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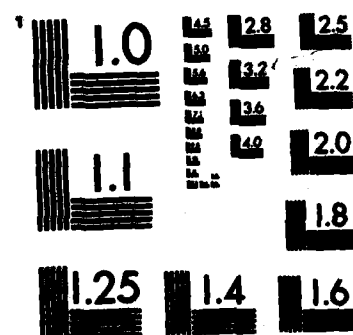
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## MICROWAVES AND HUMAN LEUKOCYTE FUNCTION

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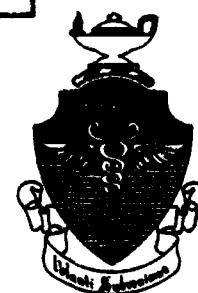
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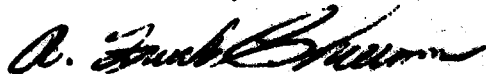
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The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.



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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number)<br>Studies were performed to determine whether human leukocytes were affected by exposure to microwave energies that equal or even exceed current safety standard recommendations. No effects were detectable on viability or on function of human mononuclear leukocytes resulting from exposure to microwave energy at specific absorption rates up to 4 mW/ml. In contrast to the results of similar studies in other laboratories, our results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate insofar as leukocyte function is concerned. |                                            |                                                                                        |

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## MICROWAVES AND HUMAN LEUKOCYTE FUNCTION

### INTRODUCTION

All individuals are exposed to radiofrequency and/or microwave energies to variable degrees. Studies by several investigators have raised the possibility that the immunocompetent cells of humans are particularly susceptible to microwaves (1-3). These studies were admitted to be poorly reproducible and nonquantitative. Nonetheless, they are frequently cited; and they have provided the limited data, on exposure of human leukocytes, for use by those who develop environmental health standards (4-6). Many animal systems have been studied; but the species, microwave power intensities, environmental conditions, and other factors have varied so widely that extrapolation to humans would be exceedingly difficult, even if appropriate (7,8).

The studies reported here have been performed to determine whether human leukocytes are affected by exposure to microwave energies that equal or exceed current safety standard recommendations. Exposure to microwave energy at specific absorption rates up to 4 mW/ml resulted in no detectable effects on viability, on unstimulated or stimulated DNA, RNA, total protein, or interferon synthesis by human mononuclear leukocytes. In contrast to the earlier studies cited, our results have been highly reproducible and have provided no evidence that current safety standard recommendations are inappropriate.

### MATERIALS AND METHODS

#### Cell Source and Collection of Blood

Peripheral venous blood was obtained by venipuncture from 21 healthy young adult donors (i.e., 14 male, and 7 female) who were taking no medication at the time of the study. Mononuclear leukocytes were obtained from the heparinized whole blood by Ficoll-Hypaque sedimentation (9). Mononuclear leukocytes obtained by this method consisted of 70% to 80% lymphocytes and 20% to 30% monocytes (10); both cell types are required for optimal responses to mitogens and antigens.

Except as noted in the following, leukocyte cultures were maintained in medium 199 (Gibco, Grand Island, N.Y.) with modified Earle's salts with glutamine, aqueous penicillin G (100 units/ml), and streptomycin (50 µg/ml). The medium was further supplemented with 10% autologous serum. For studies of total protein synthesis, leukocyte cultures were maintained in leucine-free MEM (Gibco, Grand Island, N.Y.).

#### Exposure and Sham-Exposure to Microwaves

The mononuclear leukocytes were exposed in a waveguide system to 2450 MHz (CW) microwaves at specific absorption rates (SARs) from 0.5 to 4 mW/ml. The

waveguide system used in these studies has previously been described in detail (11, 12). Exposure and sham-exposure waveguides are located within a water-jacketed, 37°C CO<sub>2</sub> incubator. Temperature inhomogeneity within the cultures is prevented by continuous shaking of the shelf upon which the waveguides rest. In addition to leukocyte cultures enclosed within waveguides for exposure or sham-exposure, we included control cultures--located within the same incubator but external to the waveguides--which rested upon a stationary shelf. Exposures and sham-exposures were monitored continuously by use of Vitek Electrothermia nonperturbing probes (Vitek, Inc., Boulder, Colo.). No attempt was made to counteract microwave-induced heating of the leukocyte cultures, since we wished to observe any potential microwave-induced effects, thermal or otherwise.

