

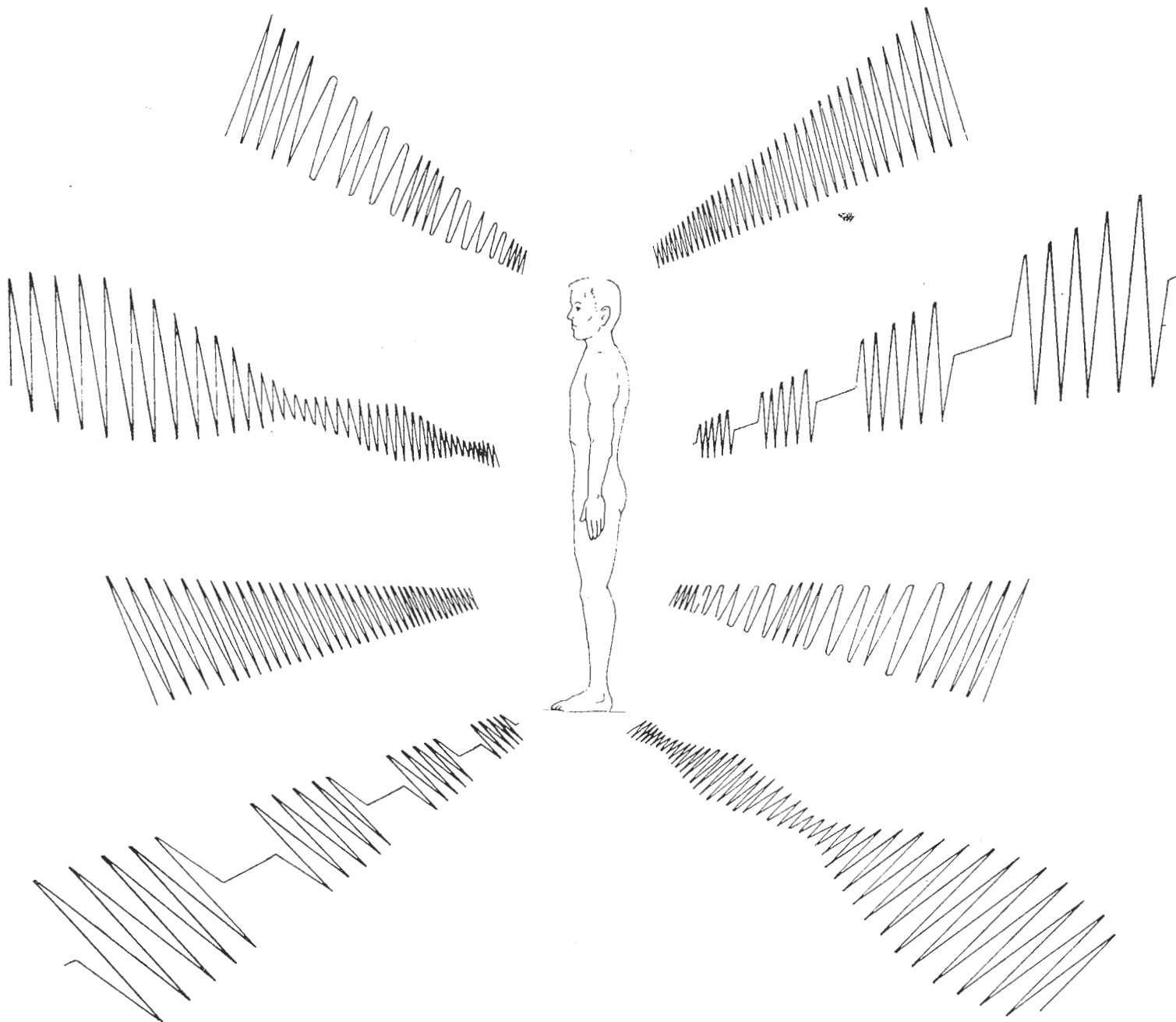
Research and Development

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# Biological Effects of Radiofrequency Radiation

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## 5.7 Endocrine, Physiological and Biochemical Effects

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### 5.7.1 Endocrine Effects

The endocrine system in coordination with the nervous system is a major regulatory system in the body. Alterations in the function of the neuroendocrine system often reflect the efforts on the part of the animal to maintain homeostasis when subjected to stressful internal and external stimuli. Detection of changes in the endocrine system is a sensitive means of analyzing the animal's responses either to direct stimulation of the endocrine organs themselves or to stimulation of the CNS.

