Effects of 2450-MHz Microwave Energy on the Blood–Brain Barrier: An Overview and Critique of Past and Present Research

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Abstract—**The dynamics and complexity of the blood–brain barrier (BBB) make it a very difficult system for study. The variety of techniques employed to assess microwave effects on the barrier all appear subject to limitations in either sensitivity or applicability. Inconsistencies in tracer characteristics, experimental design, and in the microwave parameters employed, i.e., microwave system used (pulsed or continuous wave), field orientation relative to the exposed animal, frequency, power density, and duration of exposure, anesthesia or physical restraint, all have lead to difficulties in interpreting results and replication of experiments. Therefore, the technical approach, as well as data obtained, must be carefully scrutinized to avoid misinterpretation of results that may lead to erroneous conclusions.**

Recent studies by Williams *et al.* [1]–[4] have endeavored to consolidate various technical approaches within a single study in order to more clearly interpret potential microwave-blood–brain barrier interactions and to minimize extraneous factors which may confound these effects.

Our findings, as well as those of other investigators [5], [6], fail to confirm previously published reports of increased blood–brain barrier permeability in rats following exposure to microwaves. Our findings, however, support the conclusion that suppression of BBB permeability occurs, and that this effect is mediated by temperature-dependent changes in endothelial cell function, and not by qualities unique to microwave energy.

I. Introduction

EXISTENCE OF a blood–brain barrier (BBB) was first demonstrated through the experiments of Paul Ehrlich in 1885 [7]. For years, the nature and location of the barrier was the subject of considerable controversy, and not until 1967 was the true significance of the capillary endothelium brought to light through the use of intravenously injected horseradish peroxidase (HRP). Restriction of this glycoprotein (MW 40000) from the brain was attributed to two unique characteristics of cerebral microvessels: 1) the presence of tight junctions between apposed cells; and 2) the paucity of vesicular transport [8].

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Subsequent studies have confirmed the central role of the capillary endothelium in maintaining a blood–brain barrier [9]–[12].

A. Physiological Aspects of the BBB

1) Transport Mechanisms: Substances penetrating the endothelial membrane may be transported by one of several mechanisms, including simple and facilitated diffusion, active transport, and pinocytotic or vesicular transport. The mechanism by which a molecular species permeates the blood–brain interface is the resultant of physicochemical attributes of both the molecule and the endothelial membrane.

2) Simple Diffusion: A molecule crossing the membrane by simple diffusion transgresses at a rate commensurate with its lipid partition coefficient and molecular size [13]. Nonelectrolytic diffusion is driven by concentration differences across the cell membrane, i.e., across the luminal–abluminal interface of the endothelium, while ionic species diffuse as a result of electrochemical gradients. Net transport stops once concentration or electrochemical equilibrium is attained.

3) Facilitated Diffusion: Some molecules are transported by a facilitated diffusion system. Diffusion of this type is downhill, saturable, and stereospecific. In addition, facilitated diffusion can be inhibited and may be paired with transport of a substance in the opposite direction [14].

4) Active Transport: Metabolic substrates and metabolites may be transported into and out of the brain parenchyma, respectively, by mechanisms which are energy dependent, being inhibited by ouabain, with flux in an uphill direction, against concentration and electrochemical gradients. The large energy requirement for such transport and maintenance of concentration and electrochemical gradients is apparently supplied by the large mitochondrial population within the endothelial cell [15], [16].

5) Pinocytosis and Vesicular Transport: Vesicular transport, although usually reduced in endothelial cells, may be responsible for transport of large molecules, such as proteins, down their concentration gradients. The process is relatively independent of molecular size [14]. The rate of transport may be influenced by a variety of noxious stimuli which may increase [14], [17], [18] or decrease [19], [20] vesicular transport across cerebral capillaries.

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B. Microwaves and the BBB

Evidence has been presented over the last several years suggesting that animals exposed to electromagnetic energy within the microwave domain incur altered blood–brain barrier permeability to a variety of nonpenetrating or poorly penetrating substances. Frey *et al.* [21] reported altered brain vascular permeability to intravenously injected fluorescein dye in rats exposed to 1.2-GHz microwaves. Increased levels of mannitol (MW 182) and inulin (MW 5000) have been reported to occur in the brain tissue of rats exposed to 1.3-GHz microwave energy either pulsed or continuous wave (CW) at power densities below 3 mW/cm^2 [22].

Flooding of endothelial cells with HRP, neuronal vacuolation, perivascular edema, and platelet aggregation were considered as evidence of direct effects on cellular integrity [23], [24]. Alteration of microvessel permeability to Evan's blue dye has been reported to occur in anesthetized rats exposed to 2450-MHz microwaves at an incident power density of 3000 mW/cm^2 (SAR = 240 mW/g) [25], [26]. In these experiments, a direct-contact applicator positioned over the head produced brain temperatures that exceeded 43°C. In similar experiments where incident power densities ranged from 0.5–1000 mW/cm^2 (SAR range = 0.04–80 mW/g), brain temperature nearing 41°C was recorded with no apparent alteration in membrane permeability to the tracer [27]. Correlation of increased permeability with colonic temperature has been demonstrated in adult anesthetized rats subjected to either 2450-MHz microwaves or high ambient heat (40°C) [28]. Localized microwave heating of the head, resulting in cerebral temperatures in excess of 43°C, has been found to increase BBB permeability to HRP [29], [30]. Electron microscopic evidence for increased uptake of HRP in cerebral capillaries of rats and Chinese hamsters exposed to microwaves at power densities of 10 or 25 mW/cm^2 at 2450- or 2800-MHz CW has also been reported [23], [24], [31], [32]. Interpretation of reported low-level microwave effects on the blood–brain barrier has been made difficult by the inability to replicate results of earlier studies. Attempts at replication of findings by Frey *et al.* [21] and Oscar and Hawkins [22] have not generally been successful [33]–[35]. However, statistical reanalysis of data from Merritt *et al.* [33] indicates that increased levels of fluorescein within the brain may have resulted following microwave induced hyperthermia [36]. On the other hand, studies utilizing an intravenously injected nonmetabolizable tracer (^{14}C-sucrose) to study cerebrovascular permeability [37] have revealed no significant changes in BBB function following exposure to 2450-MHz [6] and 2800-MHz [5] microwaves at incident power densities ranging from 1 to 40 mW/cm^2.

II. Analysis and Critique

The effects of microwave energy on function of the BBB are still a matter of controversy in the literature. Early reports by Albert [23], [38] and Oscar and Hawkins [22] suggesting that low-power ($\leq$10 mW/cm^2) microwave energy can significantly alter BBB permeability have not been corroborated by others [6], [33]–[35]. More recently, Spackman *et al.* [39] have reported finding increased concentrations of naturally occurring amino acids in mouse brain following exposure to 918-MHz microwaves (average power density, 10 mW/cm^2), and Albert and Kerns [24] have reconfirmed earlier observations that microwave exposure to 10 mW/cm^2 disrupts the barrier to HRP in Chinese hamsters. Variation in microwave exposure parameters and experimental techniques make conclusions concerning "low-level" microwave effects difficult. More definitive are the effects of extreme microwave-induced hyperthermia on the BBB [25], [29], [33], [40].

The inability of previously reported studies to convincingly demonstrate a true microwave-induced effect is largely the result of the nearly universal reliance upon a single technical approach, either morphological or physiological, to investigate these effects. A serious deficiency in studies utilizing intravenously injected tracers, such as NaFl and radiolabelled compounds, is the lack of adequate histological verification of results. Without such verification, conclusions must remain tentative at best. This criticism is equally applicable to studies which fail to support histological findings utilizing light and electron microscopy with physiological data.

Evaluation of studies on BBB permeability is further complicated by the prevalent use of anesthetics, commonly pentobarbital, or the use of unanesthetized, but restrained animal preparations [41], [42].

Sodium pentobarbital anesthesia has been used extensively in studies on microwave-BBB interactions [22], [23], [34], [38]. Anesthetic agents may have a marked and varied effect on cerebral blood flow and metabolism [43] and may affect the BBB to commonly used tracers [44]–[46]. Results obtained from anesthetized animals may therefore differ from those of conscious animals [47]. Furthermore, effects on cerebral function may not be uniform. For instance, pentobarbital appears to selectively reduce blood flow within the cerebral cortex, but not the hypothalamus [48].

Such interaction could either inhibit or augment BBB transfer of tracer molecules, perhaps by acting synergistically with microwave energy.

