

MICROWAVE ALTERATION OF THE BLOOD–BRAIN BARRIER SYSTEM OF RATS

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SUMMARY

Rats were exposed to 1.3 GHz microwave energy to assess the uptake of several neutral polar substances in certain areas of the brain. A quantitative, radioactive isotope method, which uses an internal standard, was employed to measure the loss of test substances to brain tissue. Single, 20 min exposure, to either pulsed or continuous wave (CW) microwave energy induced an increase in the uptake of D-mannitol at average power densities of less than 3.0 mW/sq. cm. The permeability change was greatest in the medulla, followed, in decreasing order, by the cerebellum and hypothalamus, with small or negligible changes in the hippocampus and cortex. Permeability increases were observed for mannitol and inulin but not for dextran. Increased permeability was observed both immediately and 4 h after exposure, but not 24 h after exposure. After an initial rise, the permeability of cerebral vessels to saccharides decreased with increasing microwave power. Differences in the level of uptake occurred between CW energy and pulsed energy of the same average power. Microwaves of the same average power but different pulse characteristics also produced different uptake levels. Our findings suggest that microwaves induce a temporary change in the permeability for small molecular weight saccharides in the blood–brain barrier system of rats.

INTRODUCTION

In the last few years several central nervous system (CNS) alterations have been shown to occur as a result of low power, non-ionizing electromagnetic radiation^{2,14,19,20}. These findings are significant in the study of the interaction of electromagnetic energy with the CNS both as to safety concerns and as a new research tool in the study of brain function. Amplitude modulated fields (147 MHz, 1 mW/sq. cm) have been

shown by Bawin et al.⁴ to strongly influence spontaneous and conditioned EEG patterns in the cat. Albert¹ has observed neuronal swelling, vacuolation, and chromatolysis in the hypothalamic and subthalamic brain regions of Chinese hamsters exposed to a 10 mW/sq. cm CW microwave field. Reduced calcium efflux with oscillating extremely low frequency fields³ and increased calcium efflux with modulated very high frequency fields⁵ have been demonstrated in isolated chick and cat brain. Such studies indicate that electromagnetic energy is causing changes in the CNS and the investigations which utilize quantitative techniques are very fruitful in uncovering possible mechanisms of interaction and their dependence on microwave parameters.

The blood-brain barrier (BBB) system has been used to investigate effects on the CNS of various types of physiological activity and stress. Bondy and Purdy⁶ have shown an increased penetrance of tyrosine into brain areas receiving reduced sensory input. Sabbot and Costin³¹ have shown increased uptake of ⁴⁵Ca in brain tissue as a result of cold stress conditions. Concussions have been studied experimentally by the creation of pressure pulses induced by the sudden introduction of a small volume of fluid extradurally through a parietal trephine hole³⁰. Low magnitude pressure pulses gave abnormal penetration of protein tracer within the walls of the blood vessels. Ionizing radiation, edema, anoxia, hypertension, drug induced convulsions, embolisms, osmotic imbalances, etc. have all been shown^{7,9,21,23} to cause BBB changes and increased permeability of substances to the brain. Recently, Frey et al.¹³ have reported increases in the brain tissue permeability of rats to intravenously injected fluorescein dye when exposed to low power, pulsed or CW microwave energy at 1.2 GHz.

In the present studies a quantitative radioactive isotope technique was used to corroborate and extend the work of Frey on microwave induced BBB permeability changes in brain. The technique, developed by Oldendorf^{24,26}, permits quantitative measurement of the penetration of a variety of test substances as a function of any of several variables such as microwave characteristics, brain region, time after exposure, molecular weight of test substance, etc. While this technique does not allow the detailed localization of tracers and display of the BBB alteration site that staining and observation with an electron microscope might, it does permit limited mapping of tracer penetration. This technique also lends itself well to simple statistical interpretation as to whether a certain insult produces a BBB permeability change, even if the change is only a small one.