Animals exposed to a wide variety of stimuli generally respond with a rather specific pattern of physiologic changes, usually referred to as the general adaptation syndrome, and the stimuli that can provoke the syndrome are usually called stressors. An increase in the concentration of corticosteroids in the blood above that which would normally occur at that time of day in the absence of a stimulus is considered by many to be an indicator of an animal's response to stress. Such an increase results when an internal or external stimulus, either chemical, physical, or emotional, excites neurons of the hypothalamus to release corticotropin-releasing hormone, which drives the pituitary to release adrenocorticotrophic hormone (ACTH). This hormone then stimulates the adrenal cortex to secrete corticosteroids. Among the strongest stressful stimuli are surgery, anesthesia, cold, narcosis, burning, high environmental temperature, and rough handling or restraint.

The thyroid gland plays a principal role in regulating basal metabolism, as well as in generating metabolic heat in the tissues. Changes in thyroid activity can result from changes in thyroid-stimulating hormone from the hypothalamic-hypophyseal system and/or increased metabolic activity of the thyroid gland due to heating. Direct interaction with the CNS could also produce changes in thyroid activity. Moderate or gradual heating results in a reduction of thyroid hormone; rapid or marked elevation of body temperature results in an increase in thyroid activity. The effects of RF radiation on the endocrine system are discussed below and summarized in Table 5-19.

Thyroid function of rats following exposure to 2450-MHz (CW) microwaves at 10, 20, and 25 mW/cm<sup>2</sup> (SARs estimated at 0.25 W/kg per mW/cm<sup>2</sup>) for 4 or 16 h was studied by Parker (1973). No effects on thyroid gland function were found at these exposures. However, at 15 mW/cm<sup>2</sup> (SAR estimated at 3.75 W/kg), exposure for a longer period (60 h) was reported to produce a significant decrease in serum-

protein-bound iodine, thyroxine, and thyroid/serum iodine ratio. A significant rectal temperature increase (1.7°C) was reported at 25 mW/cm<sup>2</sup>, but not at lower power densities.

Magin *et al.* (1977a,b) irradiated the surgically exposed thyroid gland of anesthetized dogs with 2450-MHz (CW) microwaves using a waveguide applicator at power densities of 72, 162, and 236 mW/cm<sup>2</sup> (SARs from 58 to 190 W/kg) for 2 h. One thyroid was irradiated while the other was used as a control. Tissue temperatures of 39, 41, and 45°C were maintained in the thyroid at the three power densities. They reported an increased release of thyroxine (TH) and triiodothyronine (T3) at all power densities, which showed that the thyroid gland in the dog can be stimulated directly by microwave heating.

Milroy and Michaelson (1972) exposed rats to 2450-MHz microwaves at 1, 10, and 100 mW/cm<sup>2</sup> (SAR = 0.25 W/kg per mW/cm<sup>2</sup>) for single exposures of 10, 20, 30, and 45 min and at 1 and 10 mW/cm<sup>2</sup>, 8 h/day, for 8 weeks, and reported no effect on T3 levels, thyroxine levels, or on the uptake of radioactive iodine. No rectal temperature increase was observed at 10 mW/cm<sup>2</sup> or less. At 100 mW/cm<sup>2</sup> there was a constant rise in rectal temperature throughout exposure, up to 42°C at the end of the exposure period. Histopathological examination of the thyroid glands also showed no effect from the exposure.

An increased production of thyroid hormone in rabbits as measured by increased incorporation of <sup>131</sup>I and increased radioactivity per gram of thyroid (verified by autoradiography) was reported by Barański *et al.* (1972). The animals were exposed for 3 h/day for 4 months to 10-cm (3-GHz, PW) microwaves at an average incident power density of 5 mW/cm<sup>2</sup> (SAR estimated at 0.25 to 0.75 W/kg). They reported no increase in body temperature or thyroid temperature. (The pulse parameters were not given, so peak power density cannot be calculated.)