The SARs were determined by analysis of steady-state temperature increments,  $\Delta T_{ss}$  (13). The SAR was the product of: the specific heat (0.97 cal/°C/g); the steady-state temperature increment (°C); and the cooling constant (0.0838/min.). The SARs (in milliwatts per milliliter) in this exposure system could be estimated by the product:  $5.67 \times \Delta T_{ss}$ . During prolonged exposures, changes in the thermal environment were expected. The relation between the SAR and the steady-state temperature increment was best represented by a constant (4.63), determined empirically by use of culture medium exposed (absorbed) between 5 and 45 mW/ml.

The changes in steady-state temperature (mean  $\pm$  S.E.) of cultures exposed to microwaves at respective SARs of 0.5, 1.0, and 4 mW/ml were  $0.10 \pm 0.03$ ,  $0.25 \pm 0.03$ , and  $0.89^\circ\text{C} \pm 0.04$ . The final culture temperatures (mean  $\pm$  S.E.) were, respectively,  $37.67 \pm 0.13$ ,  $37.72 \pm 0.15$ , and  $38.40^\circ\text{C} \pm 0.08$ .

### Assays of Leukocyte Viability

Leukocyte viability was determined, from 1 to 7 days after exposure or sham-exposure to microwaves, by use of total cell counts and assays for percent of cells able to exclude trypan blue dye or ethidium bromide (10). The assays of leukocyte function (described in the following) provided additional and even more substantial evidence of leukocyte viability. For example, mitogen-stimulated lymphocyte transformation responses of the human mononuclear leukocytes requires participation of both viable monocytes and viable lymphocytes (10), as does production of PHA-induced interferon- $\gamma$  (14). Interferon- $\alpha$  is actively produced by viable monocytes after exposure to influenza virus, and preformed interferon is not released by killed cells (14,15).

### Assays of DNA, RNA, and Total Protein Synthesis

Unstimulated and mitogen-stimulated DNA, RNA, and total protein synthesis by the mononuclear leukocytes were assayed, using established methods, by cellular incorporation of the tritiated precursors thymidine, uridine, and leucine, respectively (10, 16-19). In brief, mononuclear leukocytes were added to quadruplicate wells of sterile microtiter plates (Costar, Cambridge, Mass.) at a concentration of  $5 \times 10^5$  cells/ml ( $1 \times 10^5$  cells per well). To the cell cultures was added medium alone, or medium containing phytohemagglutinin (PHA)-M (Difco, Detroit, Mich.). The final volume of the cultures

was 0.2 ml per well. PHA was added at an optimal concentration of 160  $\mu$ g/ml, shown to yield maximum lymphocyte transformation responses with control mononuclear leukocytes (10,16), and at several suboptimal concentrations (80, 40, and 20  $\mu$ g/ml). Microtiter plates were then incubated at 37°C in 5% CO<sub>2</sub> and air. Cultures were pulsed with the tritiated precursor for the terminal 5 hr of incubation, and were harvested with a semiautomatic cell harvester (Brandel, Inc., Gaithersburg, Md.). Samples were counted with a liquid scintillation counter. Cells were pulsed and harvested from immediately ("zero" days) to 5 days after exposure or sham-exposure to microwaves. For each individual experiment, arithmetic mean counts per minute (cpm) of quadruplicate cultures were determined.

The absolute counts per minute of tritiated precursor incorporated by the normal (control) PHA-stimulated cells of different individuals varied, as previously established (16). Within each individual experiment, however, the relative responses of microwave-exposed, sham-exposed, and control mononuclear leukocytes were consistent.