The effect of stress on physiological function is often overlooked or underestimated in studies of physiological systems, such as the BBB. Pentobarbital has been shown to suppress the response to stress by the hypothalamo–pituitary–thyroid axis [49]. It has also been demonstrated that conscious rats are less prone to hypertensive effects on the BBB than are rats anesthetized with nitrous oxide [50]. Interestingly, anesthetics may also protect BBB integrity [51]. Lack of handling, novelty of experimental environment, i.e., immediate surroundings, ambient temperature, humidity, noise, etc., and restraint all contribute to a stressed animal preparation. The use of unrestrained rats is a particularly important consideration when exposure conditions involve temperatures exceeding thermoneutrality or when the animal is required to maintain a thermoregulatory balance. The use of restraint can seriously alter the physiological response of the rat to high temperature [42] and reduce survival time [52].

A. Methodological Considerations

To date, the most frequently employed methods for studying microwave effects on the BBB have been: 1) the Brain Uptake Index (BUI) method [53]; 2) the arterial-integral method [54], [55]; 3) quantitation of intracerebral NaFl by spectrophotofluorometry [33]; and 4) intravenous injection techniques using HRP as an electron dense tracer for electron microscopy [24], [38], and NaFl and Evans blue fluorescent tracers for gross visualization within the brain [25], [26], [33]. Each of these techniques presents certain advantages and limitations in their use.

Using the BUI procedure, Oscar and Hawkins [22] concluded that BBB permeability to mannitol and inulin could be increased in rats exposed to either pulsed or CW microwaves at 1.3 GHz (average power density < 3.0 mW/cm^2). A subsequent attempt to replicate these results using the identical procedure and similar exposure parameters was unsuccessful [33]. In another attempt using 2450-MHz (CW) microwave energy, Preston *et al.* [34] were unable to detect significant changes in cerebral permeability to mannitol. The BUI technique used by Oscar and Hawkins assumes that the rate of ^{3}HOH transport out of the vascular compartment remains flow-limited, and not diffusion-limited, during the course of the experiment. This condition is met if cerebral blood flow does not exceed 20 ml/100 g·min^{-1} [56]. Normal blood flow for the rat is 100 ml/100 g·min^{-1} which reduces extraction of water by 25 percent [57]. This reduction effectively decreases the denominator, artifactually increasing the numerator and therefore the BUI expression. Increases in cerebral blood flow during the course of microwave exposure would potentiate still further the reduction in water (reference tracer) extraction by brain tissue. Increased cerebral blood flow has recently been demonstrated through the use of the *in vivo* [^{14}C] iodoantipyrine technique [58]. This finding supports the observation by Preston *et al.* [34] that changes in the rate of ^{3}HOH extraction, and influence of the vertebral arterial circulation could account for apparent increases in the BUI for cerebellum and medulla [59].

Although improvements in the accuracy of the BUI technique have since been introduced [60], the arterial-integral method utilizing [^{14}C] sucrose merits consideration for the following reasons: 1) the use of a plasma–time integral eliminates the biasing effect of transient changes in blood flow; 2) the method has been estimated to be significantly more accurate than the BUI technique [61]; and 3) the technique can be easily adapted for use with unanesthetized, unrestrained animal preparations.

Of significance to the interpretation of data from tracer studies is the circulatory concentration of the tracer over the course of the experiment. In a study of [^{14}C] sucrose permeability, we [3] observed elevated plasma integrals and tracer activity in whole blood of all rats whose colonic temperature was elevated to > 41.5°C by either microwave energy or ambient heat. At these temperatures, definite changes in renal function can be expected to occur [62] including reduced cardiac output (blood flow) to the organ [63] and a reduction in glomerular filtration rate (GFR) beginning at ~ 41.3°C [64].

Sucrose metabolism is negligible in the rat [65], [66] and accumulates primarily in kidney, liver, and muscle tissue, in addition to being excreted in the urine [67]. Although changes in sucrose distribution space cannot be discounted, the principal cause of increased circulatory levels of sucrose, and of NaFl as well [1], appears to be the reduction of renal clearance [4].

Sodium fluorescein (MW 376) injected intravenously as free sodium fluorescein can be detected optically or spectrofluorometrically. Presence of dye in cerebral tissue, other than areas devoid of the BBB, has been considered as evidence of abnormal microvascular leakage resulting from exposure to microwaves at power densities ranging from 0.2 to 2.4 mW/cm^2 [21] or to severe hyperthermia induced by ambient heat [33].

Several technically derived artifacts could explain these apparent findings. The highly diffusible nature of NaFl has been documented [68]. If the dye is injected into the carotid artery, allowed to circulate, and the brain subsequently removed, traces of fluorescein can be found localized to regions devoid of the BBB and to the cerebral ventricles [Williams, unpublished observation]. Leakage from these areas, especially through the choroid plexus and ventricles, can be increased following exposure to ambient heat or microwaves which significantly increase cerebral blood flow [Williams, unpublished observation]. Diffusion of the tracer into brain tissue, especially the hippocampus and cerebral cortex adjacent to the choroid plexus and third ventricle, respectively, occurs in both sham-exposed or microwave-exposed brain slices, although to a much greater extent in brain made hyperthermic [Williams, unpublished observation]. Diffuse leakage of NaFl may also be visible on regions of the cortical surface in rats exposed to hyperthermic levels of microwave energy [Williams, unpublished observation]. It would appear from these observations that conditions which increase cerebral blood flow through areas devoid of a BBB, such as portions of the pial membrane, choroid plexus, and cerebral ventricles, might significantly increase diffusion of NaFl into adjacent tissue which does possess a BBB. In addition to augmenting diffusion of the tracer through increased blood flow in areas devoid of the barrier, diffusion might also continue to occur if brain slices are improperly fixed or not instantly frozen subsequent to fluorescein injection and brain removal [59].

Studies purporting to demonstrate increased NaFl permeability in animals made hyperthermic through whole-body exposure to microwaves or ambient heat [33] appear to have overlooked the effects of hyperthermia on cerebral blood volume and upon renal excretion of the tracer. A reduction in renal blood flow might explain data which reflect increased levels of the tracer within the brain. Comparison of NaFl plasma concentration of normothermic (colonic temperature 37 to 38°C) versus hyperthermic (colonic temperature ≥ 41.5°C) rats reveals that levels in plasma remain significantly higher for rats

made hyperthermic even though rats in both groups are injected with equivalent doses [1]. Plasma concentration-time curves for hyperthermic rats injected with NaFl are essentially identical to those obtained from hyperthermic rats injected with [^{14}C] sucrose. In our study [1] and in those of Merritt *et al.* [33], circulation of fluorescein is followed by intracardial perfusion with heparinized 0.9-percent NaCl. Of potential significance is the degree to which the tracer can be flushed from the vascular compartment. We have observed that even in sham-exposed rats, detectable levels of NaFl remain within the brain even after perfusion with a volume that should have been sufficient to clear completely the cerebrovascular compartment ($\simeq 1$ ml/cm^3 brain tissue). Since the brain normally maintains a BBB to fluorescein [68]–[70], the residual presence of the tracer would be expected to result from 1) pinocytotic uptake during the circulation period and 2) incomplete clearance of fluorescein from the lumen and walls of cerebral vessels. In fact, cerebral vessels, including capillary endothelium, exhibit distinct fluorescence following the injection of NaFl or NaFl-labelled dextrans. Fluorescence increases if the renal arteries and veins of both kidneys are ligated, although no fluorescence is seen in brain parenchyma [70].

Several sources of artifact are therefore apparent. The increased vascular volume resulting from moderate hyperthermia [71] would inherently increase the residual amount of fluorescein within the cerebrovasculature. In addition, levels could be further increased by the elevated circulating concentrations of fluorescein resulting from a reduced renal excretion rate.

B. Brain Temperature and Influencing Factors

Principal determinants of brain temperature include metabolic rate of brain tissue, temperature of arterial blood, and the rate of cerebral blood flow. In rats, the most significant parameter in determining heat dissipation within the brain is temperature of the arterial blood [72]. In recent studies by Williams *et al.* [1]–[4], whole-body heating resulted in significant elevation of both brain and colonic temperatures.

Evident in these studies employing NaFl, HRP, and [^{14}C] sucrose as tracers is the apparent correlation between altered levels of tracer within the brain and elevation of brain temperature.