MATERIALS AND METHODS

Male Wistar rats from the Walter Reed colony served as subjects in all experiments. When an individual rat had grown to a body weight of 230–270 g, it was scheduled for a single sham or microwave exposure that day between 09.00 and 15.00 h. Day of the week, time of day, and body weight were balanced among test groups in each experiment. One or more control groups were included in each test and initial procedures were designed as a double blind evaluation with the inclusion of blanks.

Exposure conditions

A microwave anechoic chamber (5 m wide by 5 m high by 10 m long) maintained

at 22 ± 1 °C was used for exposures. The inside surfaces were covered with wedge absorber (EC, WC-4) and areas of possible specular reflection were covered with pyramidally shaped absorber (EC, VHP-45). The resulting performance is such that reflected energy is approximately 40 dB below the direct rays.

All exposures were at a frequency of 1.3 GHz (wavelength of 23 cm). For CW exposures the transmitter consisted of a sweep frequency generator (Hewlett-Packard 8690B) and an amplifier (Alfred 5020) coupled to a standard gain horn (Narda Model 646). Only the horn was mounted in the anechoic chamber. The output was monitored by a calibrated directional coupler and power meter (Hewlett-Packard 423A). A leveling loop circuit from the meter back to the amplifier was established to maintain a constant level of transmitted power.

Pulsed microwave exposures were produced by a 5 kW pulsed microwave generator (Applied Microwave Laboratory PG5), coupled to the same standard gain horn used for CW radiation. Output was monitored by the same calibrated directional coupler and power meter as in the CW case. The average rms (root mean square) power was monitored by the power meter, and pulse width and number of pulses/sec (pps) by an oscilloscope (Tektronic 454). All pulsed transmissions were of square wave form.

The system was calibrated through the use of the standard gain horn to calculate the field intensity in the far field with a dipole antenna as a transfer standard. The field intensity was further checked with both a National Bureau of Standards field intensity meter and a Narda Model 8300 isotropic radiation monitor. Overall accuracy of reported peak and average power density measurements is estimated to be better than $\pm 20\%$.

The rats were exposed individually for 20 min on a styrofoam pedestal. One of two different exposure procedures was used. In the recovery time study, the rats were irradiated without anesthesia. The rats were placed in a pie shaped, well ventilated styrofoam enclosure. The box was placed on the styrofoam platform with the rat facing the center of the emitting horn and aligned with the longitudinal axis of the horn. The rat could move his head and lick his paws but could not turn around or move laterally. The animals were then anesthetized at 8 min, 4 h, or 24 h, after exposure, injected with the radiolabeled test substances and sacrificed.

In all other tests the animals were anesthetized before microwave exposure. The anesthetized rat was placed directly on the styrofoam platform facing the mouth of the horn. After the 20 min microwave exposure, the rats were then injected with the test substance and sacrificed, using the same procedure as above.

Brain uptake measurement

The method used to measure the brain uptake of radiolabeled substances was based on Oldendorf's^{24,25} double indicator technique and is briefly summarized below.

A mixture containing approximately 0.75 μCi of a ^{14}C -labeled test substance and 1.0 μCi of ^3HOH (spec. act. = 0.25 mCi/g) was used with a diluent to make up a total volume of 0.2 ml solution. The diluent was a Ringer's solution buffered to pH 7.55. The test substances used in this study were D- ^{14}C mannitol (spec. act. = 10–150

mCi/mmole) with a molecular weight (MW) of 182.2, [^{14}C]inulin (spec. act. = 2–3 mCi/mg) with MW 5000–5500, and [^{14}C]dextran (spec. act. = 0.5–2 mCi/g) with MW 60,000–75,000. Isotopes were from ICN Isotope and Nuclear Division, Cleveland, Ohio or New England Nuclear, Boston, Mass.

The rats were anesthetized with intraperitoneal pentobarbital (i.p., 60 mg/kg). The right common carotid artery was surgically exposed and cannulated using a 27-gauge needle. The entire 0.2 ml of solution (temperature 22–25 °C) was injected rapidly and the animal was sacrificed by decapitation 15 sec after injection.