Lu *et al.* (1977) reported serum thyroxine levels in rats exposed to 2450-MHz microwaves at 1, 5, 10, and 20 mW/cm<sup>2</sup> (0.25, 1.25, 2.5, and 5 W/kg) for 1, 2, 4, or 8 h. Decreased thyroxine levels were observed at 20 mW/cm<sup>2</sup> following 4- and 8-h exposures. The thyroxine values at shorter exposures and lower power densities were not significantly different from the sham values, except for an increase after 4 h at 1 mW/cm<sup>2</sup>, which appears to be a chance variation, since at both higher and lower power densities and at longer and shorter periods of exposure no effect was detected. There were small but statistically significant rectal temperature increases at 1 mW/cm<sup>2</sup> after 4 h, at 5 mW/cm<sup>2</sup> after 1 and 2 h, and at 10 mW/cm<sup>2</sup> after 2 and 4 h of exposure. The increases were in the range of 0.2 to 0.56°C. The lack of correlation between power density, exposure time, and rectal temperature increase, along with the small rectal

Paff *et al.* (1963), working with the isolated embryonic chicken heart, were unable to detect changes in heart rate during exposure to 24,000-MHz(PW) radar fields. They did, however, detect effects on the electrocardiogram (ECG), including abnormal P and T waves from 3-min exposures at 74 mW/cm<sup>2</sup>.

Frey and Seifert (1968) showed that 10- $\mu$ s pulses at a carrier frequency of 1.425 GHz given at a synchronous period with the ECG (220 ms after the P wave) resulted in tachycardia or heart arrhythmia in the isolated frog heart. The peak power density was 60 mW/cm<sup>2</sup> (average power density  $\sim$  0.6  $\mu$ W/cm<sup>2</sup>). Liu *et al.* (1976) reported no effect on heart rate with isolated frog hearts or in hearts irradiated *in situ* in a similar study. The *in situ* hearts were exposed to 100- $\mu$ s pulses of either 1.42 or 10 GHz, and the isolated frog hearts were exposed to 100- $\mu$ s pulses of 1.42 GHz. The pulse was delivered on the rising phase of the R-wave from the ECG, which as somewhat similar to, but not exactly the same as, the 200-ms delay following the P-wave used by Frey and Seifert. (The R-wave follows the P-wave by about 200 ms.) The peak and average power densities of 320 mW and 32  $\mu$ W were also considerably higher than those used by Frey and Seifert. These factors, plus differences in the manner of preparing the isolated hearts (Liu *et al.* curarized the frogs, whereas Frey and Seifert decapitated the frogs), make it difficult to compare the results of the two studies.

Clapman and Cain (1975), however, tried to replicate the study of Frey and Seifert using similar pulse widths (10  $\mu$ s), peak and average power densities (60 mW/cm<sup>2</sup> and 0.6  $\mu$ W/cm<sup>2</sup>), carrier frequency (1.42 GHz), and method of isolating the frog heart; they reported no change in heart rate. Also, no heart rate changes were found when they conducted studies with a different peak power (5.5 W/cm<sup>2</sup>), frequency (3 GHz), and pulse widths (2 and 150  $\mu$ s). Clapman and Cain were able to produce an increased heart rate with 20-mA current pulses synchronized 200 ms after the P-wave peak.

The results of microwave exposure on the cardiovascular system (Table 5-22) indicate that whole-body exposure of sufficient intensity to produce heating also produces an increase in heart rate similar to that which would be expected from heating alone. In the isolated heart there appears to be a stimulation of the autonomic nervous system from microwave exposure at levels where very little heating would be expected (1 to 2 W/kg). Low levels of synchronized PW microwaves (0.6 to 32 mW/kg) apparently are ineffective in producing detectable alterations in heart rate.

### 5.7.5 Biological Effects of Low Frequency Modulation of RF Radiation

Interest in the biological effects of low frequency modulation of RF radiation stems from reports of

changes caused by exposure to electric and magnetic fields in the sub-ELF range (0 to 30 Hz). It has been reported that exposure to low-frequency electric fields changes the reaction time in humans (Konig and Anker-muller 1960; Hamer 1968; Konig 1971) and in monkeys (Gavalas; *et al.* 1970; Gavalas-Medici and Day-Magdaleno 1976), and alters circadian activity in human beings (Wever 1973). Friedman *et al.* (1967) observed that magnetic fields modulated at low frequencies also change reaction time in human beings.

