single 2-h exposure to 27 or 2450 MHz RF radiation in the SAR range 5 to 200 W/kg induced dose-dependent biphasic alterations in the rate of incorporation of $^3$H-TdR and $^3$H-UdR. Comparison of incorporation rates of these radioactive nucleic acid precursors 1, 3, or 5 days after exposure revealed the response to be dependent on time postexposure. Precursor uptake was in most cases determined 3 or 5 days postexposure.

### *2450 MHz RF Radiation*

A 2-h exposure of LN71 glioma cells to 2450 MHz continuous-wave radiation, at 37 ± 0.1°C, induced dose-dependent increases in the rate of cellular incorporation of $^3$H-TdR and $^3$H-UdR. Table I summarizes $^3$H-TdR incorporation rates, expressed as mean uptake ratios, as a function of SAR. The table also includes the results of ANOVA of the sample radioactive disintegration rates (i.e., raw data) normalized in terms of cell concentration. These data were for samples pulse-labeled 3 days after RF exposure. For the same cell suspensions, Table II summarizes the effect of 2 h 2450 MHz RF exposure on the $^3$H-UdR incorporation rate 3 days postexposure. Uptake of $^3$H-UdR at an SAR of 5 W/kg was not determined. The data in Tables I and II are plotted in Fig. 1, which shows the uptake ratio of $^3$H-TdR and

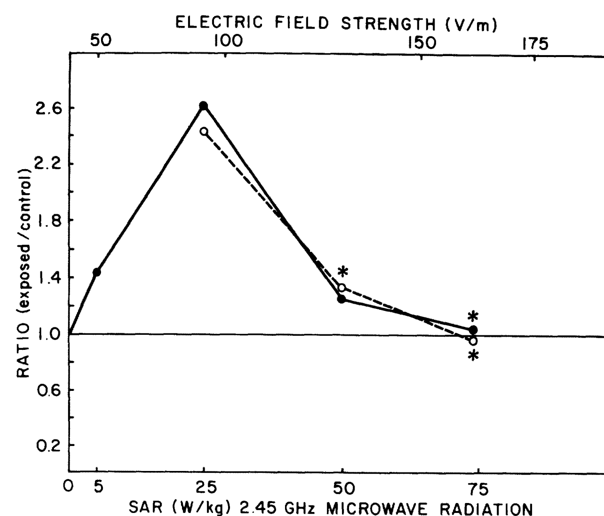


FIG. 1. Normalized weighted mean ratios of (●) $^3$H-TdR and (○) $^3$H-UdR (100 and 200 μCi/mg, respectively) uptake in RF-exposed (2450 MHz) or simultaneously sham-exposed glioma cells (LN71) ($10^6$ cells/ml) (2 h, 37 ± 0.1°C), shown as a function of RF exposure intensity [SAR (W/kg) or field strength (V/m)]. After exposure cells were incubated for 3 days in DMEM supplemented with 5% heat-inactivated fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were pulse-labeled for 2 h with radioactive precursors and uptake was determined by liquid scintillation counting. Disintegration rates (dpm) were normalized to equal cell concentrations in RF-exposed and sham-exposed cultures and the uptake ratio (exposed/control) was calculated. The results of an unbalanced randomized block analysis of variance (type III sum of squares) are summarized in Table I ($^3$H-TdR uptake) and Table II ($^3$H-UdR uptake).

This content downloaded from
158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms

TABLE III
**Incorporation Rates of $^3$H-TdR in Glioma Cells (LN71) 5 Days after *in Vitro* Exposure to Continuous-Wave 2450 MHz RF Radiation**

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | $F$ value | $P$ value | Sample size |
|---|---|---|---|---|
| 74.2 | 0.85 | 10.12 | 0.0028 | 45 |
| 50 | 1.19 | 16.39 | 0.0002 | 65 |
| 25 | 1.74 | 256.24 | <0.0001 | 12 |
| 5 | 1.49 | 233.82 | <0.0001 | 24 |

*Note.* Exposure was for 2 h at 37 ± 0.1°C.

TABLE IV
**Incorporation Rates of $^3$H-UdR in Glioma Cells (LN71) 5 Days after *in Vitro* Exposure to Continuous-Wave 2450 MHz RF Radiation**

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | $F$ value | $P$ value | Sample size |
|---|---|---|---|---|
| 74.2 | 0.85 | 13.49 | 0.0007 | 44 |
| 50 | 0.95 | 5.32[a] | 0.0273[a] | 42 |

*Note.* Exposure was for 2 h at 37 ± 0.1°C.
[a] Logarithmic transformation $F$ value (9.41), $P$ value (0.0042).