The whole brain was quickly dissected free and the tissue sections were placed rapidly into scintillation vials. Entire brain areas or large sections of brain areas were used to maintain consistency between animals and to guard against the possibility of using one area of the cortex in one animal and a different area of the cortex in another animal, where uptake may vary. The tissues were digested overnight at room temperature with 1 ml aliquots of a quaternary ammonium hydroxide tissue solubilizer (Soluene 350, Packard). Ten ml aliquots of liquid scintillation mixture (Dimilume-30, Packard) were added to each vial and the samples were subjected to routine scintillation counting. Several samples of the injected mixture were subjected to the same procedure. Radioactivity was measured with a Beckman LS-355 liquid scintillation counter which was equipped with an external standard and automatic quench control for counting dual isotope samples.

Following the procedure of Oldendorf, the ratio of ^{14}C of the test substance to ^3H of the diffusible standard in the brain tissue is divided by the same ratio in the respective injection mixture. This ratio is presented by Oldendorf as the Brain Uptake Index (BUI) and is defined as:

$$\text{BUI} = \frac{^{14}\text{C}/^3\text{H in brain tissue}}{^{14}\text{C}/^3\text{H in injected mix}} \times 100.$$

This ratio of ^{14}C to ^3H in brain tissue relative to the ratio of ^{14}C to ^3H in the original injected mixture defines the relative amount of test substance lost to the brain in a single passage through the microcirculation. This method corrects for regional differences in blood flow and should determine whether an increase in a labeled test substance is due to a blood–brain barrier alteration or to blood flow or to both. If the test substance had the same uptake as water, the BUI would be 100. If the test substance did not leave the blood vessels at all, the BUI would be zero. In practice, even neutral polar substances, which exhibit negligible penetration of the BBB during a single capillary passage, have a BUI of 1–3.

RESULTS

Microwave induced BBB alterations for different brain regions

The first test series was designed to determine if a measurable permeability change in rats could be caused by microwave exposure. D-Mannitol was chosen as the test substance because of its low molecular weight, 182.2, and because it does not normally cross the BBB system. The rats were randomly selected and individually

exposed for 20 min to one of three irradiation conditions: one sham irradiated, and two different 1.3 GHz pulsed microwave cases.

Fig. 1 shows the results of the first test series in terms of the brain uptake index (BUI) for the 3 exposure conditions and 5 different brain regions. The largest changes in uptake occurred in the medulla, followed by the cerebellum and hypothalamus. The BUI for the controls went from about 1.5 for the hippocampus to 3.2 for the medulla. For the 2.0 mW/sq. cm average power density, 200 mW/sq. cm peak power density case, the BUI varied from about 1.2 times the control value for the cortex to 2.9 times the value of the controls for the medulla. In the 0.3 mW/sq. cm average power density, 600 mW/sq. cm peak power density case, the BUI of the microwave irradiated rats varied from 1.5 times the controls in the cortex to 3.7 times the value of the controls in the medulla.

Effects of microwaves on permeability of different molecular weight substances

The second test series was conducted to determine the effects of microwaves on the permeability of 3 saccharides of different molecular weights: D-mannitol with a molecular weight of 182.2; inulin with a MW of 5000 which is similar in weight to many unbound dyes which are used for tracers; and dextran with a MW of 60,000–75,000, which would be similar in weight to dye–protein complexes. All of these substances have a negligible uptake in brain tissue under normal conditions.

Fig. 2 shows the results of this test series. The controls were sham irradiated for 20 min and the exposed were irradiated with pulsed microwaves for 20 min. The tests resulted in statistically significant permeability increases in the hypothalamus, cerebellum, and medulla for both the mannitol and inulin. With dextran there were negligible changes, with only the cerebellum showing a difference at the 0.05 level of significance.

Duration of microwave induced permeability change

The third test series was performed to determine the duration of the BBB alteration, and whether or not there is recovery. In this test series only, the alternative test procedure was used where the animals were exposed without anesthesia in a specially designed enclosure. After exposure, the animals were put back into their cages. The animals were then anesthetized, injected with the mannitol test mixture, and sacrificed at 3 different times after exposure. Separate controls (sham irradiated) were used with the microwave irradiated animals at each post-irradiation interval.