Two other studies that provide important background information are reported by Kaczmarek and Adey (1973, 1974). In the first report, they described release of calcium ions and  $\gamma$ -aminobutyric acid (GABA) from the cerebral cortex of cats in response to small changes in the extracellular concentration of calcium. In 1974, they demonstrated release of calcium ions and GABA from the cat cortex in response to low intensity electric currents, pulsed at 200 Hz, applied directly to the cerebral cortex. Thus, extracellular calcium and electric current have similar effects on the release of GABA and calcium ions from brain tissue.

The studies of (1) behavioral changes in animals and human beings induced by low frequency signals and (2) biochemical changes in the cat brain caused by electric currents led to a study of the influence of electric fields on EEG patterns associated with a conditioned behavioral response in cats (Bawin *et al.* 1973). To increase the penetration of the signals into the tissue, they chose an RF carrier wave of 147 MHz, which was amplitude modulated at sub-ELF frequencies (e.g., 3 to 14 Hz). Alterations were observed in the rate of performance, accuracy of reinforced patterns, and resistance to extinction in learned behavior of the exposed animals compared to controls, indicating that the fields were acting as reinforcers. In order to determine whether these effects were mediated via peripheral receptors or occurred as a result of changes induced directly on the CNS, experiments were designed to examine the effects of modulated RF carrier waves on brain tissue *in vitro*.

#### 5.7.5.1 Calcium Ion Efflux *In Vitro*: A Fundamental Finding

The association of calcium ions with brain tissue was selected as the biochemical marker to examine the influence of modulated RF fields because calcium ion efflux has been shown to be sensitive to electric currents applied directly to brain tissue *in vitro*, and because calcium ions have a prominent role in many biochemical and biophysical processes (e.g., cellular membrane integrity and function, enzyme cofactor, putative second messenger for the conduction of extracellular signals to the nucleus of the cell, neural tissue excitation and secretion of transmitter

substances at synapses). The first report of the influence of modulated RF fields on excised brain tissue was Bawin *et al.* (1975), who showed that a 20-min exposure of chick brain tissue *in vitro* to a 147-MHz field at 1 to 2 mW/cm<sup>2</sup> (SAR estimated at 0.002 W/kg) caused enhanced efflux of calcium ions, but only if the field was sinusoidally amplitude modulated at frequencies of 6, 9, 11, 16, or 20 Hz. Maximal efflux was measured at 16 Hz. Modulation frequencies of 0, 0.5, 3, 25, and 35 Hz were ineffective. This frequency-specific response, which occurred while the 147-MHz carrier field was maintained at the same power density, indicates that the field-induced efflux of calcium ions was not due to heating of the samples.

In another report, Bawin *et al.* (1978) exposed chick brain tissue for 20 min to 450-MHz fields, amplitude modulated at 16 Hz, at 0.75 mW/cm<sup>2</sup> (SAR estimated at 0.0035 W/kg) under a variety of chemical conditions. The results demonstrated that (a) the enhanced efflux of calcium ions is not highly sensitive to the external calcium concentration, (b) bicarbonate appears to be important for enhanced efflux, (c) lowering the pH from 7.6 to 6.8 in the presence of bicarbonate may enhance the magnitude of efflux, and (d) lanthanum causes a reversal to field-induced retardation of calcium ion efflux.