Regional brain levels of all three tracers deviated significantly from control values only when rats were made moderately or severely hyperthermic by exposure to microwaves at 65 mW/cm^2 for 30 or 90 min or to ambient heat (42 ± 2°C) for 90 min. Exposure to these conditions resulted in elevation of colonic and regional brain temperatures to approximately 41 and 40°C, respectively, or higher. A critical temperature for significantly altering tracer levels within the brain appears to lie near 41.5°C. Colonic temperatures near 41.5°C or higher are capable of seriously reducing renal excretion rate [64]. This fact very likely accounts for the increased brain NaFl levels observed in rats following 30- or 90-min exposures to microwave energy or ambient heat and most likely explains the findings reported by Merrit *et al.* [33]. This temperature also appears critical for significant hyperthermia-induced reduction in pinocytotic uptake of HRP and [^{14}C] sucrose [2], [3].

Significant effects on body physiology and cellular function may begin at temperatures exceeding 40°C [73]. At colonic temperatures ranging from 40 to 42°C, cerebral metabolic rate (CMR_{O_2}) and cerebral blood flow may initially increase proportionately, suggesting hyperthermia-induced increases in the rate of energy consumption [74]–[77]. As core body temperature exceeds 42°C, cerebral energy consumption may surpass energy production as a result of an increased metabolic rate and hypoxia [78].

C. Temperature Effects on Endocytic Processes

Transport processes, such as pinocytosis and phagocytosis, may be increased [79] or decreased [80], [81] by exposure of isolated cells to moderate or severe hyperthermia. Rate-controlling events in vesicular transport appear to involve the attachment/detachment phase of the transport process [82]. Moreover, the demonstrated temperature dependence of vesicular transport in pinocytosis [83], [84] and phagocytosis [85] is apparently affected at the plasma membrane [82]. Hyperthermic conditions may alter cell membrane permeability [40], and membrane fluidity [86], [87]. These effects are the likely result of changes in membrane lipoprotein complexes [88], surface charge, and stereo-organization of macromolecules attached to the membrane surface [87]. In fact, reduced phagocytosis of polymorphonuclear leukocytes (PMN) has been shown to correspond to the hyperthermia-induced reduction of net surface charge and membrane potential [89].

Vesicular transport is but one of several means by which substances can cross the BBB, the mode of transport being a function of multiple factors, including molecular weight, lipid solubility, charge, and stereospecificity [14]. Active transport involving specific carrier systems, facilitated diffusion, and passive diffusion, involving primarily water and electrolytes, are additional mechanisms by which molecular species may traverse the barrier [14], [90]. Since each mechanism is highly dependent upon the plasma membrane, it is not surprising that alterations of its biophysical state would be manifested in a generalized alteration of microvessel permeability. In most instances, injury to the cell membrane or exposure to noxious stimuli result in potentiation of a specific transport mechanism [17], [91]–[95], or nonspecific increase in permeability [96]–[102]. Only a few studies have been reported in the literature which deal specifically with hyperthermic effects on *in vivo* microvascular permeability. Hyperthermia has been induced in anesthetized rats by microwave exposure or ambient heat, and, in general, resulted in increased permeability to Evans blue dye [69], sucrose, and inulin [28]. However, Sutton and Nunnally [30] found that BBB integrity could be extended, even with brain temperature as high as 45°C, for limited periods, if microwave heating was conducted concurrently with body core hypothermia.

Other forms of thermal stress, such as pyrogen-induced fever [103], cold [104], and heat injury [104]–[106] may also increase BBB permeability. Barrier changes as a result of thermal injury (lesions produced by direct tissue contact with cold or heat) are produced by release of prostaglandins, as well as mechanical trauma (opening of tight junctions) of the tissue [105]. Pyrogen-induced fever is similar in some respects (i.e., prostaglandin release; effect on hypothalamic fever response center) to the inflammatory response just described [107]. This may indicate some common aspects between mechanisms underlying observed changes in permeability. However, comparison of BBB effects between hyperthermia produced by ambient heat or microwaves and the forms of thermal stress just discussed is difficult because of fundamental differences in etiology. Hyperthermia, as a result of heating, is an essentially uncontrolled event where thermoregulatory mechanisms attempt to maintain thermoneutrality. Fever is a controlled response where thermoregulation is functional [108]. Thermal injury, on the other hand, produces localized physical trauma which is accompanied by widespread edema [105].

Only a few studies have dealt with the effects of hyperthermia on pinocytosis. However, *in vitro* preparations of rat visceral yolk sac, incubated at temperatures ranging from 2 to 42°C, show a marked reduction of pinocytotic activity at temperatures above and below 37°C [19].

Even fewer studies have considered microwave effects on endocytic processes. In this regard, increased pinocytosis resulting from 1.4-cm microwave exposure of cultured cells has been reported [109]. Assessment of these observations with respect to the present study is difficult because neither SAR, incident power density, nor temperature of the cell culture are stated in the report.

Increased phagocytosis has been observed in mice following exposure to 10-cm microwaves [110]. This initial rise was followed by a reduction in activity. Suppression of phagocytic activity in perfused macrophage monolayers has also been reported following exposure to 2450-MHz microwaves at 50 mW/cm^2 [111]. Temperature of the culture medium was reportedly maintained below 37°C, even after 30 min of exposure. In similar experiments in which macrophages were incubated at varying temperatures, phagocytic activity first increased up to 38.5°C and then rapidly declined. In microwave-exposed cultures, suppression of phagocytosis occurred at 34 to 36°C, leading Mayers and Habeshaw [111] to conclude that the microwave-induced suppression was a nonthermal, temperature-independent response. However, several aspects of the study make it difficult to separate thermal from nonthermal effects. Most notable is the presence of an estimated 2.5°C temperature rise in microwave-exposed cultures and the uncertainty in determining the exact thermal conditions within the culture during the exposure. Two factors which may have contributed to this uncertainty include 1) the temperature of the perfusing solution surrounding the macrophages and 2) delaying temperature measurement until the end of exposure. Grant *et al.* [112] have shown that heating of macromolecules in a solution of high water content may occur as a result of energy absorption by bound water surrounding the molecules. Such heating very likely involves short thermal time constants [113]. For these reasons, temperatures considerably higher than those recorded from the culture medium could have been present at the macrophage plasma membrane.

D. Thermal Effects on Biological Membranes and Membrane-Mediated Reactions

It has been proposed that hyperthermically induced transitions of membrane lipids and the subsequent increase in membrane fluidity could result in altered membrane properties manifested by changes in surface charge, passive transmembrane permeability, and reorganization of macromolecules attached to the membrane surface [99]. Studies with biological membranes and intact cells have demonstrated the influence of lipid phase-transitions on the activity of membrane-bound enzymes and transport properties [114].

The presence of an optimum temperature above or below which enzymatic activity and permeability decrease is a commonly observed event in biological systems. Bělehrádek [115] terms this optimum condition the "upper biokinetic limit." Many reactions, both biological and chemical, exhibit an optimum temperature above which the velocity of the reaction decreases. For many cellular processes, function is suppressed at temperatures exceeding 40°C [73].

The mechanism by which microwave energy and ambient heat suppress vesicular activity in microvessel endothelium [2] cannot be conclusively determined from information presently available. It is apparent that appreciable hyperthermia (> 40°C), regardless of the source, is necessary before cerebral permeability is significantly altered. It may be that the rate and/or duration of heating are influencing factors in the development of hyperthermia-induced changes in cerebral permeability.

The evidence presented is supportive of the view that hyperthermia is the principal effector of reduced endothelial cell permeability. Furthermore, it has been shown that in both *in vitro* and *in vivo* systems, hyperthermia is capable of inducing thermal conditions which reduce or suppress the activity of endocytosis. This suppression may result from perturbation of membrane lipids and changes in membrane fluidity. Direct application of heat (burns) or prolonged hyperthermia resulting in core body temperatures near the upper level of survivability (~ 43°C) may disrupt membrane processes to the extent that cellular homeostasis is lost and injury or death occurs. At this point cell permeability may increase.

The apparent reduction in BBB permeability indicated by reduced uptake of HRP and [^{14}C] sucrose was an unexpected finding [2], [3]. The fact that exposure to ambient heat effected similar reduction in HRP uptake suggests that the response is mediated principally by ther-

mally induced alteration of endothelial cell function, and not by qualities unique to microwave energy.