The results of the third test series are shown in Fig. 3. The rats were sacrificed 8 min, 4 h, or 24 h after either 20 min of sham irradiation or 20 min of pulsed microwave irradiation. The 8 min and 4 h groups were very much the same and showed statistically significant increases in BBB permeability over controls. By 24 h, the uptake of mannitol was almost back to normal with only the medulla showing a statistically significant difference.

Brain uptake as a function of microwave parameters

The next test series was conducted to determine if CW microwave energy would

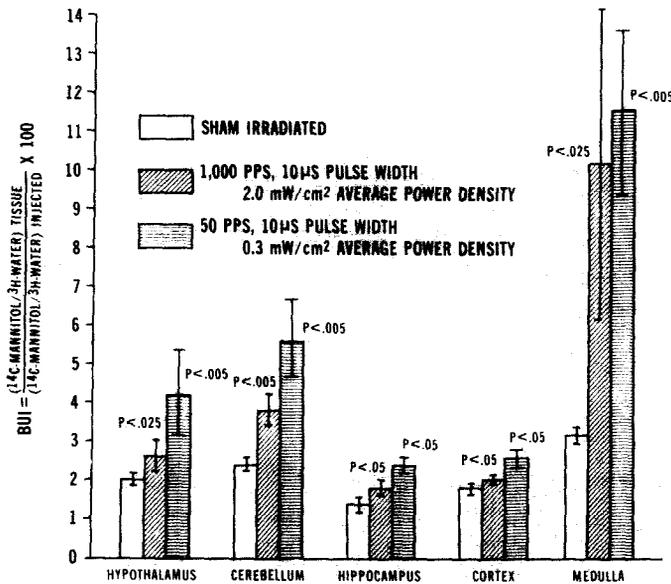


Fig. 1. Brain uptake of [^{14}C]mannitol for 3 irradiation conditions and 5 different brain regions. Each bar represents the mean \pm S.D. of the mean for 5 animals. The P values represent the statistical significance of the difference between means of the microwave irradiated animals and the controls using the Student's t -test.

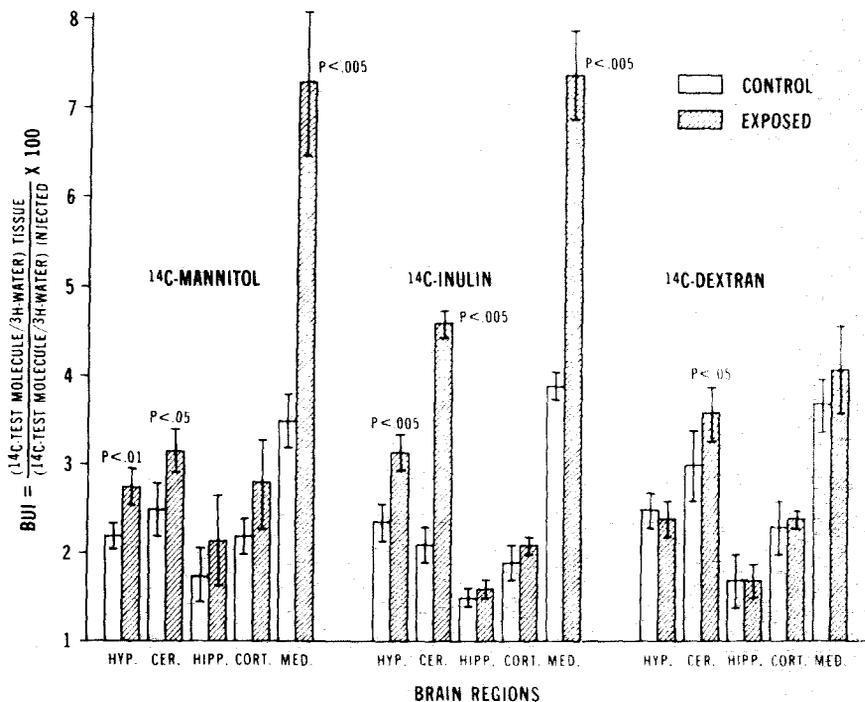


Fig. 2. Brain uptake of mannitol, inulin and dextran for 5 different brain regions in controls and exposed animals. Controls were sham irradiated and exposed, were irradiated for 20 min with 1.3 GHz, 1000 pps, 0.5 μsec , pulsed microwaves of 0.3 mW/sq. cm average and 600 mW/sq. cm peak power density. Each bar is the mean \pm S.D. of 5 rats. The P values represent the statistical significance that the means of the exposed are different from the controls.