Corroboration of the frequency-specific response described by Bawin and co-workers was provided by Blackman *et al.* (1979), who showed that 16-Hz amplitude modulation of 147-MHz carrier waves caused enhanced efflux in chick brain tissue *in vitro*, whereas modulation frequencies of 3, 9, and 30 Hz did not. Although the data had large variances, an unusual intensity response was described, i.e., only 0.83 mW/cm<sup>2</sup> (SAR estimated at 0.0014 W/kg) produced a statistically significant efflux enhancement (intensity values are corrected based on discussion in Blackman *et al.* 1980a); power densities (0.11, 0.55, 1.11 and 1.38 mW/cm<sup>2</sup>) below and above the effective value did not cause efflux. In a later report, Blackman *et al.* (1980a) used a revised statistical model and experimental procedure to reduce the influence of the large sample variance. An intensity response identical to their earlier result was found. However, when the distance between samples was halved, the range of intensities that produced enhanced efflux increased to include 0.55, 0.83, 1.11 and 1.38 mW/cm<sup>2</sup>, whereas lower and higher values of 0.11 and 1.66 mW/cm<sup>2</sup> were ineffective. In addition, an intensity region from 0.55 to 1.11 mW/cm<sup>2</sup> caused enhanced efflux when 9 Hz was used as the modulation frequency. These data, obtained with a more rigorous experimental protocol, provided additional support for the results of Bawin *et al.* (1975) and Blackman *et al.* (1979); however, the explanation for the dependence on sample spacing awaited further developments.

Joines *et al.* (1981) examined the dependence on sample spacing by calculation of the electrical coupling between the samples; for simplicity the samples were modeled as spheres. They found that increased electrical interaction between the more closely packed spheres produced a broader range of internal field strengths within each sphere. Thus, if a given internal field strength were necessary to cause enhanced efflux, the chance would be greater for that internal field strength to occur in closely coupled samples exposed to a specific range of incident intensities. Joines *et al.* (1981) found this result to be consistent with the experimental findings in Blackman *et al.* (1980a). Thus a potential artifact was shown to be a logical result of the experimental procedures.

The intensity response observed by Blackman *et al.* (1979) with modulated 147-MHz carrier waves was confirmed by Sheppard *et al.* (1979) with 450-MHz carrier waves, modulated at 16 Hz; calcium-ion efflux was enhanced at 0.1 and 1.0 mW/cm<sup>2</sup> but not at 0.05, 2.0, or 5.0 mW/cm<sup>2</sup>. (The estimated SAR at 1.0 mW/cm<sup>2</sup> is 0.0047 W/kg.) The results of these two reports show that the intensities producing calcium-ion efflux from chick brain tissue *in vitro* are within the range of 0.1 to 1.38 mW/cm<sup>2</sup> for modulated 147-MHz and 450-MHz carrier waves.

The apparent carrier-frequency independence of effective intensities was tested with a 50-MHz carrier wave, amplitude modulated at 16 Hz. Enhanced efflux of calcium ions occurred within two intensity regions (between 1.44 and 1.67, and at 3.64 mW/cm<sup>2</sup>; SARs were 0.0013 and 0.0035 W/kg, respectively) separated by intensities of no effect, including 0.72 mW/cm<sup>2</sup> (Blackman *et al.* 1980b). These effective intensity values were different from the corresponding values of 147-MHz radiation; thereby indicating a dependence on carrier frequency. In addition this result revealed the existence of more than one range of effective intensities.

The apparent discrepancy in effective power densities at the three different carrier frequencies (50, 147, and 450 MHz) has been resolved by the finding that efflux is dependent on the electric field strength within the tissue and not on incident intensity (Joines and Blackman 1980). The calculation to transform the incident intensity to internal field strength was based on an empirical model described by Joines *et al.* (1981). With the data available at 50 and 147 MHz, the model was used to predict intensities that would produce both alterations and no alterations in calcium-ion efflux; some predictions were tested and found to be valid (Blackman *et al.* 1981). These reports described two intensity ranges that appear effective for enhanced efflux at both 50 and 147 MHz, identified the internal electric field strength rather than incident intensity as the important exposure parameter, and showed the

importance of frequency-dependent complex permittivity values of brain tissue in the conversion of incident intensity to internal field strength. The exposures at 50 and 147 MHz caused no generalized heating of the sample. The maximum temperature rise was calculated to be  $<0.0004^{\circ}\text{C}$ , and SAR calculated at each carrier frequency was  $<0.0014$  W/kg (Blackman *et al.* 1980b).

Subsequent to the critique by Athey (1981) that the simple spherical model used by Joines and Blackman (1980) was too idealized, these authors showed that a layered sphere model produced relationships between incident intensities at 50, 147, and 450 MHz and internal field strengths that were also consistent with the experimental results (Joines and Blackman 1981). The success of the initial, simple models to predict intensity regions of both field-induced efflux enhancement and no enhancement demonstrates the utility of the approach. More refinements in the models are necessary before the experimental situation is realistically described.