### Assays of Interferon Production

The interferon activity in supernatant culture fluids of unstimulated, influenza virus-stimulated, and PHA-stimulated mononuclear leukocytes was assayed by the inhibition of plaque formation by vesicular stomatitis virus in human foreskin fibroblast cultures, as previously described (14,15). Production of interferon- $\alpha$  was induced by exposure of the leukocytes to influenza A/AA/Marton/43 HONI at multiplicities of infection (MOI) of 10, 1.0, and 0.1. Production of interferon- $\gamma$  was induced by exposure of the leukocytes to PHA at a concentration of 160  $\mu$ g/ml. The minimum interferon titer detected in these studies was 5 units/ml. Results are presented in mean log<sub>2</sub> units/ml  $\pm$  S.E.

### Statistical Analyses

Analysis of variance and Student's t-test were selected for analysis of the data.

## RESULTS

### Leukocyte Viability

Exposure of the leukocytes at an SAR of 4 mW/ml produced no significant changes in cell viability for up to one week after exposure (Table 1). Results were similar, with exposures at lower SARs.

Table 1. TOTAL VIABLE MONONUCLEAR LEUKOCYTES AFTER EXPOSURE TO MICROWAVE ENERGY AT SAR = 4 mW/ml

| Exposure  | Days of Exposure <sup>a</sup> |       |      |       |       |       |
|-----------|-------------------------------|-------|------|-------|-------|-------|
|           | 1                             | 2     | 4    | 5     | 6     | 7     |
| Microwave | 58±7 <sup>b</sup>             | 60±9  | 41±4 | 47±16 | 39±10 | 40±10 |
| Sham      | 65±11                         | 63±15 | 39±2 | 72±36 | 41±9  | 37±8  |
| Control   | 54±6                          | 56±6  | 41±8 | 46±13 | 36±7  | 38±11 |

<sup>a</sup>Insufficient numbers of observations (<5) were available 3 days after exposure. Viability was assessed by the ability of the cells to exclude trypan blue dye and ethidium bromide.

<sup>b</sup>Data represent mean total number of viable cells (total cells X percent viable X 10<sup>-4</sup>, ± S.E.

#### DNA, RNA, and Total Protein Synthesis

Unstimulated and mitogen-stimulated DNA, RNA, and total protein synthesis were examined after exposure of the mononuclear leukocytes to microwaves at SARs of 4 mW/ml or less. No significant differences existed between microwave-exposed (4 mW/ml), sham-exposed, and control leukocytes in unstimulated DNA, RNA, or protein synthesis (Figs. 1-3, respectively), or in responses of the leukocytes to an optimal concentration of mitogen (Figs. 1-3). Results were similar using suboptimal concentrations of mitogen, and using lower SARs (0.5 and 1.0 mW/ml) for the microwave-exposed cultures (data not shown). Microscopic inspection of Wright-Giemsa-stained cytospin preparations did not reveal any discrepancies between morphologic lymphocyte blastogenesis (used in some of the studies cited earlier) and determinations using incorporation of the radiolabelled precursors.

#### Interferon Synthesis

In addition to our determinations of total protein synthesis, we measured spontaneous production of interferon (none detected in any cultures), as well as production of influenza virus-induced interferon- $\alpha$  and PHA-induced interferon- $\gamma$ , at 1 and 3 days after induction (14, 15). By 24 hr, virtually all detectable virus-induced interferon- $\alpha$  was present, with equivalent amounts being produced by microwave-exposed (SAR = 4 mW/ml), sham-exposed, and control leukocytes (Fig. 4A). Equivalent interferon titers were produced by the leukocytes, whether influenza virus was added at an MOI of 10 (Fig. 4A), or at an MOI of 1.0 or 0.1 (data not shown). PHA-induced interferon- $\gamma$ , usually produced by 48-72 hr, was not detected in any culture supernatant fluid at 24 hr.