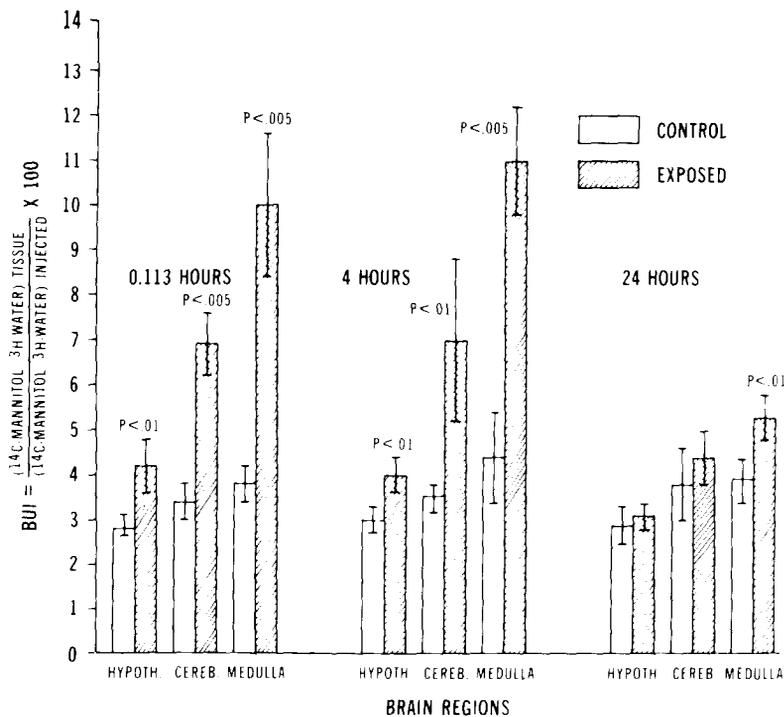


Fig. 3. Brain uptake of mannitol 8 min, 4 h and 24 h after 20 min of sham irradiation (control) or 20 min of exposure to 1.3 GHz, 1000 pps, 0.5 μ sec, pulsed microwaves of 0.3 mW/sq. cm average and 600 mW/sq. cm peak power density for 3 different brain regions. Each bar represents the mean \pm S.D. of 5 animals and the *P* values are the statistical significance that the means of the exposed are different from the controls.

affect the uptake of mannitol and, if so, to compare the magnitude of change to that produced by pulsed microwave irradiation. Also, the functional relationship between BBB permeability and different microwave modulation conditions was explored. One hundred and five male Wistar rats served as subjects. They were exposed to one of 19 different irradiation conditions for 20 min.

Fig. 4 gives the results for the medulla. The uptake of mannitol was a very definite function of exposure parameters. For the CW microwave case, the uptake of mannitol increased with increasing power density up to about 1.0 mW/sq. cm and then started to decrease. The pulsed microwave cases produced similar changes but at different average power densities. There was a definite difference (statistically significant at the overlap points of 0.2, 0.4 and 0.6 mW/sq. cm) between the permeability change produced by the CW and pulsed microwaves of the same average power density. There was also a definite difference in mannitol uptake produced by pulsed microwaves of the same average power density but different pulsed characteristics. The graphs also show that there was a statistically significant increase in the uptake of mannitol in the medulla at an average power density of only 0.03 mW/sq. cm. The results for the cerebellum and hypothalamus were similar, but there was less of a

