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# Exposure of Rabbit Erythrocytes to Microwave Radiation

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Rabbit erythrocytes were exposed to microwave radiation at 2450 and 3000 MHz in an attempt to duplicate reported changes in the erythrocyte membrane permeability to potassium and changes in osmotic resistance. However, our results show no significant difference between exposed and control erythrocyte suspensions in either potassium efflux or in osmotic resistance.

Several reports[1] (*1*, *2*) indicate that the *in vitro* exposure to continuous-wave (CW) microwave radiation has a very noticeable effect on the membrane permeability and structural integrity of erythrocytes. These reported effects have been observed on both human and rabbit erythrocytes. Wavelengths of 3 and 10 cm and exposure intensities of 1–45 mW/cm² were effective in causing accelerated potassium and hemoglobin efflux from erythrocytes and in decreasing the osmotic resistance of the cells. The magnitude of these effects increased with intensity and with time of exposure. Exposure times of up to 3 hr have been employed with very definite effects being reported for times of 1 hr or more even for intensities as low as 1 mW/cm². Stodolnik-Baranska (*1*) has concluded from these studies that the rate of active transport as well as the rate of diffusion through the membrane is affected by microwave radiation.

The changes reported in the membrane function were highly significant. For example, Baranski (*2*) reported for a 3-hr exposure at 5 mW/cm² and 10-cm wavelength (CW) that the efflux of potassium and hemoglobin from the erythrocyte is increased by factors of the order of 15–20 times. In the same study, the saline concentration at which 100% hemolysis was observed changed by 0.2% saline indicating a reduced osmotic resistance. In all of the above-mentioned studies the temperature of exposed and control solutions were maintained equal.

On the basis of the dramatic results reported in these experiments, we decided the erythrocyte system would be excellent for investigating the mechanism of

[1] S. Baranski and S. Szmigrelski. Effect of Microwave Irradiation *in vitro* on Cell Permeability. Paper presented at the International Symposium on Biological Effects and Health Hazards of Microwave Radiation, Warsaw, Poland, 1973. (Organized by Scientific Council to the Minister of Health and Social Welfare of the Polish Peoples Republic.)

interaction of microwave with biological systems. However, we have been unable to verify these experiments.

## EXPERIMENTAL METHODS

Freshly drawn, heparinized rabbit blood was used in the preparation of the erythrocytes [heparin was the anticoagulant used by Baranski (*2*)]. The erythrocytes were spun down by centrifugation and washed three times in the medium which was to be used for the final suspension (resuspended to the initial blood volume). The medium was sodium phosphate-buffered saline (pH 7.4). Three concentrations of saline were used, 0.9%, 0.66%, and 0.54% for the suspending medium. In one case, glucose, calcium, and magnesium ions were added to the medium. A series of buffered saline solutions of varying concentrations were prepared for use in determining the osmotic resistance of the cells (*3*). Before use, the osmolarity of the various saline concentrations was determined with a Model 3L Osmometer (Advanced Instruments, Inc.).

Two milliliters of the erythrocyte suspension were placed in a test tube and irradiated for various times. The temperature was monitored during the exposure by a thermistor in a similar tube containing 2 ml of erythrocyte suspension which was also placed in the microwave field. Controls held at the same temperature in a water bath were treated the same way. The exposure intensities were measured with a Narda exposure meter for 2450 MHz and a National Bureau of Standards meter for 3000 MHz. The absorbed power was determined from the heating and cooling rates of the exposed solutions. The monitoring system and method of determining absorbed power is treated in more detail in a previous article (*4*).

After exposure, the cells were spun down in a centrifuge and the supernatant diluted by a factor of 20. The potassium content of the solution was determined with a Model 143 flame photometer (Instrumentation Laboratory, Inc.). Twenty

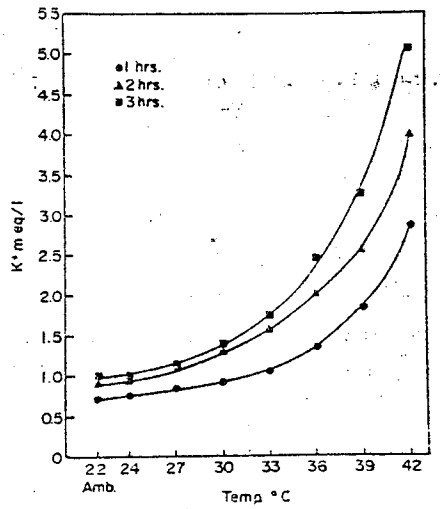


FIG. 1. Temperature dependence of potassium efflux for 1-, 2-, and 3-hr holding times.