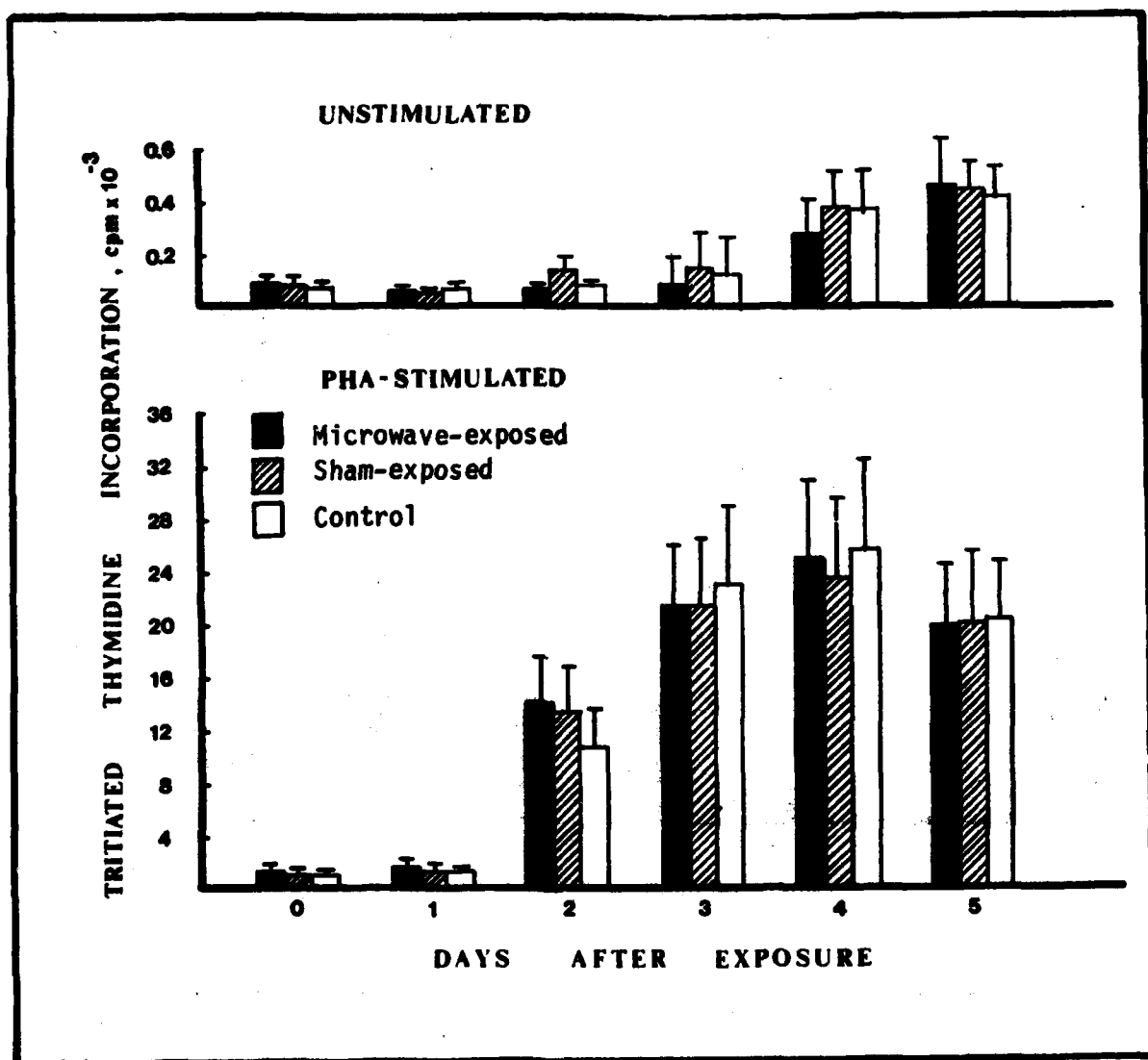


Figure 1. DNA synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes. SAR = 4 mW/ml. DNA synthesis by unstimulated cells and by cells stimulated by an optimal concentration of PHA (160  $\mu$ g/ml) is shown. Columns indicate mean counts per minute tritiated thymidine incorporated  $\pm$  S.E., from immediately to 5 days after exposure.

