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# MEMBRANE DAMAGE OF HUMAN RED BLOOD CELLS INDUCED BY LOW-POWER MICROWAVE IRRADIATION

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## ABSTRACT

The effect of low-level, 2.45 GHz microwaves on human erythrocyte membrane was studied by measuring the induced hemolysis of the exposed erythrocytes at different power densities (0.025–10.000 mW/cm$^2$). A significant increase of the hemoglobin loss by the microwave-exposed erythrocytes compared to controls was observed. Red blood cell count was essentially the same in irradiated and control samples while the mean cellular hemoglobin concentration decreased in the exposed samples. These observations indicate that the hemoglobin loss from the microwave-irradiated cells is due to the membrane permeabilization of the exposed erythrocytes rather than to their lysis.

## INTRODUCTION

Previously reported data on the irradiation of human blood with microwaves of low-power density have produced conflicting results. They are difficult to interpret and compare because of the different experimental designs (frequencies, power densities, times of exposure, as well as temperature and solvents used) described in different papers.

Baranski *et al.* (1) observed increased hemoglobin (Hb) and potassium ion ($K^+$) leakage from rabbit erythrocytes irradiated for 3 h with low-power density (5.00 $mW/cm^2$) 3 GHz radiation at room temperature. Ismailov (2) obtained similar results on human erythrocytes exposed to 45 $mW/cm^2$ of 1 GHz radiation.

In contrast, Hamrick and Zinkl (3) found no increase in Hb and $K^+$ loss from rabbit and human red blood cells (RBC) exposed to different power densities (4 $mW/cm^2$, 10 $mW/cm^2$, and 75 $mW/cm^2$) of 2.45 GHz radiation; however, they observed a change in the osmotic resistance of the erythrocytes. Peterson *et al.* (4) have also reported no significant increase in Hb and $K^+$ leakage from rabbit and human RBC irradiated with 2.45 GHz, 10 $mW/cm^2$ radiation. These authors argue for an absence of any nonthermal effect of microwave irradiation. The same idea was recently claimed by Marani and Feirabend (5).

Olcerst *et al.* (6) found an increased $Na^+$ and $Rb^+$ efflux, but no Hb leakage from rabbit erythrocytes irradiated with 2.45 GHz, specific absorption rate (SAR) of 100 mW/g, at different temperatures; Galvin *et al.* (7) also found no hematological effects (Hb level, RBC count, etc.) in rats exposed for 8 h to 2.00 and 10.00 $mW/cm^2$ of 2.45 GHz continuous-wave (CW) radiation.

The purpose of the present study was to examine the effects of long-term exposure of human blood to 2.45 GHz CW radiation at power densities that are supposed not to induce thermal effects; it was shown theoretically by Keilmann (8) for biological 1-$\mu$m-sized enitities (cells) that heating induced by power densities below 10 $mW/cm^2$ is of the order of $10^{-5}$ °C. This is why we suppose that the effects expected in the present study are nonthermal.

The microwave frequency of 2.45 GHz was chosen for use in this study due to its technological predominance. This frequency is used extensively in domestic, industrial, and therapeutic applications and is the proposed transmission frequency for future power satellites.

The effects on the erythrocyte membrane have been characterized by measuring Hb loss (expressed as hemolysis degree $\alpha$) during and after irradiation at different power levels.

## MATERIALS AND METHODS

Blood samples have been collected in isotonic ACD (52 mmol/L citric acid; 119 mmol/L sodium citrate; 136 mmol/L glucose) from six different individuals at a 1:5 v/v dilution. The samples were divided into 1-ml aliquots. In each experiment, 10 of these aliquots were exposed to the selected CW fields and 10 were reserved as concurrent controls.

Irradiation was performed with 2.45 GHz microwave radiation at different power densities (0.025–10.000 $mW/cm^2$) in a controlled water bath (4°C). Except for the kinetic measurements, time of irradiation was 60 h. At the end of the irradiation time each sample was washed in phosphate buffered saline (PBS) (0.122 M NaCl, 0.030 M $KH_2PO_4$ + $Na_2HPO_4$, pH 7.4, 2 $g.L^{-1}$ glucose, 310 $mOsmol.kg^{-1}$) by diluting it with 3 ml PBS and centrifugating for 30 min at 500 × $g$ at 4°C.

The degree of hemolysis ($\alpha$) was determined spectrophotometrically as the ratio of the optical absorbance of supernatant from each sample at 420 nm to the absorbance of a control sample which was totally hemolyzed by osmotic shock.