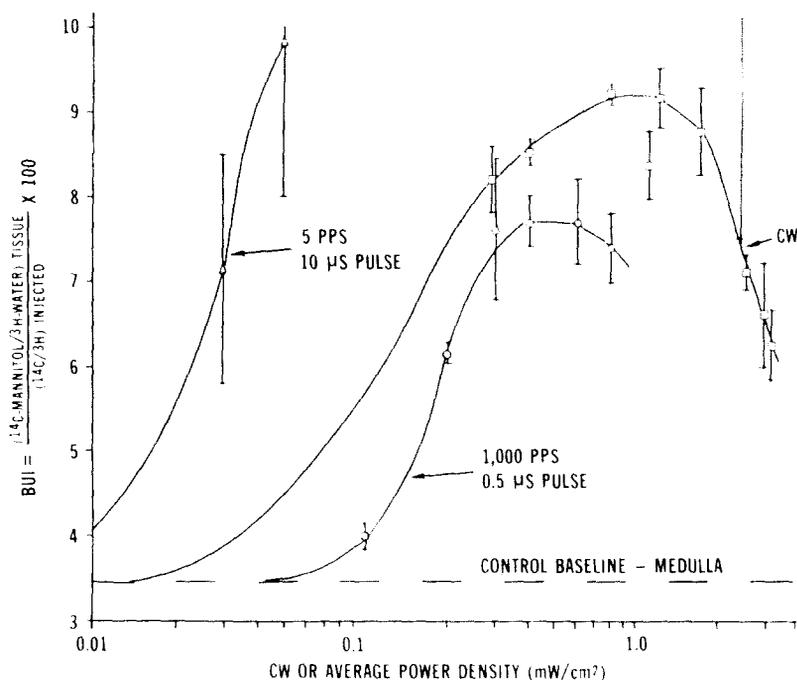


Fig. 4. Uptake of mannitol in the medulla as a function of average power density for 3 types of microwave irradiation. Each point represents the mean \pm S.D. of 3-13 rats.

TABLE I

Uptake of $[^{14}\text{C}]$ mannitol for 12 different 1.3 GHz, 20 min, pulsed microwave exposure conditions and 3 different brain regions

Each BUI value represents the mean \pm S.D. of 3-13 animals. The value for controls is approximately 2.2 for the hypothalamus, 2.5 for the cerebellum, and 3.4 for the medulla.

Microwave parameters					Brain uptake index			
No.	x_1 (pulses/sec)	x_2 pulse width (μsec)	x_3 peak pd. (mW/sq. cm)	$x_1x_2x_3$ ave pd. (mW/sq. cm)	x_2x_3 energy/pulse ($\mu\text{J/sq.cm}$)	Hypothalamus	Cerebellum	Medulla
1	1000	0.5	200	0.1	0.1	2.3 \pm 0.1	2.5 \pm 0.4	4.0 \pm 0.1
2	1000	0.5	400	0.2	0.2	2.4 \pm 0.2	3.1 \pm 0.1	6.2 \pm 0.1
3	1000	0.5	600	0.3	0.3	2.9 \pm 0.4	3.6 \pm 0.6	7.6 \pm 0.9
4	1000	0.5	800	0.4	0.4	2.8 \pm 0.1	4.0 \pm 0.5	7.7 \pm 0.5
5	1000	0.5	1200	0.6	0.6	3.2 \pm 0.1	4.5 \pm 0.3	7.7 \pm 0.9
6	1000	0.5	1600	0.8	0.8	3.3 \pm 0.2	4.9 \pm 1.3	7.4 \pm 0.5
7	5	10.0	600	0.03	6.0	3.0 \pm 0.1	3.8 \pm 0.2	7.2 \pm 1.7
8	5	10.0	1000	0.05	10.0	3.5 \pm 0.6	4.0 \pm 0.7	9.8 \pm 4.2
9	50	10.0	600	0.3	6.0	3.9 \pm 1.3	5.8 \pm 2.1	10.0 \pm 5.7
10	250	20.0	60	0.3	1.2	3.3 \pm 0.4	4.2 \pm 0.6	7.0 \pm 0.3
11	250	2.0	600	0.3	1.2	4.5 \pm 1.2	4.1 \pm 1.8	7.5 \pm 2.1
12	1000	10.0	200	2.0	2.0	2.6 \pm 0.4	3.8 \pm 0.5	9.1 \pm 4.2

magnitude of change. The cortex and hippocampus results were erratic and changes in permeability were small.