$^3$H-UdR as a function of SAR. In this and subsequent figures, mean uptake ratios for difference in incorporation rates of RF-exposed versus sham-exposed cells that were not statistically significant (i.e., $P > 0.05$), as determined by ANOVA, are indicated by an asterisk. Mean uptake ratios of incorporation rates of $^3$H-TdR or $^3$H-UdR are plotted without standard deviations or standard errors of random variables of differing sample sizes. As noted data were analyzed statistically by ANOVA; the results are given in both tables and figures. Data points were connected by straight-line segments solely to illustrate data trends. No attempts were made to determine dose–response curves analytically, principally because of the limited independent variable set.

Exposure at an SAR of 74.2 W/kg did not significantly affect incorporation of either radiolabeled precursor 3 days after exposure, whereas exposure to lower SARs (i.e., 5, 25, or 50 W/kg) resulted in a statistically significant increased uptake, except for $^3$H-UdR at 50 W/kg. ANOVA of logarithmic transformed data (i.e., log[dpm]) generally resulted in smaller P values than untransformed data, which suggested the data were log normally distributed. As indicated in Table II, logarithmic transformation of the 50 W/kg $^3$H-UdR data resulted in a $P$ value of 0.052, in general agreement with responses to other SARs of less than 74.2 W/kg. Comparison of the mean ratios for $^3$H-TdR and $^3$H-UdR at an SAR of 50 W/kg (i.e., 1.24 and 1.21, respectively) indicates a similar response, even though the differences in the untransformed $^3$H-UdR data were not statistically significant. In general, these data suggest a dose-dependent biphasic response of glioma cells to 2450 MHz continuous-wave RF radiation 3 days postexposure.

Relative incorporation rates of $^3$H-TdR and $^3$H-UdR in glioma cells 5 days after a single 2-h exposure to 2450 MHz continuous-wave radiation, expressed as mean uptake ratios, and ANOVA results, are summarized in Tables III and IV, respectively. In Fig. 2, the mean uptake ratio of $^3$H-TdR and $^3$H-UdR is shown as a function of SAR of 2450 MHz continuous-wave radiation. These data indicate a statistically significant biphasic dose-dependent response of glioma cells to 2450 MHz radiation 5 days after exposure.

The time dependence of the relative uptake of $^3$H-TdR and $^3$H-UdR in glioma cells is shown in Fig. 3, a graph of

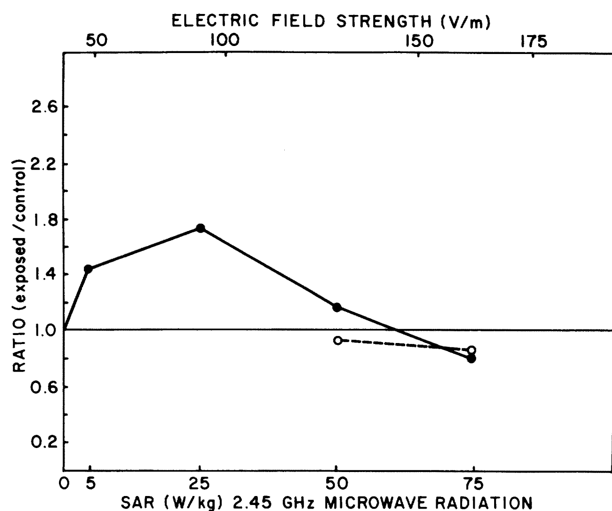


FIG. 2. Normalized weighted mean ratios of (●) $^3$H-TdR and (○) $^3$H-UdR (100 and 200 μCi/mg, respectively) uptake in RF-exposed (2450 MHz) or simultaneously sham-exposed glioma cells (LN71) ($10^6$ cells/ml) (2 h, 37 ± 0.1°C) 5 days postexposure, shown as a function of RF exposure intensity [SAR (W/kg) or field strength (V/m)]. For experimental details refer to legend to Fig. 1. ANOVA results are summarized in Table III ($^3$H-TdR uptake) and Table IV ($^3$H-UdR uptake).