In order to check for the reversibility of the radiation damage, the pellets were washed by centrifugation, resuspended in 3 ml PBS, and left for 24 h, at the end of which the hemolysis degrees of all samples were determined again. By "reversibility" we mean the postirradiation recovery of the erythrocyte membrane permeabilized for hemoglobin during microwave irradiation.

For the kinetic measurements, the hemoglobin loss of the irradiated and control samples belonging to the same blood unit was measured at different exposure times, using a constant power level.

## RESULTS

### The Hemoglobin Loss

The hemoglobin (Hb) loss from irradiated and control samples was measured as hemolysis degree ($\alpha$); $\alpha$ is given in Table 1 for different power densities. Due to the large intrinsic variability of the blood samples collected from different individuals, global statistical analysis for $\alpha$ at one power density was not possible; this is why the results are given for each different blood unit (different experimental runs) separately. Since only three horn antennae were available, it was not possible to irradiate the same blood sample at all nine power densities given in the table. Thus the set of chosen powers varied from one experiment to the other in order to cover the behavior of the blood at all mentioned power levels.

One can see (Table 1) that the obvious discrepancies between irradiated and control samples appear already at 0.10 mW/cm$^2$, 60 h irradiation.

**Table 1.** The Hemolysis Degree of 6 Blood Units Exposed for 60 h at Different Power Densities

| | D(mW/cm$^2$) | | | | |
|---|---|---|---|---|---|
| Blood unit | 0.000 (control) | 0.025 | 0.050 | 0.100 | 0.250 |
| 1 | 1.97 ± 0.12 | | 2.33 ± 0.15 | | |
| 2 | 1.25 ± 0.14 | | 1.39 ± 0.09[b] | | |
| 3 | 1.92 ± 0.15 | 1.97 ± 0.12[a] | | | 2.09 ± 0.07 |
| 4 | 2.01 ± 0.14 | 2.61 ± 0.25 | | | 3.00 ± 0.19 |
| 5 | 1.33 ± 0.16 | | 1.44 ± 0.13[c] | | |
| 6 | 2.00 ± 0.15 | | | 2.29 ± 0.09 | |
| | D(mW/cm$^2$) | | | | |
| Blood unit | 0.500 | 1.000 | 2.500 | 5.000 | 10.000 |
| 1 | 2.88 ± 0.19 | | | 3.45 ± 0.25 | |
| 2 | 1.63 ± 0.11 | | | 2.07 ± 0.10 | |
| 3 | | | 2.39 ± 0.07 | | |
| 4 | | | 4.61 ± 0.23 | | |
| 5 | 1.69 ± 0.13 | | | 2.09 ± 0.18 | |
| 6 | | 2.77 ± 0.15 | | | 3.71 ± 0.25 |

Each value is expressed as mean ± SD calculated for 10 different samples. The level of significance for the difference from controls is $p < 0.05$, excepting cases [a]$p < 0.5$; [b]$p < 0.1$; [c]$p < 0.3$.