Table I is a summary of the pulsed microwave data from several of the test series. Three primary microwave parameters (pulses/sec, pulse width, and peak power density) are denoted as x_1 , x_2 and x_3 . Other microwave parameters (except frequency and exposure time, which were fixed) are secondary parameters and are derivable from x_1 , x_2 and x_3 . For example, the table lists average power density, which is x_1 times x_2 times x_3 , and energy per pulse which is x_2 times x_3 . There are not enough data to rigidly determine the functional dependence of mannitol uptake on the respective microwave parameters, but certain trends are suggested.

The data in the table suggest that the BUI is a function of peak power density, pulse width and the number of pulses per second. As each of these primary parameters is increased, with the other two held constant, the BUI goes up. However, the effect of peak power on the uptake of mannitol seems greater than the effect of pulse width and both seem to have a greater influence on permeability than the number of pulses per second. This implies that for a given average power density one can get a higher permeability change by raising the peak power and lowering the number of pulses per second than by raising the pulses per second and lowering the peak power. Such phenomena would give rise to a family of curves of BUI versus average power which vary depending on the x_1 , x_2 and x_3 characteristics. Fig. 4 gives preliminary indications of such a family of curves.

DISCUSSION

The results of the present study indicate that low power, pulsed and CW microwave exposures affect brain tissue permeability, thus confirming Frey et al.'s findings¹³. It does not appear that pulsed energy is always more effective in causing BBB alterations than CW energy, but that depending on certain pulsing characteristics, pulsed energy can be more or less effective than CW energy of the same average power density. From Fig. 4 it can be observed that pulsed energy with a high peak power, large pulse widths and few pulses per second affected the uptake of mannitol at an average power density of only 0.03 mW/sq. cm, where it took CW energy of approximately 0.3 mW/sq. cm to cause the same magnitude of change.

In general, the uptake of mannitol increased with increasing power density for both pulsed and CW microwave signals up to 0.5–2.0 mW/sq. cm, where the BUI started to level off and then, decrease. A similar amplitude 'tuning curve' or inverted U shaped function has been observed by Bawin and Adey³ for calcium efflux with weak low frequency electric fields of similar amplitude. Equipment used in the present study did not allow for power density exposures above 3.1 mW/sq. cm, so it is not known whether the permeability change continues to decrease or rise again as the power is increased further. Such a complex function may be the reason that other researchers have not observed BBB alterations, as the tendency of most researchers is to start at high power levels looking for an 'effect' and then to work down in power.

Exposed and sham irradiated rats exhibited similar regional differences in the

uptake of the 3 neutral polar substances in the present experiments. The magnitude of the uptake of the radiolabeled substances was lowest in the cortex and hippocampus, with higher uptakes in the hypothalamus, followed by the cerebellum and the highest in the medulla. These regional differences agree with recent literature^{31,32}, but are difficult to interpret. The cerebellum and medulla always exhibited more uptake than the hypothalamus, which is considered to be an area of diminished BBB. In turn, the uptake of the hypothalamus was similar to that of the hippocampus and cortex, areas of supposedly, uniformly well developed BBB. The short interval (15 sec) between isotope injection and sacrifice was felt to minimize the distribution and spread throughout the brain of any tracer that had entered the brain tissue. Perhaps the BBB is more developed in the hypothalamus than previously thought or perhaps the site of leakage is concentrated in the area postrema. In experiments with cold stress³¹, brain concussions³⁰ and ionizing radiation¹⁰, it has been observed that the brain stem and upper cervical cord are the regions of the CNS which are most effected.