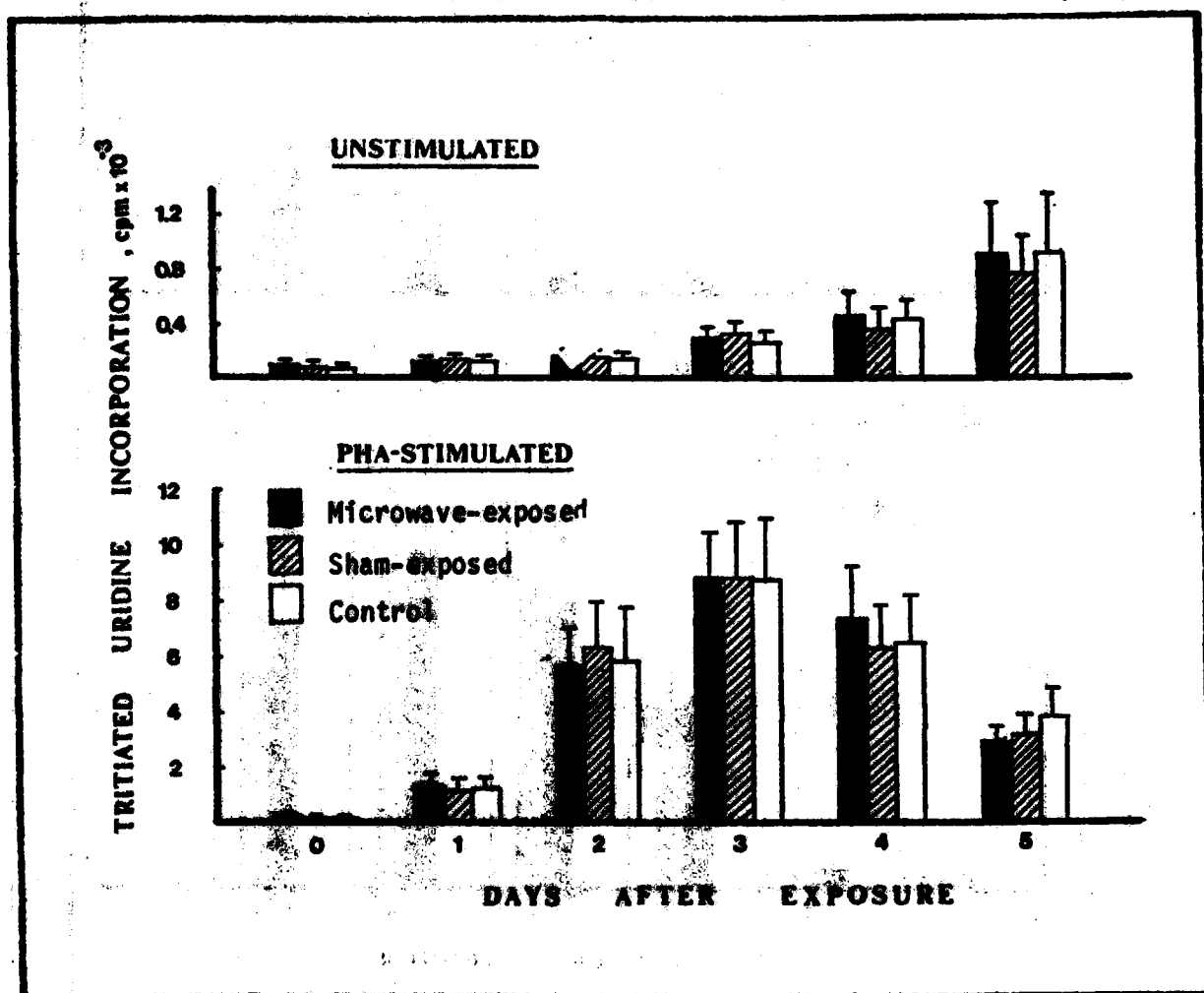


Figure 2. RNA synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes. SAR = 4 mW/ml. RNA synthesis by unstimulated cells and by cells stimulated by an optimal concentration of PHA (160 µg/ml) is shown. Columns indicate mean counts per minute tritiated uridine incorporated ± S.E., from immediately to 5 days after exposure.