In our studies [2], [3], significantly reduced vesicular uptake of HRP and [^{14}C] sucrose was observed in regions of the brain exhibiting temperatures near 41°C to slightly over 43°C. Very distinct membrane effects have been shown to occur at these temperatures [88], [89], [116]–[119]. The mechanism by which microwave and ambient hyperthermia suppress vesicular uptake, and perhaps other transport processes as well, cannot be determined from our data. However, available evidence suggests that the endothelial cell membrane might be the principal site for heat effects. This would be especially true for microwave energy where field-induced rotation of polar molecules, such as proteins and phospholipids, residing in or on the membrane, would occur. An induced torque of bound water and membrane protein would also result. With a sufficiently high energy absorption rate, detectable levels of tissue heating could be produced. Heating may also occur as a result of microwave-induced rotational movement of free cytoplasmic water [120]. These membrane interactions probably explain why microwave exposure appeared to be somewhat more effective than ambient heat in eliciting observed effects on the BBB.

As suggested previously, hyperthermia-induced changes in membrane fluidity could effectively disrupt lipoprotein stability, and, in so doing, alter membrane-bound enzyme systems [88], surface charge, and transmembrane permeability [87]. This disruptive nature of heat could therefore effectively reduce enzyme-dependent transport systems, facilitated transport, and pinocytotic activity. Because of the nearly total energy independence of the pinocytotic process, it does not appear likely that energy depletion would account for the apparent suppression of vesicular activity. In addition, the suppression of pinocytotic activity apparent in rats exposed to 20 mW/cm^2 occurs at elevated brain temperatures far below levels that would inhibit metabolism [2]. Blood pressure, although elevated in severely hyperthermic rats [2], does not appear sufficiently raised to have an effect on transport function [14].

A tentative hypothesis for the influence of microwave and ambient heat-induced effects on pinocytotic activity and perhaps on other transport systems can be stated as follows: It is known that pinocytosis in macrophages can be inhibited by nicotine, and tetramethylammonium (TMA) and hexamethonium ions. These compounds are approximately 80-percent ionized with a positive charge at physiological pH [121]. The means by which these substances suppress endocytic activity appears to lie in their ability to displace membrane calcium [20]. The presence of membrane calcium has been demonstrated to be an essential element for a number of membrane-mediated events, including pinocytosis and membrane permeability [19], [59], [122], [123]. The importance of multivalent cations, especially Ca^{++} and Mg^{++}, may lie in the fact that these cations can interact with negatively charged sites forming stabilizing bridges at membrane locations prone to forming pinocytotic caveolae [20], [124], [125]. Inducers of pinocytosis might effectively destabilize a region of the membrane by displacing calcium [126]–[128], thereby initiating vesicle formation [124].

On the other hand, formation of pinocytotic vesicles may be coupled with increased membrane permeability to Ca^{++} [129], [130]. Inhibition of Ca^{++} influx has been shown to reduce, but not completely inhibit, vesicular activity [130]. Moreover, some mammalian cells, including those that exhibit phagocytic activity, have been shown to possess Ca^{++} [131] or (Ca^{++} Mg^{++})-dependent ATPase [132], [133] which is involved in the phagocytic process [20], [134]. A Ca^{++}-dependent ATPase has recently been isolated from rat liver plasma membrane [131]. It is not improbable therefore that hyperthermia induced by microwaves or ambient heat could effectively destabilize the microenvironment of vesicle forming regions of the membrane through inactivation of enzymes involved with the process and/or by causing the release of calcium. In this regard, evidence has been reported suggesting that brain tissue exposed to radio frequencies (RF) [135]–[137], and ambient heating to 41°C [138] resulted in significantly altered efflux of $^{45}Ca^{++}$. Johansson and Josefsson [139] have shown that pinocytosis increases when extracellular Ca^{++} concentrations are maintained at a certain level, but decreases when this optimum concentration is exceeded.

Whether RF field effects on calcium efflux are real or apparent is still open to controversy [59], [140]. Evidence that increased efflux occurs as a result of ambient heat [138] adds further credence to the proposition that hyperthermia per se and not microwave field effects is the essential factor behind changes in BBB permeability. In this regard, changes in membrane permeability to K^+ in CHO cells made hyperthermic (43°C) by microwave exposure or ambient heat (water bath) were found to be a function of temperature and not of the mode of treatment [141]. Furthermore, Ward *et al.* [28] have recently suggested that an apparent change in permeability to [^{14}C] sucrose in anesthetized rats is the result of hyperthermia induced by either 2450-MHz microwaves at 10 to 30 mW/cm^2 or ambient heat at 22 to 40°C. After correcting their data for temperature effects, no significant change in permeability to sucrose was evident.

III. Comments

Technical considerations employed by Williams *et al.* [1]–[4] warrant special note: 1) possible microwave influences on cerebral microvessel permeability were assessed through comparison of results obtained from a variety of tracer techniques; 2) unanesthetized, unrestrained animal (rat) preparations were used for all brain temperature and tracer studies; 3) physiological stress was minimized prior to all exposures through the use of cage conditioning and a controlled environment during experiments; and 4) physiological effects of whole-body exposure to microwave energy or ambient heat and systemic effects of intravenously

injected tracers were considered with respect to possible influences on experimental results.

Results of this work demonstrate the inability of whole-body hyperthermia induced by 2450-MHz CW microwave energy or ambient heat ($42 \pm 2°C$) to compromise the BBB in the conscious, unrestrained rat. Studies reporting disruption of the BBB to a variety of intravascularly administered tracers very likely have misinterpreted technically derived artifacts for increases in barrier permeability. The lack of histological scrutiny on the part of some investigations and lack of functional confirmation by others makes assessment of results difficult. Results of our study do not preclude the possibility that heat applied locally to the head may increase BBB permeability through induction of brain temperatures exceeding those observed here. However, it is conceivable that cerebral temperatures in excess of 43°C might disrupt the barrier by overt thermal injury to the microvessel endothelium [142]. Such thermal injury, including cerebrovascular fragility and stasis, are known to occur with prolonged hyperthermia [143], heat stroke [142], and in various tumors exposed to extreme temperatures [73]. These thermal effects may or may not be reversible.

The decreased entry of HRP and [^{14}C] sucrose into the microvessel endothelium of hyperthermic rats [2], [3] is consistent with experimental evidence reporting hyperthermia-induced disruption of membrane function. It is unfortunate that the effects of hyperthermia on other modes of transport, such as active and facilitated transport mechanisms, cannot presently be evaluated. Most studies to date have relied solely upon nonphysiological tracers that 1) have low permeability coefficients, and 2) are not carried by active or facilitated transport systems. For this reason, statements concerning alterations of BBB permeability must be limited to those aspects of the BBB by which the tracer is subject to transfer, such as vesicular transport and leakage through tight junctions in the case of HRP, NaFl, and sucrose. Studies employing the use of substrate analogs, such as 2-deoxyglucose, would yield useful information concerning microwave effects on active and facilitated transport mechanisms. One such study [144] employing [^{3}H] thymidine has clearly demonstrated the ability of hyperthermia ($\geqslant 43.5°C$) to inhibit membrane transport by reducing facilitated diffusion of the substrate into Chinese hamster ovary cells *in vitro*. These findings are in agreement with those recently reported by Williams *et al.* [4] and support the conclusion that suppression of BBB permeability is a temperature-dependent phenomenon.

REFERENCES

[1] W. M. Williams, M. Formaniak, W. Hoss, and S. M. Michaelson, "Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. A. Effect on the permeability to sodium fluorescein," *Brain Res. Rev.*, in press.

[2] W. M. Williams, M. del Cerro, and S. M. Michaelson, "Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. B. Effect on the permeability to HRP," *Brain Res. Rev.*, in press.

[3] W. M. Williams, J. Platner, and S. M. Michaelson, "Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. C. Effect on the permeability to [^{14}C] sucrose," *Brain Res. Rev.*, in press.

[4] W. M. Williams, S.-T. Lu, and S. M. Michaelson, "Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. D. Brain temperature and blood-brain barrier permeability to hydrophilic tracers," *Brain Res. Rev.*, in press.

[5] S. P. Gruenau, K. J. Oscar, M. T. Folker, and S. I. Rapoport, "Absence of microwave effect on blood-brain barrier permeability to [^{14}C] sucrose in the conscious rat," *Exp. Neurol.*, vol. 75, pp. 299–307, 1982.

[6] E. Preston and G. Prefontaine, "Cerebrovascular permeability to sucrose in the rat exposed to 2,450-MHz microwaves," *Appl. Physiol. Respirat. Environ. Exercise Physiol.*, vol. 49, pp. 218–223, 1980.

[7] P. Ehrlich, Das Sauerstoff-Bedurfnis Des Organismus. Eine Farbenanalytische Studie. Berlin: Herschwald 1885, pp. 69–72, cited to D. H. Ford, S. Ehrenpreis, and I. J. Kopin, Eds., *Reviews of Neuroscience 2*. New York: Raven Press, 1976, pp. 1–41.