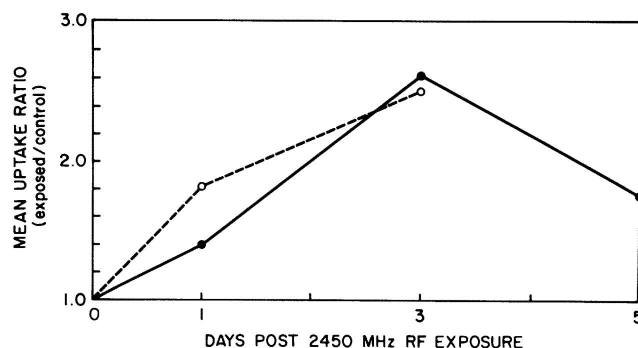


FIG. 3. Normalized weighted mean ratios of (●) $^3$H-TdR and (○) $^3$H-UdR uptake in RF-exposed (2450 MHz) or sham-exposed glioma cells (LN71) ($10^6$ cells/ml) as a function of time after exposure. Cells were exposed to 25 W/kg RF radiation for 2 h at 37 ± 0.1°C.

This content downloaded from
158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms

TABLE V
Incorporation Rates of $^{3}$H-TdR in Glioma Cells (LN71) 3 Days after *in vitro* Exposure to Continuous-Wave 27 MHz RF Radiation

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | F value | P value | Sample size |
|---|---|---|---|---|
| 200 | 0.56 | 202.68 | <0.0001 | 64 |
| 80 | 0.86 | 3.17 | 0.0897 | 23 |
| 50 | 0.80 | 7.51 | 0.0208 | 12 |
| 25[a] | 1.47 | 7.67 | 0.0122 | 23 |
| 25 | 1.96 | 0.31[b] | 0.58[b] | 63 |
| 5 | 1.13 | 9.05 | 0.0053 | 36 |

*Note.* Exposure was for 2 h at 37 ± 0.2°C.
[a] $^{3}$H-TdR incorporation 1 day postexposure.
[b] Logarithmic transformation *F* value (32.21), *P* value (0.0001).

TABLE VI
Incorporation Rates of $^{3}$H-UdR in Glioma Cells (LN71) 3 Days after *in Vitro* Exposure to Continuous-Wave 27 MHz RF Radiation

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | F value | P value | Sample size |
|---|---|---|---|---|
| 200 | 0.69 | 7.45 | 0.0102 | 38 |
| 80 | 0.78 | 38.91 | <0.0001 | 23 |
| 50 | 0.98 | 0.05 | 0.8281 | 12 |
| 25[a] | 1.60 | 4.92 | 0.0389 | 23 |
| 25 | 2.57 | 12.30 | 0.0014 | 40 |

*Note.* Exposure was for 2 h at 37 ± 0.2°C.
[a] Uridine incorporation 1 day postexposure.

mean uptake ratios as a function of the number of days after exposure to 25 W/kg, 2450 MHz radiation. There was a statistically significant monotonic increase in both $^{3}$H-TdR and $^{3}$H-UdR during the first 3 days after RF exposure. Thymidine uptake (uridine uptake was not determined) decreased on Day 5, relative to the maximum uptake detected on Day 3. However, 5 days postexposure there was a highly statistically significant increase (74%) in $^{3}$H-TdR uptake in RF-exposed cells at this SAR. Uptake of $^{3}$H-TdR by glioma cells exposed to 50 W/kg exhibited a statistically significant increase of 20% 5 days postexposure, whereas cells exposed to 74.2 W/kg had a statistically significant reduction in uptake of 18%.

A comparison of dose–response curves for 3 and 5 days postexposure (Figs. 1 and 2) suggests that the largest change, an approximate 90% reduction in the incorporation rate of $^{3}$H-TdR over this 2-day period, occurred at an SAR of 25 W/kg. Incorporation of $^{3}$H-UdR 5 days postexposure was reduced by 26 or 12% at SARs of 50 and 74.2 W/kg, respectively, relative to incorporation rates 3 days postexposure. Five days after exposure, $^{3}$H-UdR incorporation was suppressed in cells exposed to either 50 or 74.2 W/kg. The incorporation of $^{3}$H-UdR, which at 3 days postexposure to 50 W/kg was increased significantly by 21%, became significantly suppressed (5%) by Day 5.