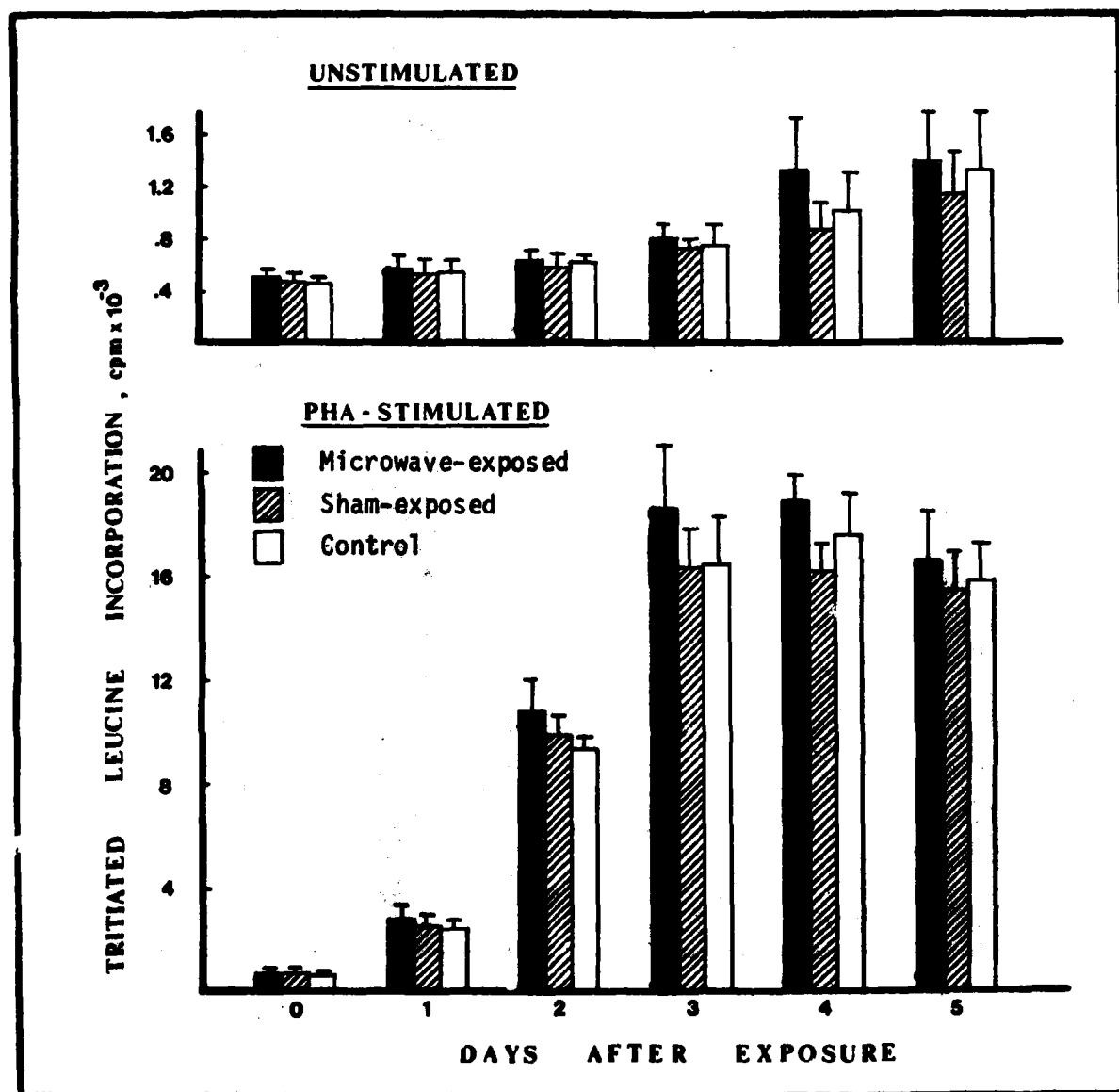


Figure 3. Protein synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes. SAR = 4 mW/ml. Protein synthesis by unstimulated cells and by cells stimulated by an optimal concentration of PHA (160  $\mu$ g/ml) is shown. Columns indicate mean counts per minute tritiated leucine incorporated  $\pm$  S.E., from immediately to 5 days after exposure.