[8] T. S. Reese and M. S. Karnovsky, "Fine structural localization of a blood-brain barrier to exogenous peroxidase," *J. Cell Biology*, vol. 34, pp. 207–216, 1967.

[9] T. S. Bodenheimer and M. W. Brightman, "A blood-brain barrier to peroxidase in capillaries surrounded by perivascular spaces," *Am. J. Anat.*, vol. 122, pp. 249–268, 1968.

[10] C. Crone and A. M. Thompson, "Permeability of brain capillaries," in *Capillary Permeability*, C. Crone and N. A. Lassen, Eds. Copenhagen: Munksgaard, 1970, pp. 447–453.

[11] G. D. Pappas, "Some morphological considerations of the blood-brain barrier," *J. Neurol. Sci.*, vol. 10, pp. 241–246, 1970.

[12] S. C. Sorensen, "The permeability to small ions of tight junctions between cerebral endothelial cells," *Brain Res.*, vol. 70, pp. 177–178, 1974.

[13] W. M. Pardridge, J. D. Conner, and I. L. Crawford, "Permeability changes in the blood-brain barrier: Causes and consequences," *CRC Critical Reviews in Toxicology*, vol. 3, pp. 159–199, 1975.

[14] S. I. Rapoport, *Blood-Brain Barrier in Physiology and Medicine*. New York: Raven Press, 1976, pp. 1–316.

[15] G. W. Goldstein, "Metabolism of brain capillaries in relation to active ion transport," in *Pathology of Cerebrospinal Microcirculation, Advances in Neurology 20*, J. Cervos-Navarro, E. Betz, G. Ebhart, R. Ferszt and R. Wullenweber, Eds. New York: Raven Press, pp. 11–16.

[16] W. H. Oldendorf and W. J. Brown, "Greater number of capillary endothelial cell mitochondria in brain than in muscle," *Proc. of the Society for Experimental Biology and Medicine*, vol. 149, 1975, pp. 736–738.

[17] H. Hansson and B. B. Johansson, "Induction of pinocytosis in cerebral vessels by acute hypertension and by hyperosmolar solutions," *J. Neurosci. Res.*, vol. 5, pp. 183–190, 1980.

[18] H. Reyners, E. Gianfelici de Reyners, J. M. Jadin, and J. R. Maisin, "An ultrastructural quantitative method for the evolution of the permeability to horseradish peroxidase of cerebral cortex endothelial cells of the rat," *Cell Tiss. Res.*, vol. 157, pp. 93–99, 1975.

[19] R. Duncan and J. B. Lloyd, "Pinocytosis in the rat visceral yolk sac: Effects of temperature, metabolic inhibitors and some other modifiers," *Biochim. Biophys. Acta*, vol. 544, pp. 647–655, 1978.

[20] S. L. Schwartz, D. E. Evans, J. E. Lundin, and J. C. Bond, "Inhibition of pinocytosis by nicotine," *J. Pharmacol. Exp. Ther.*, vol. 183, no. 2, pp. 370–377, 1972.

[21] A. H. Frey, S. R. Feld, and B. Frey, "Neural function and behavior: Defining the relationship," *Ann. N.Y. Acad. Sci.*, vol. 247, pp. 433–438, 1975.

[22] K. J. Oscar and D. Hawkins, "Microwave alteration of the blood-brain barrier system of rats," *Brain Res.*, vol. 126, pp. 281–293, 1977.

[23] E. N. Albert, "Ultrastructural pathology associated with microwave induced alterations in blood-brain barrier permeability," in *URSI, Proc. Int. Symp. on Biological Effects of Electromagnetic Radiation*, (Helsinki), 1978, p. 58.

[24] E. N. Albert and J. M. Kerns, "Reversible microwave effects on the blood-brain barrier," *Brain Res.*, vol. 230, pp. 153–164, 1981.

[25] J. C. Lin and M. F. Lin, "Microwave hyperthermia-induced blood-brain barrier alterations," *Radiation Res.*, vol. 89, pp. 77–87, 1982.

[26] J. C. Lin and M. F. Lin, "Power-time relations of microwave-induced blood-brain barrier permeation," *Bioelectromagn.*, vol. 1, p. 207, 1980.

[27] J. C. Lin and M. F. Lin, "Studies on microwave and blood-brain barrier interaction," *Bioelectromagn.*, vol. 1, pp. 313–323, 1980.

[28] T. R. Ward, J. A. Elder, and M. D. Long, "A comparative study of microwave and high ambient temperature exposures on the blood-brain barrier," *Bioelectromagn.*, vol. 1, no. 2, p. 207, 1980.