*27 MHz RF Radiation*

The effects of 2 h exposure of LN71 glioma cells to 27 MHz continuous-wave RF radiation at 37 ± 0.1°C on incorporation of $^{3}$H-TdR and $^{3}$H-UdR 3 days postexposure are summarized in Tables V and VI, respectively. Exposure at SARs of 5 or 25 W/kg resulted in a statistically significant increase in $^{3}$H-TdR and $^{3}$H-UdR incorporation, whereas exposure to 50 W/kg caused a 20% reduction in $^{3}$H-TdR without affecting $^{3}$H-UdR uptake. Exposure to 200 W/kg led to a 44% reduction in $^{3}$H-TdR incorporation and a 31% reduction in $^{3}$H-UdR; both effects were statistically significant. The biphasic response of glioma nucleic acid precursor incorporation as a function of 27 MHz RF dose is shown in Fig. 4. As in the case of 3 days postexposure to 2450 MHz CW radiation, maximum effect of 27 MHz RF SAR radiation (a 96% increase in $^{3}$H-TdR uptake) occurred following exposure of 25 W/kg. Exposure to 25 W/kg 2450 MHz CW radiation resulted in a 161% increase in $^{3}$H-TdR and a 151% increase in $^{3}$H-UdR. At this SAR, 27 MHz radiation exposure enhanced $^{3}$H-UdR incorporation by 157%. A statistically significant ($P$ = 0.005) 13% increase in $^{3}$H-TdR occurred in samples exposed at 5 W/kg ($^{3}$H-UdR uptake was not determined at this SAR).

The relative rates of incorporation of $^{3}$H-TdR and $^{3}$H-UdR into glioma cells 5 days after exposure to 27 MHz RF

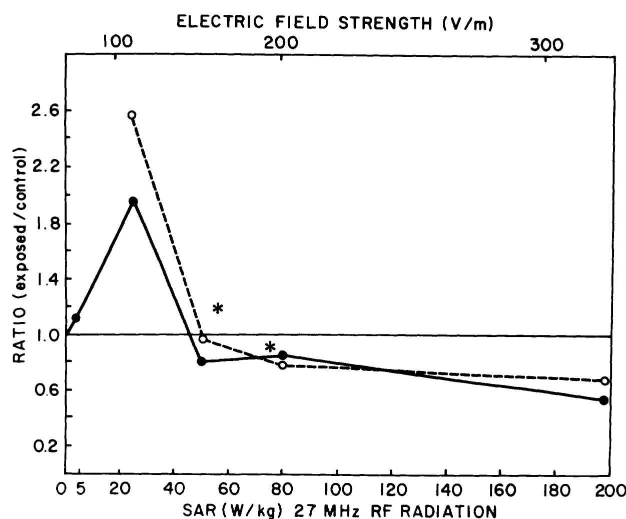


FIG. 4. Normalized weighted mean ratios of (●) $^{3}$H-TdR and (○) $^{3}$H-UdR (100 and 200 μCi/mg, respectively) uptake 3 days postexposure in RF-exposed (27 MHz) or simultaneously sham-exposed glioma cells (LN71) ($10^6$ cells/ml) (2 h, 37 ± 0.1°C), shown as a function of RF exposure intensity [SAR (W/kg) or field strength (V/m)]. For experimental details refer to legend to Fig. 1. ANOVA results are summarized in Table V ($^{3}$H-TdR) and Table VI ($^{3}$H-UdR).

This content downloaded from
fff:ffff:ffff:ffff:ffff:ffff:ffff on Thu, 01 Jan 1976 12:34:56 UTC
All use subject to https://about.jstor.org/terms

TABLE VII
**Incorporation Rates of $^3$H-TdR in Glioma Cells (LN71) 5 Days after *in Vitro* Exposure to Continuous-Wave 27 MHz RF Radiation**

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | *F* value | *P* value | Sample size |
|---|---|---|---|---|
| 200 | 1.27 | 35.63 | <0.0001 | 40 |
| 80 | 0.95 | 0.34 | 0.5645 | 22 |
| 50 | 1.33 | 23.24 | 0.0013 | 10 |
| 25 | 2.58 | 12.76 | 0.0009 | 48 |
| 5 | 1.10 | 18.26 | 0.0002 | 36 |

*Note.* Exposure was for 2 h at 37 ± 0.2°C.

radiation are summarized in Tables VII and VIII, respectively, together with results of ANOVA. Exposure to 5, 25, or 50 W/kg resulted in statistically significant enhanced uptake of $^3$H-TdR; a maximum increase of 158% occurred at an SAR of 25 W/kg. Uridine uptake at these SARs was also increased. The 112% increase at 25 W/kg was not statistically significant ($P = 0.16$); but the 33% increase at 50 W/kg was significant ($P < 0.001$). Temporal data trends for $^3$H-TdR and $^3$H-UdR uptake by glioma 1, 3, or 5 days postexposure to 25 W/kg 27 MHz RF radiation are shown in Fig. 6. During this period of observation $^3$H-TdR uptake increased in an apparently linear fashion. Maximum incorporation of $^3$H-UdR occurred 3 days after exposure (157% increase).