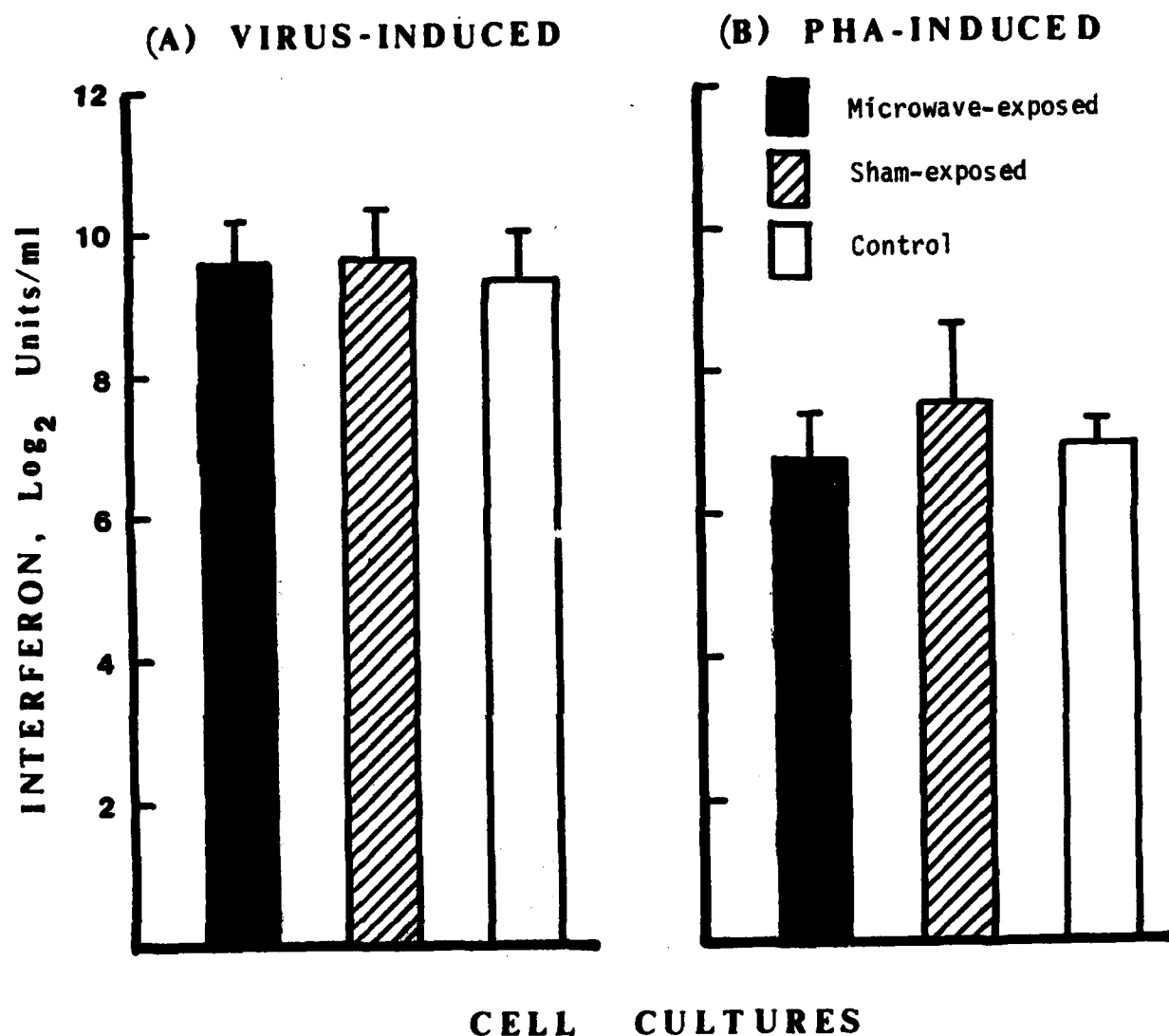


Figure 4. Interferon- $\alpha$  and interferon- $\gamma$  synthesis by microwave-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes.

SAR = 4 mW/ml. (A) Interferon- $\alpha$  titers present 24 hr after induction with influenza virus; and (B) interferon- $\gamma$  titers present 72 hr after induction with PHA. Columns indicate mean log<sub>2</sub> units/ml  $\pm$  S.E.

By 72 hr, interferon- $\gamma$  was detected in all PHA-induced cultures, with no significant differences appearing between the microwave-exposed (SAR=4mW/ml), sham-exposed, and control leukocytes (Fig. 4B). Results were similar to exposures to microwaves at lower SARs.

## DISCUSSION

The U.S. safety standard limit, proposed by Committee C-95.4 of the American National Standards Institute, is 0.4 mW/g --which is equivalent to 0.4 mW/ml in the cultures of our studies. This standard incorporates at least a 10-fold safety factor relative to bioeffects reported using animal models. In the current studies, exposure of human mononuclear leukocytes to microwave energy at SARs up to 4 mW/ml resulted in no detectable effects on cell viability, or unstimulated or stimulated DNA, RNA, total protein, interferon- $\alpha$ , or interferon- $\gamma$  synthesis.

Our current studies provide the first clear and reproducible data concerning exposure of human leukocytes to microwave energies relevant to current public safety recommendations. Direct extrapolation to the in vivo setting--with many physiological, homeostatic, integrated systems--is not appropriate. However, these data do suggest that earlier reports of possible microwave effects on human leukocytes, at such energy levels, remain poorly reproducible and should not form a basis for the re-setting of safety standards. Most studies of environmental physical factors examine effects on resting cell populations