TABLE 1

POTASSIUM EFFLUX (meq/liter ± p.e.) FROM RABBIT ERYTHROCYTES EXPOSED TO MICROWAVE RADIATION

| Suspending medium | Exposure intensity | | | | | |
|---|---|---|---|---|---|---|
| | 4 mW/cm²[a] (2,450 MHz) | | 10 mW/cm²[b] (2,450 MHz) | | 75 mW/cm²[c] (2,450 MHz) | |
| | Control | Exposed | Control | Exposed | Control | Exposed |
| | 2-hr exposure | | 1-hr exposure | | 2-hr exposure | |
| 0.9% Buffered saline | 0.53 ± .01 | 0.55 ± .01 | 0.55 ± .01 | 0.55 ± .01 | 1.25 ± .01 | 1.24 ± .01 |
| | 4-hr exposure | | 3-hr exposure | | 4-hr exposure | |
| | 0.92 ± .02 | 0.91 ± .01 | 0.67 ± .02 | 0.70 ± .01 | 1.71 ± .01 | 1.77 ± .05 |
| 0.66% Buffered saline | — | | — | | 2-hr exposure | |
| | | | | | 1.46 ± .01 | 1.39 ± .12 |
| 0.54% Buffered saline | — | | — | | 2 hr exposure | |
| | | | | | 1.51 ± .02 | 1.40 ± .05 |
| 0.9% Buffered saline and glucose | — | | — | | 2-hr exposure | |
| | | | | | 0.88 ± .01 | 0.87 ± .01 |
| | 5 mW (3000 MHz) | | | | 3-hr exposure | |
| | | | | | 1.05 ± .05 | 1.02 ± .01 |
| | 1-hr exposure | | | | | |
| 0.9% Saline unbuffered | 0.34 ± .01 | 0.35 ± .01 | — | | — | |
| | 3-hr exposure | | | | | |
| | 0.39 ± .01 | 0.38 ± .01 | | | | |

[a] Temperature rise <0.2 C above ambient, no compensation made in temperature of control.
[b] Temperature rise <1.5 C above ambient, temperature of control increased by 1.5 C by water bath.
[c] Temperature of exposed and control 30 C. Control temperature maintained by water bath.

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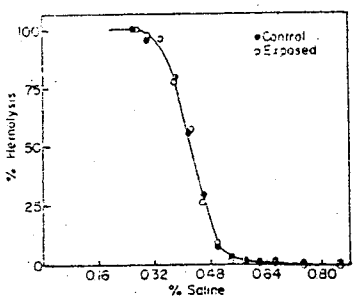


FIG. 2. Percent hemolysis as a function of saline concentration for exposed and control erythrocytes.

microliters of erythrocytes were added to each of a series of 13 tubes containing 5 ml of buffered saline with concentrations ranging from 0.26 to 0.85%. Hemolysis was allowed to proceed for 1 hr. The tubes were cleared of lysed and unlysed cells by centrifugation and the supernatant used to determine the percentage of hemolysis. The optical density of the supernatant was determined at 540 nm with a Gilford 300 N spectrophotometer.

## RESULTS AND DISCUSSION

We concentrated our work on two parameters, the potassium efflux and the osmotic resistance. Figure 1 shows the results of a controlled-temperature study on potassium efflux. This study without microwave exposure shows that potassium efflux is highly temperature dependent. In similar studies on the temperature dependence of osmotic resistance, the osmotic resistance was not found to be highly dependent on temperature.

Our initial studies with microwaves were made at 2450 MHz(CW). Exposures were made at three exposure intensities; 4 mW/cm², 10 mW/cm², and 75 mW/cm² with absorbed power densities of 3 mW/g, 7.6 mW/g, and 57 mW/g, respectively. Since the potassium efflux varied so much with temperature, we ran three temperature controls for each exposure, one at the temperature of exposure, another 1 C above, and the third 1 C below the exposure temperature in order to bracket the exposure temperature. The temperature in the exposed tube varied $\pm\frac{1}{2}$ C. Only control data corresponding to the temperature recorded for the exposed tube is presented. Table I contains the results of our study on potassium efflux. Values are given in milliequivalents per liter $\pm$ the probable error. Four suspending media were used. We began the study using 0.9% buffered saline. When we found no effects using the 0.9% buffered saline, we tried lowering the saline concentration to increase the sensitivity. When we still obtained negative results, we decided to employ a more physiological buffer system since Stodolnik-Baranska (*1*) had indicated active transport was affected. Again no differences could be detected between exposed and control solutions. All cases were tested for statistically significant difference using a paired $t$ test and none was statistically significant at the $p = 0.1$ level.

For each case in Table I, the osmotic resistance was also determined. In none of the cases was any difference detected of the magnitude reported by Baranski

(2). Rather than present the curves for all the negative cases, Fig. 2 is presented as typical of the curves obtained. Although we concentrated our work on the potassium efflux and the osmotic resistance, on several occasions we looked for differences in hemoglobin efflux between control and exposed solutions but saw none.

Since we found no differences between exposed and control solutions, we were concerned that our procedure was in some way different from that of Baranski's (2). We were fortunate to have an opportunity to discuss the experiment with Dr. Pryemyslaw Czerski of Poland who has done some work with Baranski and was familiar with the experiment. He suggested that we try unbuffered saline for washing and resuspending the cells and to expose at 3000 MHz rather than 2450 MHz. Following these suggestions, we repeated our work exposing at 5 mW/cm$^2$ (5.2 mW/g absorbed power) for 1 and 3 hr but again found no statistically significant differences (see bottom of Table I).

We realize it is almost impossible to duplicate in every detail another's experiment and we may have omitted just the factor needed to reproduce the results. However, we felt our results should be reported and would appreciate hearing from others who have tried to repeat these experiments with either positive or negative results.

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