[29] C. H. Sutton and F. B. Carroll, "Effects of microwave induced

hyperthermia on the blood-brain barrier of the rat," *Radio Sci.*, vol. 14, no. 63, pp. 329–334, 1979.
[30] C. H. Sutton and R. L. Nunnally, "Protection of the microwave-irradiated brain with body-core hypothermia," in *Abstracts—Tenth Ann. Meet. Cryobiology*, vol. 10, 1973, pp. 513–514.
[31] E. N. Albert, D. L. Brainard, J. D. Randall, and F. S. Janatta, "Neuropathological observations of microwave-irradiated hamsters," in *URSI, Proc. Int. Symp. on Biological Effects of Electromagnetic Radiation*, (Helsinki), 1978, p. 59.
[32] E. N. Albert, "Reversibility of microwave-induced blood-brain barrier permeability," *Radio Sci.*, vol. 14, pp. 323–327, 1979.
[33] J. H. Merritt, A. F. Chamness, and S. J. Allen, "Studies on blood-brain barrier permeability after microwave-radiation," *Rad. and Environ. Biophys.*, vol. 15, pp. 367–377, 1978.
[34] E. Preston, E. J. Vavasour, and H. M. Assenheim, "Permeability of the blood-brain barrier to Mannitol in the rat following 2450 MHz microwave irradiation," *Brain Res.*, vol. 174, pp. 109–117, 1979.
[35] D. H. Spackman and V. Riley, "Studies of RF radiation effects on blood-brain barrier permeability using fluorescein and amino acids," in *URSI, Proc. Int. Symp. on Biological Effects of Electromagnetic Radiation*, (Helsinki), 1978, p. 75.
[36] A. H. Frey, "Lab notes: On microwave effects at the blood-brain barrier," *Bioelectromagn. Soc. Newsletter*, vol. 1, p. 4, 1980.
[37] S. I. Rapoport, K. Ohno, W. R. Fredericks, and K. D. Pettigrew, "Regional cerebrovascular permeability to [^{14}C] sucrose after osmotic opening of the blood-brain barrier," *Brain Res.*, vol. 150, pp. 653–657, 1978.
[38] E. N. Albert, "Light and electron microscopic observations on the blood-brain barrier after microwave irradiation," in *Proc. Symp. on Biological Effects*, (Rockville, MD), 1977, pp. 294–304.
[39] D. H. Spackman, V. Riley, A. W. Guy, and C. K. Chou, "The elevation of natural amino acids in brain following exposure of mice to low-level microwave (RFR) radiation in blood-brain barrier studies," in *Abstract Fed. Proc.*, vol. 39, 1980, p. 1903.
[40] S. Szmigielski, M. Luczak, M. Janiak, M. Kobus, B. Laskowska, E. de Clercq, and P. de Somer, "*In vitro* and *in vivo* inhibition of virus multiplication by microwave hyperthermia," *Arch. Virology*, vol. 53, pp. 71–77, 1977.
[41] R. D. Philips, E. L. Hunt, R. D. Castro, and N. W. King, "Thermoregulatory, metabolic, and cardiovascular response of rats to microwaves," *J. Appl. Physiol.*, vol. 38, no. 4, pp. 630–635, 1975.
[42] R. S. Spielman and C. P. Lyman, "Thermal bradycardia in the mildly stressed cat," *Am. J. Physiol.*, vol. 221, no. 3, pp. 948–951, 1971.
[43] A. L. Smith and H. Wollman, "Cerebral blood flow and metabolism: Effects of anesthetic drugs and techniques," *Anesthesiology*, vol. 36, no. 4, pp. 378–400, 1972.
[44] J. I. Sage and T. E. Duffy, "Pentobarbital anesthesia: Influence on amino acid transport across the blood-brain barrier," *J. Neurochem.*, vol. 33, pp. 963–965, 1979.
[45] B. B. Johansson, "Effect of an acute increase of the intravascular pressure on the blood-brain barrier. A comparison between conscious and anesthetized rats," *Stroke*, vol. 9, no. 6, pp. 588–590, 1978.
[46] A. Gjedde and M. Rasmussen, "Pentobarbital anesthesia reduces blood-brain glucose transfer in the rat," *J. Neurochem.*, vol. 35, no. 6, pp. 1382–1387, 1980.
[47] J. E. Hardebo and B. B. Johansson, "Effect of an anion transport inhibitor on blood-brain barrier lesions during acute hypertension," *Acta Neuropathol. (Berl.)*, vol. 51, pp. 33–38, 1980.
[48] W. I. Cranston and C. Rosendorff, "Local blood flow, cerebrovascular autoregulation and CO_2 responsiveness in the rabbit hypothalamus," *J. Physiol. (Lond.)*, vol. 215, pp. 577–590, 1971.
[49] M. R. Brown and G. A. Hedge, "Thyroid secretion in the unanesthetized, stress-free rat and its suppression by pentobarbital," *Neuroendocrinology*, vol. 9, pp. 158–174, 1972.
[50] B. B. Johansson, L. Linder, and L. I. Persson, "Hypertension-induced protein leakage in the brain in ethanol-intoxicated conscious and anesthetized rats," *Acta Neurol. Scand.*, vol. 57, pp. 333–339, 1978.
[51] J. McClendon and G. Medes, *Physical Chemistry in Biology and Medicine*. Philadelphia: W. B. Saunders, 1925, pp. 17–413.
[52] H. M. Frankel, "Effects of restraint on rats exposed to high temperature," *J. Appl. Physiol.*, vol. 14, no. 6, pp. 997–999, 1959.
[53] W. H. Oldendorf, "Measurement of brain uptake of radiolabeled substances using tritiated water internal standard," *Brain Res.*, vol. 24, pp. 372–376, 1970.
[54] S. I. Rapoport, K. Ohno, and K. Pettigrew, "Blood-brain barrier permeability in senescent rats," *J. Gerontol.*, vol. 34, no. 2, pp. 162–169, 1979.
[55] S. I. Rapoport, K. Ohno, W. R. Fredericks, and K. D. Pettigrew, "A quantitative method for measuring altered cerebrovascular permeability," *Radio Sci.*, vol. 14, no. 65, pp. 345–348, 1979.
[56] M. E. Raichle, J. D. Eichling, and R. L. Grubb, "Brain permeability of water," *Arch. Neurol.*, vol. 30, pp. 319–321, 1974.
[57] M. E. Raichle, J. D. Eichling, M. G. Straatmann, M. J. Welch, K. G. Larson, and M. M. Ter-Pogossia, "Blood-brain barrier permeability of ^{11}C-labeled alcohols and ^{15}O-labeled water," *Am. J. Physiol.*, vol. 230, no. 2, pp. 543–552, 1976.
[58] K. J. Oscar, S. P. Gruenau, M. T. Folker, and S. I. Rapoport, "Local cerebral blood flow after microwave exposure," *Brain Res.*, vol. 204, pp. 220–225, 1981.
[59] R. D. Myers and D. H. Ross, "Radiation and brain calcium: A review and critique," *Neuroscience and Biobehavioral Reviews*, vol. 5, pp. 503–543, 1981.
[60] W. H. Oldendorf, "Clearance of radiolabeled substances by brain after arterial injection using a diffusible internal standard," in *Research Methods in Neurochemistry 5*, N. Marks and R. Rodnight, Eds. New York: Plenum, 1981, pp. 27–35.
[61] R. G. Blasberg, "Problems of quantifying effects of microwave irradiation on the blood-brain barrier," *Radio Sci.*, vol. 14, no. 65, pp. 335–344, 1979.
[62] M. C. Kew, C. Abrahams, N. W. Levin, H. C. Seftel, A. H. Rubenstein, and I. Bersohn, "The effects of heatstroke on the function and structure of the kidney," *Quart. J. Med.*, vol. 36, no. 143, pp. 277–390, 1962.
[63] J. P. Knochel, "Environmental heat illness: An eclectic review," *Arch. Intern. Med.*, vol. 133, pp. 841–864, 1974.
[64] G. S. Kanter, "Glomerular filtration and renal plasma flow during hyperthermia," *Am. J. Physiol.*, vol. 198, no. 5, pp. 1044–1048, 1960.
[65] L. S. Schanker and A. M. Hogben, "Biliary excretion of inulin, sucrose, and Mannitol: Analysis of bile formation," *Am. J. Physiol.*, vol. 200, pp. 1087–1090, 1961.
[66] K. Ohno, K. D. Pettigrew, and S. I. Rapoport, "Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat," *Am. J. Physiol.*, vol. 235, pp. N299–N307, 1978.
[67] M. Flores, E. Weser, and E. A. Young, "Comparative metabolism of intravenously injected sucrose and trehalose in the rat," *Comp. Biochem. Physiol.*, vol. 50B, pp. 221–224, 1975.
[68] H. J. Hoffman and J. Olszewski, "Spread of sodium fluorescein in normal brain tissue," *Neurology*, vol. 11, pp. 1081–1085, 1961.
[69] M. Wolman, I. Klatzo, E. Chui, F. Wilmes, K. Nishimoto, K. Fujiwara, and M. Spatz, "Evaluation of the dye-protein tracers in pathophysiology of the blood-brain barrier," *Acta Neuropathol.*, vol. 54, pp. 55–61, 1981.
[70] T. Tervo, F. Joó, A. Palkama, and L. Salminen, "Penetration barrier to sodium fluorescein and fluorescein-labelled dextrans of various molecular sizes in brain capillaries," *Experientia*, vol. 35, no. 2, pp. 252–254, 1979.
[71] C. W. Song, "Effect of hyperthermia on vascular functions of normal tissues and experimental tumors: Brief communication," *J. Natl. Cancer Inst.*, vol. 60, no. 3, pp. 711–713, 1978.
[72] R. M. Abrams, J. A. J. Stolwyk, H. T. Hammel, and H. Graichen, "Brain temperature and brain blood flow in unanesthetized rats," *Life Sciences*, vol. 4, pp. 2399–2410, 1965.
[73] S. Arrhenius, *Quantitative Laws in Biological Chemistry*. London: G. Bell and Sons, 1915, pp. 1–164.
[74] C. Carlsson, M. Hägerdal, and B. K. Siesjö, "The effect of hyperthermia upon oxygen consumption and upon organic phosphates, glycolytic metabolites, citric acid cycle intermediates and associated amino acids in rate cerebral cortex," *J. Neurochem.*, vol. 26, pp. 1001–1006, 1976.
[75] E. M. Nemoto and H. M. Frankel, "Cerebral oxygenation and metabolism during progressive hyperthermia," *Am. J. Physiol.*, vol. 219, no. 6, pp. 1784–1788, 1970.
[76] E. M. Nemoto and H. M. Frankel, "Cerebrovascular response during progressive hyperthermia in dogs," *Am. J. Physiol.*, vol. 218, no. 4, pp. 1060–1064, 1970.
[77] B. K. Siesjö, L. Berntman, and B. Nilsson, "Regulation of microcirculation in the brain," *Microvascular Res.*, vol. 19, pp. 158–170, 1980
[78] L. Nilsson, K. Kogure, and R. Busto, "Effects of hypothermia and hyperthermia on brain energy metabolism," *Acta Anaesth. Scand.*, vol. 19, pp. 199–205, 1975.
[79] A. Siflinger, K. Parker, and C. G. Caro, "Uptake of ^{125}I albumin by the endothelial surface of the isolated dog common carotid artery: Effect of certain physical factors and metabolic inhibitors," *Cardiovascular Res.*, vol. 9, pp. 478–489, 1975.

[80] P. K. Peterson, J. Verhoef, L. D. Sabath, and P. G. Quie, "Extracellular and bacterial factors influencing staphylococcal phagocytosis and killing by human polymorphonuclear leukocytes," *Infect. Immun.*, vol. 14, no. 2, pp. 496–501, 1976.

[81] E. Philipsborn, "Ergebnisse der wichtigsten phagocytoseversuche der letzten jahre. Eine beitrag zur normalen und pathologischen physiologie der weissen blutzellen," *Klin. Wschr.*, vol. 5, pp. 373–377, 1926.

[82] B. T. Rubin, "A theoretical model of the pinocytotic vesicular transport process in endothelial cells," *J. Theor. Biol.*, vol. 64, pp. 619–647, 1977.