even though, under normal conditions, man is commonly exposed to more than one environmental stress at a time (20). Thus, our results are notable further for indicating that human leukocytes exposed to microwaves, as a potential physical stress factor, can respond normally to a second biological factor, such as the commonly encountered infectious agent, influenza virus.

The current studies do not exclude the existence of microwave-induced effects on human leukocytes resulting from exposures at greater SARs. Such exposures commonly produce effects that can be related to the degree and/or the rate of heating of the cell cultures or tissues in vivo (7,21). Furthermore, the current studies do not completely exclude potential microwave-induced effects, resulting from exposure at similar SARs but applied by almost innumerable different possible wave forms (e.g., frequencies, modulations, etc.).

The ubiquitous distribution of microwave energy and the potential differences between animal models and humans suggest that further investigations with human leukocytes and other cells may be warranted. The literature regarding microwaves includes animal studies reporting deleterious effects attributed to exposure as well as animal studies reporting beneficial effects, over a broad range of SARs. Fever or moderate hyperthermia, including microwave-induced hyperthermia, has been shown to enhance human leukocyte and animal leukocyte functions, and has been shown to enhance survival in animals challenged with bacteria, viruses, or tumors (7,8,17,18, 22,23). Thus, further investigations regarding microwave exposure should not only serve to define and limit health hazards for humans, but also to

define and expand potential health benefits, such as the use of microwave-induced hyperthermia in the treatment of cancer.

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