## DISCUSSION

These data demonstrate modulation of DNA and RNA synthesis by RF radiation. Obligate involvement of these events in mitogenesis indicates that RF radiation, as used in this study, modulated cell proliferation. Although $^3$H-TdR and $^3$H-UdR incorporation are standard assays of cell proliferation, since mitotic indices were not determined per se, we cannot rule out the possibility of other cellular effects of RF exposure such as disruption of spindle fiber formation. To our knowledge, modulation of cell proliferation has not been demonstrated previously to be a direct nonthermal effect of RF radiation of the frequencies and SARs used in this *in vitro* study, although there was indirect evidence from *in vivo* studies.

TABLE VIII
**Incorporation Rates of $^3$H-UdR in Glioma Cells (LN71) 5 Days after *in Vitro* Exposure to Continuous-Wave 27 MHz RF Radiation**

| SAR (W/kg) | Mean uptake ratio (Exposure/control) | *F* value | *P* value | Sample size |
|---|---|---|---|---|
| 200 | 1.78 | 79.23 | <0.0001 | 16 |
| 80 | 0.97 | 0.36 | 0.5557 | 24 |
| 50[a] | 1.33 | 34.65 | 0.0002 | 12 |
| 25 | 2.12 | 2.11 | 0.1589 | 30 |

*Note.* Exposure was for 2 h at 37 ± 0.2°C.
[a] $^3$H-UdR uptake 1 day postexposure.

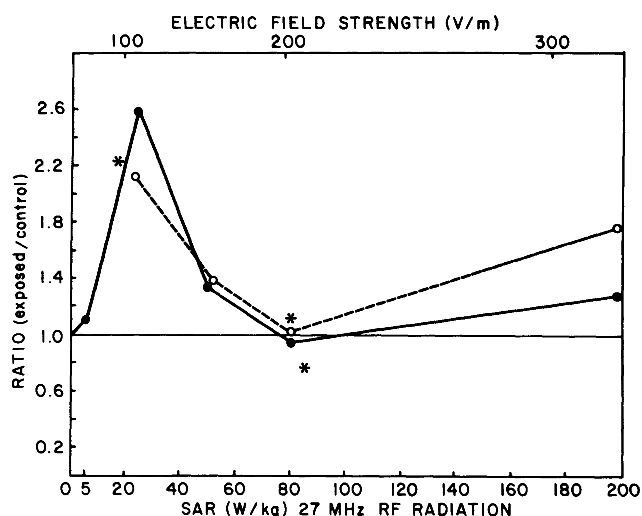


FIG. 5. Normalized weighted mean ratios of (●) $^3$H-TdR and (○) $^3$H-UdR (100 and 200 μCi/mg, respectively) uptake 5 days postexposure in RF-exposed (27 MHz) or simultaneously sham-exposed glioma cells (LN71) ($10^6$ cells/ml) (2 h, 37 ± 0.1°C), shown as a function of RF exposure intensity [SAR (W/kg) or field strength (V/m)]. For experimental details refer to legend to Fig. 1. ANOVA results are summarized in Table VII ($^3$H-TdR) and Table VIII ($^3$H-UdR).

The potential significance of these results is:

(a) Cells were exposed isothermally; therefore, effects were not induced indirectly by heating.

(b) RF exposure at either frequency resulted in biphasic dose responses; SARs of 50 W/kg or less stimulated DNA and RNA synthesis and higher SARs suppressed precursor incorporation.

(c) Statistically significant effects on glioma nucleic acid synthesis occurred 1, 3, and 5 days after a single 2-h exposure.

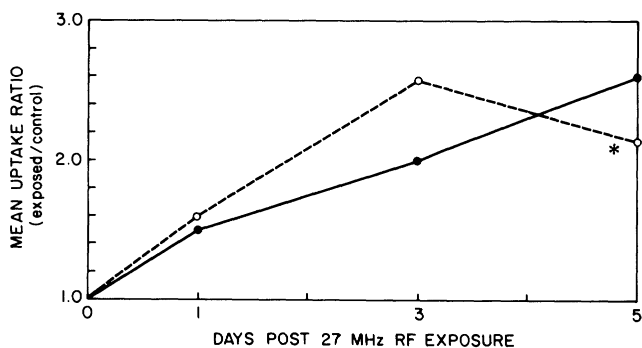


FIG. 6. Normalized weighted mean ratios of (●) $^3$H-TdR and (○) $^3$H-UdR uptake in RF-exposed (27 MHz) or sham-exposed glioma cells (LN71 ($10^6$ cells/ml) as a function of time after exposure. Cells were exposed to 25 W/kg RF radiation for 2 h at 37 ± 0.2°C.