[83] N. deTerra and R. C. Rustad, "The dependence of pinocytosis on temperature and aerobic respiration," *Exp. Cell Res.*, vol. 17, pp. 191–195, 1959.

[84] S. K. Williams, M. A. Matthews, and R. C. Wagner, "Metabolic studies on the micropinocytotic process in endothelial cells," *Microvascular Res.*, vol. 18, pp. 175–184, 1979.

[85] T. Madsen and O. Wulff, "Influence de la tempirature sur la phagocytose," *Annls. Inst. Pasteur*, vol. 33, pp. 437–447, 1919, cited to E. Philipsborn, *Klin. Wschr.*, vol. 5, pp. 373–377, 1926.

[86] B. Bowers, "Comparison of pinocytosis and phagocytosis in Acanthamoeba castellanii," *Exp. Cell. Res.*, vol. 110, pp. 409–417, 1977.

[87] M. B. Yatvin, C. Cree, C. E. Elson, J. J. Gipp, I.-M. Tegmo, and J. W. Vorpahl, "Correspondence—Probing the relationship of membrane "fluidity" to heat killing cells," *Radiation Res.*, vol. 89, pp. 644–646, 1982.

[88] K. Bowler, C. J. Duncan, R. T. Gladwell, and T. F. Davison, "Cellular heat injury," *Comp. Biochem. Physiol.*, vol. 45A, pp. 441–450, 1973.

[89] K. Redmann, J. Burmeister, and H.-L. Jenssen, "The influence of hyperthermia on the transmembrane potential, zeta-potential and metabolism of polymorphonuclear leukocytes," *Acta Biol. Med. Germ.*, vol. 33, pp. 187–196, 1974.

[90] E. M. Renkin, The Microcirculatory Society Eugene M. Landis Award Lecture, *Microvascular Res* , vol. 15, pp. 123–135, 1978.

[91] B. B. Johansson, "Indomethacin and cerebrovascular permeability to albumin in acute hypertension and cerebral embolism in the rat," *Exp. Brain Res.*, vol. 42, pp. 331–336, 1981.

[92] B. B. Johansson, "Pharmacological modification of hypertensive blood-brain barrier opening," *Acta Pharmacol. et Toxicol.*, vol. 48, pp. 242–247, 1981.

[93] B. B. Johansson and M. Henning, "6-Hydroxydopamine and the blood-brain barrier in adult conscious rats," *Acta Physiol. Scand.*, vol. 110, pp. 1–4, 1980.

[94] S. M. Mueller and D. D. Heistad, "Effect of chronic hypertension on the blood-brain barrier," *Hypertension*, vol. 2, no. 6, pp. 809–812, 1980.

[95] B. van Deurs, "Observations on the blood-brain barrier in hypertensive rats, with particular reference to phagocytic pericytes," *J. Ultrastruct. Res.*, vol. 56, pp. 65–77, 1976.

[96] J. E. Hardebo, "Vasodilatation augments the blood-brain barrier lesions induced by an acute rise in intracarotid pressure," *Blood Vessels*, vol. 18, pp. 9–15, 1981.

[97] A. McCall, B. S. Galeser, W. Millington, and R. J. Wurtman, "Monosodium glutamate neurotoxicity, hyperosmolarity, and blood-brain barrier dysfunction," *Neurobehavioral Toxicology*, vol. 1, pp. 279–283, 1979.

[98] Z. Nagy, H. Peters, and I. Hüttner, "Endothelial surface charge: Blood-brain barrier opening to horseradish peroxidase induced by the polycation protamin sulfate," *Acta Neuropathol. (Berl.), Suppl.* vol. VII, pp. 7–9, 1981.

[99] Z. Nagy, G. Mathieson, and I. Hüttner, "Opening of tight junctions in cerebral endothelium. II. Effect of pressure-pulse induced acute arterial hypertension," *J. Comp. Neur.*, vol. 185, pp. 579–586, 1979.

[100] ——, "Blood-brain barrier opening to horseradish peroxidase in acute arterial hypertension," *Acta Neuropathol. (Berl.)*, vol. 48, pp. 45–53, 1979.

[101] M. P. Remler and W. H. Marcussen, "Time course of early delayed blood-brain barrier changes in individual cats after ionizing radiation," *Experimental Neurology*, vol. 73, pp. 310–314, 1981.

[102] L. A. Wade, R. J. Majeste, and H. M. Brady, "Osmotically induced increase in cerebrovascular permeability to [^{3}H] sucrose," *Brain Res.*, vol. 209, pp. 485–490, 1981.

[103] P. M. Schlievert and D. W. Watson, "Group A streptococcal pyrogenic exotosin: Pyrogenicity, alternation of blood-brain barrier, and separation sites for pyrogenicity and enhancement of lethal endotoxin shock," *Infect. Immun.*, vol 21, no. 3, pp. 753–763, 1978.

[104] H. M. Pappius, "Local cerebral glucose utilization in thermally traumatized rat brain," *Ann. Neurol.*, vol. 9, pp. 484–491, 1981.

[105] G. Arturson, "Microvascular permeability to macromolecules in thermal injury," *Acta Physiol. Scand., Suppl.*, vol. 463, pp. 111–122, 1979.

[106] F. Moati, M. Miskulin, G. Godeau, and A. M. Robert, "Blood-brain barrier permeabilizing activity in sera of severe-burn patients," *Neurochemical Res.*, vol. 4, no. 3, pp. 377–383, 1979.

[107] X. J. Musacchia, "Fever and hyperthermia," *Fed. Proc.*, vol. 38, no. 1, pp. 27–29, 1979.

[108] J. T. Stitt, "Fever versus hyperthermia," *Fed. Proc.*, vol. 38, no. 1, pp. 39–43, 1979.

[109] S. Heller, "Experiments on the effect of electromagnetic waves on the reactivity of cells and tissues. III. Communication: Effect of irradiation with red-light and microwaves on pinocytosis in FL cell cultures," *Zentralblatt Bakteriologie erste abt., Originale part A*, vol. 221, pp. 386–397, 1972.

[110] G. Plurien, H. Sentenac-Roumanou, R. Joly, and J. Drouet, "Influence du rayonement électromagnétique d'un émetteur radar sur la fonction phagocytaire des cellules du système réticulo-endothelial au contact du sang chez la Souris," *C.r. Seance Soc. Biol.*, vol. 160, pp. 597–602, 1966; cited to C. P. Mayers and J. A. Habeshaw, *Int. J. Radiat. Biol.*, vol. 24, pp. 449–461, 1973.

[111] C. P. Mayers and J A. Habeshaw, "Depression of phagocytosis: A nonthermal effect of microwave radiation as a potential hazard to health," *Int. J. Radiat. Biol.*, vol. 24, no. 5, pp. 449–461, 1973.

[112] E. Grant, R. Sheppard, and G. South, "Importance of bound water studies in the determination of energy absorption by biological tissue," in *Proc. of the 5th Eur. Microwave Conf.*, J. H. Schmitt, Ed. Sevenoaks: Microwave Exhibitions and Publishers, Ltd., 1975, pp. 366–370.

[113] L.-E. Paulsson, U. Hamnerius, and W. G. McLean, "The effects of microwave radiation on microtubules and axonal transport," *Radiation Res.*, vol. 70, pp. 212–223, 1977.

[114] D. Papahadjopoulos, K. Jacobson, S. Nir, and T. Isac, "Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measures concerning the effect of temperature and cholesterol," *Biochem. Biophys. Acta.*, vol. 311, pp. 330–348, 1973.

[115] J. Bělehrádek, "Temperature and living matter," in *Protoplasma Monographien 8.* Berlin: Verlag von Gebriider Borntraeger, 1935, pp. 1–267.

[116] F. J. Burger and F. A. Fuhrman, "Evidence of injury by heat in mammalian tissues," *Am. J. Physiol.*, vol. 206, pp. 1057–1061, 1964.

[117] E. W. Gárner, A. E. Cress, D. G. Stickney, D. K. Holmes, and P. S. Culver, "Factors regulating membrane permeability alter thermal resistance," *Ann. N.Y. Acad. Sci.*, vol. 335, pp. 215–230, 1980.

[118] B. Gwóźdź, A. Dyduch, H. Grzybek, and D. Panz, "Structural changes in brain mitochondria of mice subjected to hyperthermia," *Exp. Path. Bd.*, vol. 15, pp. 124–126, 1978.

[119] G. L. Mandell, "Effects of temperature on phagocytosis by human polymorphonuclear neutrophils," *Infect. Immun.*, vol. 12, pp. 221–223, 1975.