There is a remote possibility that the increase in BUI produced by microwave exposure occurs because of the reduced uptake of tritiated water, rather than because of the increased uptake of mannitol and inulin. The lack of change in the dextran uptake due to microwave exposure mitigates this possibility and reinforces our conclusion of changes in the permeability of the BBB to mannitol and inulin. In the present study, dextran, which is similar in molecular weight to protein, did not penetrate the barrier, but inulin, which is similar in molecular weight to many dyes used as tracers did. This suggests that in earlier microwave studies, which used dyes as tracers, a penetration of unbound dye was being observed rather than a penetration of dye-protein complexes.

Microwaves can be characterized by 4 fundamental or first order parameters: frequency, pulse width, pulses/sec and peak power density (amplitude). Other microwave parameters such as average power density, energy per pulse, total energy, duty cycle, etc. are second order parameters and are derivable from the 4 fundamental ones. Dealing with secondary parameters, or a mixture of secondary and primary parameters, may not uncover functional dependencies. Several investigators^{13,18} in an effort to determine the cause or at least the functional dependence of microwave induced biological effects have conducted numerous experiments holding some secondary parameters constant and varying others. It seems that a more rigorous approach, and the one attempted in this study, would be to isolate the primary microwave parameters and investigate their effect on the biological phenomena. With this type of analysis, the functional dependence of brain permeability on the microwave parameters can be examined.

In 1961, the same year Frey¹² discovered RF sound (microwave induced auditory sensations), White³³ independently demonstrated that pulsed electromagnetic energy could induce pressure pulses in material. As further developed by White³⁴ and Gournay¹⁷, it was shown that these elastic stress waves were a function, for a single pulse, of peak power density for long pulse widths and peak power density times pulse width for short pulse widths¹¹. Since in the present study CW microwaves were very effective in creating BBB alterations, it would seem unlikely, even though pressure

pulses and brain concussions are known to cause BBB alteration, that such a phenomenon is the major cause of the permeability change. It does not exclude, however, the possibility of two different mechanisms, one for pulsed microwaves and one for CW microwaves.

Another possibility may be local heating due to 'hot spots' or focusing of energy, since the greatest BBB alteration occurs in the cerebellum and medulla or close to the neck region of rats. A theoretical prediction of such neck heating has been made by Gandhi¹⁵ and Ghandi et al.¹⁶. Trace metal content and neurotransmitter changes^{8,22} have also been observed due to whole body heating and to 10 min, 1.6 GHz microwave exposures at an average power density of 80 mW/sq. cm. This work demonstrated large amplitude power deposition in the floor of the brain of rats. It was hypothesized that microwave induced hyperthermal environments were the cause. Because of the lower powers that were involved in the present study, 0.03 mW/sq. cm average power density for pulsed microwaves and 0.3 mW/sq. cm for CW microwaves, the possibility of the BBB alteration being caused by direct heating seems remote. The present data do not address the question of whether the microwave exposure interacts directly to alter the BBB system or whether the microwave exposure causes an indirect effect. The data also do not address the currently debated question of whether BBB alterations are due to lesions or increases in micropinocytotic vesicle transfer^{27-29,31}. Future work is planned to address these questions.

Comparison of the uptake of mannitol for the case of sacrifice 8 min after irradiation (Figs. 2 and 3) demonstrates that neither the head movement nor the anesthesia seems to affect the uptake level of the sham or microwave irradiated rats. With anesthesia, the animals were always facing the emitting horn and were immobile. Without anesthesia, the animals were free to move their head and paws within their enclosure. Both exposure procedures resulted in similar permeability changes.

Recent findings with several independent confirmations have demonstrated that low power microwaves can interact with animals to cause CNS changes such as auditory sensations¹⁴, calcium efflux changes^{3,5}, regional histopathology¹ and altered EEG patterns⁴. This paper demonstrating blood-brain barrier alterations is another major example of CNS changes due to microwaves at average power densities below 10 mW/sq. cm. It should be emphasized, however, that no one has determined whether or not these CNS changes are hazardous. Possibly, they may even be beneficial. For example, selective BBB changes may enhance the permeability of therapeutic pharmacological agents.

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