This content downloaded from
158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms

(d) Uptake of $^3$H-TdR or $^3$H-UdR varied with time after exposure, indicating a kinetic cellular response to RF radiation.

*Isothermal Exposure*

Since homogeneous glioma cell suspensions (LN71) were exposed isothermally *in vitro* without cell-to-cell contact, effects on DNA and RNA synthesis apparently resulted from a direct RF interaction at the cellular level. Isothermal exposure to SARs used in this study had no detectable effects on cell morphology or viability as determined by histology and cell counting.

The fact that cells were isothermally exposed must be kept in mind in evaluating these data, especially in relation to *in vivo* responses. The known thermal sensitivity of cellular processes, including mitogenesis, indicates the possibility of quantitative (and perhaps qualitative) differences in the effects of isothermal and nonisothermal RF exposure. Consequently, the effects of RF exposure *in vivo* or *in vitro* involving both direct effects and indirect heating effects might differ from the effects reported here. Since isothermal RF exposure affects cellular biosynthetic processes directly and, it can be assumed by inference, mitotic activity, there is a need to investigate the interaction of direct and indirect thermal RF radiation effects *in vitro* to ascertain the physiological significance of these results.

*Biphasic Dose Response*

The biphasic dose response of glioma cells to RF radiation under the conditions of this study indicates that for either 27 or 2450 MHz radiation the threshold SAR for eliciting increased mitogenic activity 1, 3, or 5 days after exposure is less than 5 W/kg. Suppression of uptake of $^3$H-TdR and $^3$H-UdR occurred at SARs in excess of 50 W/kg for 2450 MHz RF exposure, whereas 27 MHz RF radiation suppressed uptake at 50 W/kg. Although it was not possible to obtain $^3$H-UdR uptake data for all SARs in this study, the data indicate similar RF dose–response effects of $^3$H-UdR and $^3$H-TdR uptake. This is anticipated on the basis of coupled synthesis of DNA, RNA, and proteins during mitogenesis. The similarity of the biphasic dose response for 27 or 2450 MHz RF radiation suggests similar mechanisms of the effect, although the mechanisms are unknown. Herein, exposure-dependent effects are generally referred to in terms of dose. However, since exposures were for a fixed period of 2 h, it is not possible to distinguish dose (SA) from dose-rate (SAR) dependent alterations.

*Persistence of the Latent Period*

Modulation of glioma mitogenesis by isothermal RF exposure was detected 1, 3, and 5 days after exposure. Although there was evidence of reversibility of effects, especially in cells exposed to 27 MHz at 200 W/kg, the highest SAR used in this study, it may be concluded that the effects of a 2-h RF exposure persisted for at least 5 days. This finding is significant since it suggests the possibility of cumulation of effects of RF radiation when exposure occurs at intervals on the order of 5 days or less. Cumulation of exposure effects of RF radiation on cells *in vitro* is currently being investigated. The persistence of the effect of isothermal RF radiation detected in this study may contribute to an understanding of mechanisms of interaction, as discussed below.

Comparisons of 3- and 5-day 2450 MHz dose responses shown in Figs. 1 and 2, respectively, or for 27 MHz radiation shown in Figs. 4 and 5, indicate time-dependent alterations in the rate of incorporation of $^3$H-TdR and $^3$H-UdR. The time-dependent trend of the data suggests reversibility of RF exposure effects on precursor uptake, although statistically significant alterations still persisted 5 days postexposure, the maximum period of observation. Reversibility of exposure effects is most pronounced in cells exposed to 27 MHz RF radiation at 200 W/kg. Three days after exposure $^3$H-TdR uptake was suppressed 44% ($P < 0.001$) and $^3$H-UdR uptake was 31% less than in sham-exposed cultures ($P = 0.01$). Two days later (5 days postexposure), the mean $^3$H-TdR incorporation in 27 MHz exposed cells was 27% greater than in sham-exposed cells ($P < 0.001$); the mean rate of $^3$H-UdR uptake was 78% ($P < 0.001$) greater in RF-exposed than in sham-exposed cells. Consequently, relative rates of $^3$H-TdR incorporation at this SAR changed by 71% and the $^3$H-UdR incorporation rate increased by 109% over a 2-day postexposure period, a finding consistent with the reversal or repair of an effect on cell proliferation rate induced by exposure to 27 MHz.