[120] M. A. Stuchly, "Interaction of radiofrequency and microwave radiation with living systems: A review of mechanisms," *Rad. and Environ. Biophys.*, vol. 16, pp. 1–14, 1979.

[121] R. C. Rustad, "Molecular orientation of the surface of amoebae during pinocytosis," *Nature*, vol. 183, no. 4667, pp. 1058–1959, 1959.

[122] R. C. Wagner and J. R. Casley-Smith, "Review. Endothelial vesicles," *Microvascular Res.*, vol. 21, pp. 267–298, 1981.

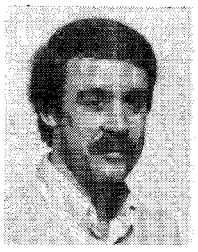
[123] D. J. Wilkins and A. D. Bangman, "The effect of some metal ions on *in vitro* phagocytosis," *J. Reticuloendothel. Soc.*, vol. 1, pp. 233–242, 1964.

[124] W. Gawlitta, W. Stockem, T. Wehland, and K. Weber, "Pinocytosis and locomotion of amoebae. XV. Visualization of Ca^{++}-dynamics by chlorotetracycline (CTC) fluorescence during induced pinocytosis in living Amoeba proteus," *Cell Tissue Res.*, vol. 213, pp. 9–20, 1980.

[125] M. W. Smith and K. A. Burton, "Calcium dependence of protein transport by the small intestine of the new-born pig," *Experientia*, vol. 28, no. 6, pp. 667–669, 1972.

[126] P. W. Brandt and A. R. Freeman, "Plasma membrane: Structural changes correlated with electrical resistance and pinocytosis," *Science*, vol. 3, pp. 582–585, 1967.

[127] K. Braatz-Schade, "Effects of various substances on cell shape, motile activity and membrane potential in Amoeba proteus," *Acta Protozool.*, vol. 17, pp. 163–176, 1978.
[128] K. Braatz-Schade, M. Haberey, and W. Stockem, "Correlation between membrane potential, cell shape, and motile activity in Amoeba proteus," *Exp. Cell Res.*, vol. 80, pp. 456–458, 1973.
[129] R. D. Prusch, "Calcium and sucrose movements in Amoeba proteus induced with calcium ionophore A 23187," *J. Gen. Physiol.*, vol. 74, pp. 10a, 1979.
[130] W. Stockem and H.-P. Klein, "Pinocytosis and locomotion in amoebae. XV. Demonstration of Ca^{++}-binding sites during induced pinocytosis in Amoeba proteus," *Protoplasma*, vol. 100, pp. 33–43, 1979.
[131] Y. Iwasa, T. Iwasa, K. Higashi, K. Matsui, and E. Miyamoto, "Demonstration of a high affinity Ca^{2+}-ATPase in rat liver plasma membranes," *Biochem. Biophys. Res. Communicat.*, vol. 105, no. 2, pp. 488–494, 1982.
[132] R. J. North, "The localization by electron microscopy of nucleoside phosphatase activity in guinea pig phagocytic cells," *J. Ultrastruct. Res.*, vol. 16, pp. 83–95, 1966.
[133] A. M. Woodin and A. A. Wieneke, "Composition and properties of cell membrane function from the polymorphonuclear leucocyte," *Biochem. J.*, vol. 99, pp. 493–500, 1966.
[134] R. J. North, "The uptake of particulate antigens," *RES J. Reticuloendothel. Soc*, vol. 5, pp. 203–229, 1968.
[135] S. Bawin, W., Adey, and I. Sabbot, "Ionic factors in release of $^{45}Ca^{+2}$ from chicken cerebral tissue by electromagnetic fields," *Proc. Nat. Acad. Sci. USA*, vol. 75, pp. 6314–6318, 1975.
[136] C. F. Blackman, J. A. Elder, C. M. Weil, S. G. Benane, and D. C. Eichinger, "Two parameters affecting radiation-induced calcium efflux from brain tissue," in *Abstracts of Scientific Papers, Int. Symp. on the Biological Effects of Electromagnetic Waves*, (Washington, DC), 1977, p. 101.
[137] L. K. Kaczmarek and R. Adey, "The efflux of $^{45}Ca^{+2}$ and [^{3}H]-aminobutyric acid from cat cerebral cortex," *Brain Res.*, vol. 63, pp. 331–342, 1973.
[138] C. F. Blackman, S. G. Benane, J. A. Elder, D. E. House, J. A. Lampe, and J. M. Faulk, "Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effect of sample number and modulation frequency on the power-density window," *Bioelectromagn.*, vol. 1, pp. 35–43, 1980.
[139] P. Johansson and J. Josefsson, "Evidence for a dual effect of intracellular Ca^{++} on pinocytosis," *Acta Physiol. Scand.*, vol. 102, pp. 71A–72A, 1978.
[140] W. W. Shelton, Jr., and J. H. Merritt, "*In vitro* study of microwave effects on calcium efflux in rat brain tissue," *Bioelectromagn.*, vol. 2, pp. 161–167, 1981.
[141] E. Balcer-Kubiczek, J. E. Robinson, and D. McCulloch, "Membrane injury in CHO cells exposed to microwave hyperthermia at 37.5°C and at 43°C, 4th Annual Scientific Session," in *Proc. Bioelectromagn. Soc.*, (Los Angeles, CA), 1982, p. 83.
[142] R. S. Sohal, S. C. Sun, H. L. Colcolough, and G. E. Burch, "Heat stroke. An electron microscopic study of endothelial cell damage and disseminated intravascular coagulation," *Arch. Intern. Med.*, vol. 122, pp. 43–47, 1968.
[143] S. Shibolet, M. C. Lancaster, and U. Danon, "Heat stroke: A review," *Aviat. Space Environ. Med.*, vol. 47, pp. 280–301, 1976.
[144] H. Slusser, L. E. Hopwood, and M. Kapiszewska, "Inhibition of membrane transport by hyperthermia," in *Third Int. Symp.: Cancer Therapy by Hyperthermia, Drugs, and Radiation*, Monograph No. 61, U.S. Dept. of Health and Human Services, 1982, pp. 85–87.



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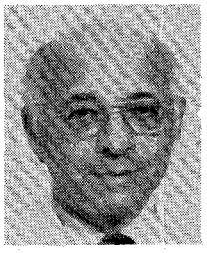

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A High-Power Automatic Network Analyzer for Measuring the RF Power Absorbed by Biological Samples in a TEM Cell

JOHN R. JUROSHEK AND CLETUS A. HOER

Abstract—**A device for measuring the radiofrequency (RF) power absorbed by biological samples while they are being irradiated in a transverse electromagnetic (TEM) cell is described. The report discusses the design, calibration, and performance of this automated measurement system. The power absorption analyzer is based on a six-port type of automatic network analyzer, and operates at an incident power to the TEM cell of 1 to 1000 W, over a frequency range of 100 to 1000 MHz. Experiments show that an absorbed power of 0.02 to 0.05 percent of the incident power can be measured. Measurements of the power absorbed by a 1-percent saline solution were made using the power absorption analyzer and by an independent calorimetric measurement. The two measurement techniques show excellent agreement.**

Key Words—**Automatic network analyzer, biological effects, impedance measurements, power absorption analyzer, power measurements, six-port network analyzer, and TEM cell.**

I. Introduction

THIS REPORT describes an RF power-absorption analyzer developed by the National Bureau of Standards for the National Institute for Occupational Safety and Health. The analyzer is designed specifically to measure the power absorbed by biological samples while they are being irradiated with continuous-wave RF energy in a transverse electromagnetic (TEM) cell. The analyzer is based on a six-port automatic network analyzer which makes it possible to detect very small amounts of absorbed power [1]–[3]. Typically, the incident power to the TEM cell is of the order of 1 to 1000 W over a frequency range of 100 to 1000 MHz. The goal of the power absorption analyzer is to be able to detect absorbed power levels of the order of 0.05 percent of the incident power (0.002 dB in insertion loss). Some of the problems typically encountered in making these measurements are discussed in a publication by Hill [4].

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II. Theory

A simple expression for the RF power absorbed by any biological sample irradiated in a TEM cell is derived as follows. Referring to Fig. 1 for a definition of terms, the RF power absorbed by the empty cell can be written

$$P_{\text{cell}} = P_1 - P_L = P_1(1-\eta) \tag{1}$$

where

P_1 = net power into the empty TEM cell measured at the input to the TEM cell,

P_L = net power into the load measured at the output of the TEM cell,

$\eta = P_L/P_1$ = efficiency of the empty TEM cell.

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