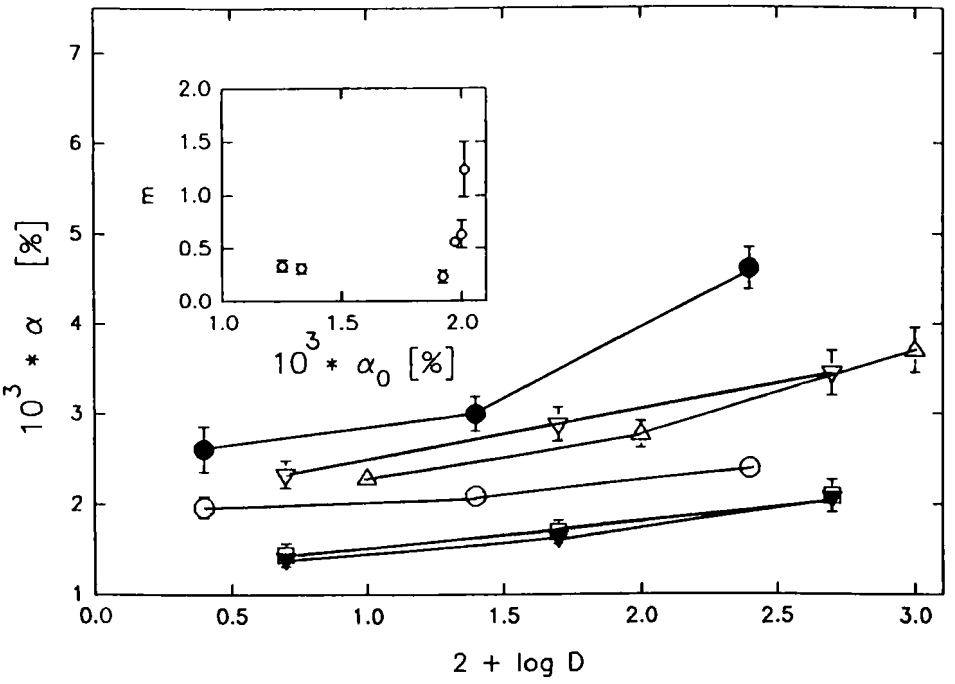


FIGURE 1. Semilogarithmic representation of the degree of hemolysis versus power density for six different blood units. (Inset) The dependence of the slope (m) on the initial degree of hemolysis of each blood unit. Blood unit number 1: ∇; 2: ▼; 3: ○; 4: ●; 5: □; 6: Δ.

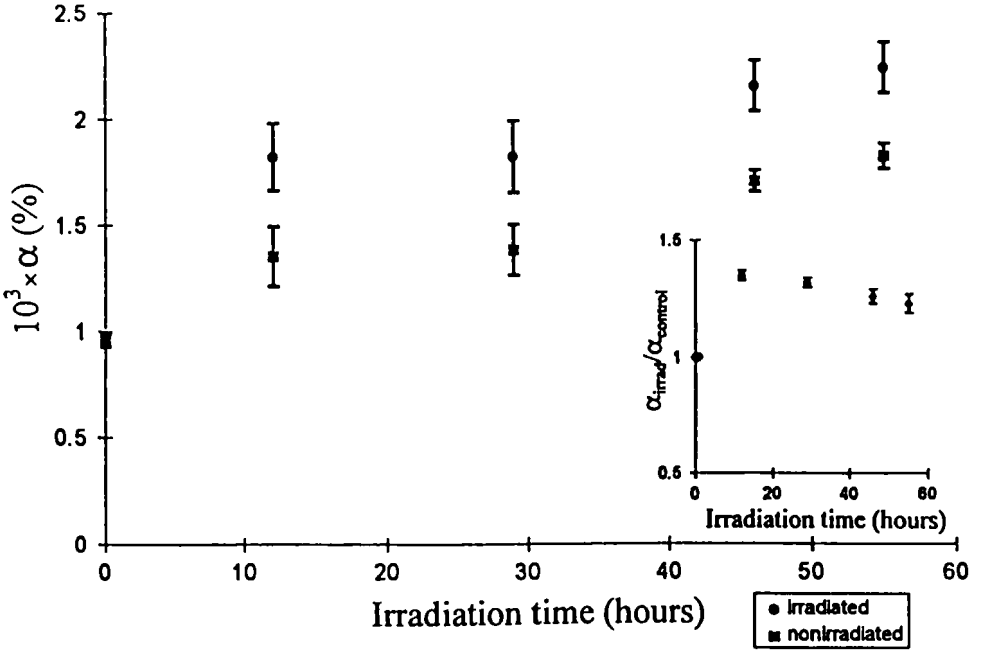


FIGURE 2. Kinetics of hemolysis degree $\alpha$ at irradiation with 5 mW/cm$^2$. (Inset) $\alpha_{irrad}/\alpha_{control}$ versus irradiation time ($\alpha_{irrad}$ and $\alpha_{control}$ represent the mean hemolysis degrees of the irradiated and control samples, respectively).

In Figure 1, the hemolysis degree $\alpha$ versus log power density is represented for six different blood units. Each of these plots shows a linear dependence (correlation coefficients $r \geq 0.94$). It appears from Figure 1 that the rate of the $\alpha$ increase (reflected by slope $m$) is strongly dependent on the initial hemolysis degree $\alpha_0$ of the blood unit (see inset of Fig. 1).

The kinetics of $\alpha$ at irradiation with 0.84, 1.36, and 5.00 mW/cm$^2$ was also recorded. At irradiation with 5.00 mW/cm$^2$ and different exposure times, the Hb loss becomes evident after 10 h exposure (Fig. 2).

### The Blood Count and MCHC

The blood count provided by a Coulter Counter for buffer-washed samples indicated that the number of RBCs is essentially the same in irradiated and control samples (Table 2).