Comparison of 27 and 2450 MHz 3- and 5-day postexposure incorporation rates of labeled nucleic acid precursors suggests a difference in the cellular kinetic response to exposures to these frequencies. Over this 2-day period, cells exposed to 27 MHz radiation at SARs of 50 W/kg or greater had a 45% increase in the average $^3$H-TdR uptake and a 54% increase in the average $^3$H-UdR incorporation rate. Conversely, cells exposed to 2450 MHz at 50 W/kg or greater had 13 and 19% reductions in incorporation rates of $^3$H-TdR and $^3$H-UdR, respectively, over this same 2-day postexposure period. Excluding the effect of 200 W/kg exposure at 27 MHz (an SAR that was not investigated for 2450 MHz radiation), average percentage changes during the 3- to 5-day postexposure period for 27 MHz radiation at SARs of 50 or 80 W/kg were 32 and 27% for $^3$H-TdR and $^3$H-UdR, respectively. Compared with reductions of 13 and 19% for $^3$H-TdR and $^3$H-UdR, respectively, for cells exposed to 2450 MHz, the 45 and 46% increase from Day 3 to Day 5 postexposure in $^3$H-TdR and $^3$H-UdR, respectively, in cells exposed to 27 MHz radiation suggests differences in the time between exposure and the effect for these radiation frequencies.

This content downloaded from
158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms

If it was assumed that the cell membrane was the site of interaction of RF radiation which led to effects on proliferation, frequency dependence would be predicted on the basis of differences in RF-induced field strength in the cell medium. Due to the frequency-dependent conductivity of the glioma cell suspension medium, RF-induced transmembrane potential at 27 MHz was 20% greater than at 2450 MHz. Thus, if altered proliferation was a field strength-dependent phenomenon mediated by a direct membrane effect, frequency-dependent differences would occur. However, uncertainty about the involvement of transmembrane potential per se in altered proliferation precludes the conclusion that the responses resulted from differences in the frequency of the radiation.

### *Mechanisms*

Mechanisms for the effects of isothermal RF radiation on glioma cell DNA and RNA synthesis are unknown. However, consideration of the experimental conditions and outcome of this study provide a basis for speculation. Since $^3$H-TdR and $^3$H-UdR incorporation rates were normalized in terms of cell number, RF-induced differences between exposed and sham-exposed cellular uptake indicate alteration in mitotic activity. It should be noted that there was no fetal calf serum (FCS) in the culture medium during RF exposure. Thus effects on cell proliferation were not caused by direct RF interaction with serum-borne extrinsic growth factors that led to altered receptor binding. After RF exposure, cells were separated from exposure medium and resuspended in fresh medium supplemented with 5% FCS. Since FCS containing extrinsic cell growth factors was added 1 to 2 h after exposure, RF effects on growth factor-receptor binding leading to altered cell proliferation could only have occurred if RF-induced receptor alteration persisted for an hour or more. Effects of RF exposure on cell receptors are being investigated.

The biphasic 27 or 2450 MHz dose response indicated that a 2-h exposure at SARs of 50 W/kg or less (i.e., electric field strengths for 27 or 2450 MHz of 160 or 134 V/m, respectively) caused more than a 100% increase in nucleic acid synthesis as indicated by the uptake of precursors 3 to 5 days after exposure. Exposure to SARs of 80 W/kg decreased synthesis 3 days postexposure by 40% or less, an effect that was reversed by 5 days postexposure. These results are not inconsistent with cell cycle stage-dependent variations in sensitivity of mitogenesis. The significantly greater stimulatory effect of RF radiation at SARs of 50 W/kg or less, compared to the suppressive effect of higher SARs, may be caused by differences in the fraction of cells sensitive to modulation by RF radiation. The lower magnitude of the response to high SARs suggests the possibility of RF induction of mitotic delay in a specific phase of the cell cycle. Stimulation of glioma cells at lower SARs, on the other hand, may be a more general RF effect on cells in various stages of the cycle. Indeed, Peters *et al.* (*8*) reported specific cell cycle inhibitory effects on L60T cells exposed *in vitro* to high-intensity 2450 MHz RF radiation during $G_1$ or M phase.

Since the doubling time of glioma LN71 cells is on the order of 9 h, an RF exposure of 2 h would be of sufficient duration to alter cells selectively in a sensitive cell cycle phase, or to affect a fraction of cells in more than one or all phases. Lacking data on the mitogenic status of the glioma (LN71) cells during RF exposure, other than the fact that the cells were in log phase, this would suggest that the cells were asynchronous. However, since the cells were treated with trypsin 2 to 3 h prior to RF exposure to facilitate transfer of adherent cells from culture flasks, cells were most probably synchronized to an undetermined extent. The induction of such parasynchronous cell growth was described by Newton and Wildy (*9*) and Engelberg (*10*).

The postexposure time-dependent variation in biosynthetic activity detected in this study may be caused by the effect of the RF exposure intensity on the progression of RF-altered cells through the cell cycle. Indeed, preliminary studies of the effects of a 2-h exposure of synchronized Chinese hamster ovary (CHO) cells to 25 or 50 W/kg, 27 or 2450 MHz continuous-wave RF radiation, indicated cell-cycle-specific exposure effects of the type reported here (Cleary, unpublished data). The sensitivity of cells in various phases of the cell cycle to RF-induced modulation is currently being investigated.

In summary, the biphasic dose- and time-dependent responses of glioma LN71 cells suggest that RF radiation may selectively affect cells in specific cell cycle phases, resulting in altered rates of progression through the cell cycle. The biphasic response suggests more than one cellular interaction site, such as the plasma membrane or the genome, depending upon RF dose or induced field strength. Similar biphasic, dose-dependent alterations in mitogenesis have been detected in human lymphocytes exposed to RF radiation under the same conditions as used in this study (*1*). These findings suggest that this may be a general cellular response to RF radiation.

## ACKNOWLEDGMENTS

We acknowledge Dr. Guanghui Cao for his input regarding the effects of RF radiation on cell synchronization. This research was supported by NIH Grant 1R01OH02148 and Office of Naval Research, Contract N00014-84-K-0539.

RECEIVED: March 3, 1989; ACCEPTED: August 16, 1989

## REFERENCES

1. S. F. CLEARY, L. M. LIU, and R. E. MERCHANT, Lymphocyte proliferation modulated *in vitro* by isothermal radiofrequency radiation exposure. *Bioelectromagnetics*, in press.

This content downloaded from 158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms

2. S. F. CLEARY, L. M. LIU, R. GRAHAM, and J. EAST, *In vitro* fertilization by mouse spermatogonia exposed isothermally to radiofrequency radiation. *Bioelectromagnetics* **10,** 361–369 (1989).

3. S. F. CLEARY, L. M. LIU, and F. GARBER, Erythrocyte hemolysis by radiofrequency fields. *Bioelectromagnetics* **6,** 313–322 (1985).

4. S. F. CLEARY, L. M. LIU, and F. GARBER, Viability and phagocytosis of neutrophils exposed *in vitro* to 100 MHz radiofrequency radiation. *Bioelectromagnetics* **6,** 53–60 (1985).

5. L. M. LIU and S. F. CLEARY, Effects of 2.45 GHz microwaves and 100 MHz radiofrequency radiation on liposome permeability at the phase transition temperature. *Bioelectromagnetics* **9,** 249–258 (1988).

6. L. M. GREEN, J. L. READ, and C. F. WARE, Rapid colormetric assay for cell viability: Application to the quantitation of cytotoxic and growth inhibitory lymphokines. *J. Immunol. Methods* **70,** 257–268 (1984).

7. SAS/STAT, *Guide for Personal Computers,* Version 6, Chap. 8. SAS Institute, Cary, NC, 1985.

8. W. J. PETERS, R. W. JACKSON, and K. IWANO, Effects of controlled electromagnetic radiation on the growth of cells in tissue culture. *J. Surg. Res.* **27,** 8–13 (1979).

9. A. A. NEWTON and P. WILDY, Parasynchronous division of HeLa cells. *Exp. Cell Res.* **16,** 624–635 (1959).

10. J. ENGELBERG, A method of measuring the degree of synchronization of cell populations. *Exp. Cell Res.* **23,** 218–227 (1961).

This content downloaded from
158.111.236.95 on Mon, 19 Sep 2022 18:32:23 UTC
All use subject to https://about.jstor.org/terms