The Hb content determined spectrophotometrically in washed erythrocytes was lower however in irradiated than in control samples. The same is indicated by the mean cellular hemoglobin concentration (MCHC) value provided by the Coulter Counter (Table 2). This suggests that Hb release induced by irradiation is due to membrane permeabilization rather than to the lysis of the RBC.

### The Reversibility of the Membrane Damage

Determining the hemolysis degree of each sample after 24 h postirradiation storage at 4°C showed that irradiated samples are losing the same amount of Hb as controls (the level of significance for the difference between irradiated and control samples was $p > 0.04$). Thus, at least at the temperature, power densities, and exposure times used in these particular experiments, the membrane permeabilization caused by irradiation seems to be reversible.

There are two ways of interpreting this result, depending on two different assumptions:

1. The microwave-induced hemolysis is due to a hemoglobin leakage of the irradiated erythrocyte via permeabilization of its membrane.
2. The increase of $\alpha$ in the irradiated blood reflects the complete lysis (membrane disruption) of a fraction of irradiated erythrocytes.

In our opinion the first hypothesis is closer to reality, since no depression in RBC count of the irradiated blood was observed. This is in agreement with recent studies

**Table 2.** Blood Parameters of Irradiated and Control Samples (Blood Unit 1)

| RBC[a] $10^{12}$ ($l^{-1}$) | Hct (l/l) | MCHC (mmol/l) | Probes |
|---|---|---|---|
| 1.08 ± 0.02 | 0.91 ± 0.02 | 28.4 ± 0.03 | Irradiated |
| 1.04 ± 0.03 | 0.87 ± 0.02 | 33.4 ± 0.30 | Nonirradiated |

RBC, number of red blood cells/volume unit; Hct, hematocrit; MCHC, mean cellular hemoglobin concentration.

[a]Blood samples were diluted 4 times in PBS.

on liposomes irradiated with 2.45 GHz microwaves, which have shown that the lipid membrane becomes leaky for entrapped carboxyfluorescein (9,10). Other studies show the microwave-induced modification of the receptor-binding properties of the erythrocyte membrane (11) as well as its permeabilization to low-molecular-weight proteins (12).

## DISCUSSION

Summarizing our observations we may draw the following conclusions:

1. The microwave-induced increase of Hb loss by irradiated erythrocytes is up to 80% (at maximal powers used) greater than the spontaneous Hb loss by the controls.

2. The rate of microwave-induced increase of Hb loss reaches saturation below 10 h for irradiation with 5.00 $mW/cm^2$ (Fig. 2) and requires longer times for lower power densities (about 30 h for 1.36 $mW/cm^2$ and more than 60 h for 0.84 $mW/cm^2$).

3. The rate of the increase of Hb loss with increasing power density is highly dependent on the initial level of spontaneous hemolysis. It seems that the membrane is more sensitive to the radiation power if it was leakier at the start (Fig. 1).

4. The logarithmic dependence of $\alpha$ (which may be designated as "the rate of the microwave effect," being a measure of the microwave-induced hemolysis after irradation time $\Delta t$) versus the rate of the power increase is in agreement with Fröhlich's prediction concerning the triggering effect of nonthermal microwave levels (13). The manner of the interaction of the microwaves with the erythrocyte is still a "black box"; however, the formal resemblance of our data with Fröhlich's curve (which gives the theoretical dependence of the response rate of the biological system vs. the rate of the microwave energy supply) advocates the excitation of coherent modes of vibration in the erythrocyte membrane by the incident radiation.

The mechanism by which microwave radiation causes an increase of Hb loss is, of course, of primary interest. As a result of this study, we believe that the hemoglobin is lost via permeabilization of the plasma membrane and is not due to cell lysis. The main argument for this is the drastic decrease of MCHC while the average RBC count remains essentially the same for irradiated and control blood.

It must be noted that the induced hemolysis we observe is negligible ($\alpha$ is of the order of 0.1% of the total Hb content of the samples) at the power levels we used. However, it may become important if the initial hemolysis is high, so that the erythrocyte membrane is already destabilized and leaky.

Probably, *in situ*, the natural protection mechanisms would defend/repair the red cell membrane insulted by prolonged interaction with high-frequency radiation. This may be the reason why a reliable reproducible effect of the microwave irradiation *in vivo* could hardly be observed (14). We think, however, that our results may be of some interest and have to be taken into consideration in the case of persons living and working quasi-permanently in the vicinity of microwave sources.

## ACKNOWLEDGMENTS

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