Shelton and Merritt (1981), who used different procedures from those described by Bawin *et al.* (1975), Blackman *et al.* (1979, 1980a,b), and Sheppard *et al.* (1979) reported no change in calcium-ion efflux from rat brain. Brain tissue, labeled *in vitro* with radioactive calcium, was irradiated at 1 GHz, pulse-modulated with square waves at 16 or 32 Hz (0.5, 1.0, 2.0, and 15 mW/cm<sup>2</sup>). In a second report, Merritt *et al.* (1982) exposed rat brain tissue labeled *in vivo* with radioactive calcium to microwave radiation, pulse modulated at 16 Hz (20-ms pulse width). The intensities for the 1-GHz carrier frequency were 1 mW/cm<sup>2</sup> (SAR = 0.29 W/kg) and 10 mW/cm<sup>2</sup> (SAR = 2.9 W/kg); and for the 2.45-GHz carrier frequency, 1 mW/cm<sup>2</sup> (SAR = 0.3 W/kg). In addition, animals labeled with radioactive calcium were exposed for 20 min to 2.06-GHz radiation at one of 17 different combinations of intensity and pulse repetition rate: 0, 0.5, 1.0, 5.0, 10.0 mW/cm<sup>2</sup> (SAR was 0.24 W/kg per mW/cm<sup>2</sup>); and 0, 8, 16, 32 Hz (pulse width was 10 ms). After exposure, brain tissue was analyzed for radioactivity. No statistically significant field-induced enhancement of calcium-ion efflux or change of calcium content in the brain tissue was found. The reason for these negative findings is not known; however, the use of square wave rather than sine wave modulation, the different biological preparation, and different medium composition are factors that may have influenced the outcome.

#### 5.7.5.2 Additional CNS Studies

The reports of field-induced calcium-ion efflux from chick brain tissue *in vitro* have led to other CNS studies. Synaptosomes, prepared from rat cerebra and labelled with radioactive calcium, were exposed for 10 min at 0.5 mW/cm<sup>2</sup> to 450-MHz fields, amplitude modulated at 0, 16, or 60 Hz (Lin-Liu and

Adey 1982). Only 16 Hz affected the efflux kinetics of calcium ions. Although the SAR can be estimated as low, an exact value cannot be unequivocally established because the exposure chamber may have been operated in a multimodal condition. (See Weil *et al.* 1981.) Nevertheless, this result is modulation dependent, and it is unlikely that heating is involved as a causative agent.

Similar field-induced efflux enhancement has been reported in a live animal. Adey *et al.* (1982) exposed awake, immobilized cats to 450-MHz fields, amplitude modulated at 16 Hz, at 3.0 mW/cm<sup>2</sup> (SAR = 0.29 W/kg). The release of calcium ions from the cortex was observed as a function of time. Irradiation for 60 min caused episodes of enhanced efflux lasting 20 to 30 min and extending into the postexposure period. Although focusing on a different component of the efflux kinetics than that studied by Lin-Liu and Adey (1982), these results demonstrate that RF fields modulated at 16 Hz can cause changes in both a subcellular membrane system and in the live mammal. Thus, the field-induced phenomenon is not restricted to an avian species nor to *in vitro* preparations.

Recently, Dutta *et al.* (1984) observed field-induced enhancement of calcium ions from cells of human origin. Monolayer cultures of human neuroblastoma cells were exposed for 30 min at ten SARs from 0.01 to 5.0 W/kg to 915-MHz fields, with or without sinusoidal amplitude modulation (80 percent) at frequencies between 3 and 30 Hz. Significant increases in the efflux of calcium ions occurred at two SARs (0.05 and 1.0 W/kg). The increased efflux at 0.05 W/kg was dependent on the presence of 16-Hz modulation but not at the higher value. Exposure at modulation frequencies between 3 and 30 Hz (SAR = 0.05 W/kg) revealed a peak in the response at 16 Hz. Although the effective SAR (0.05 W/kg) for 16-Hz modulation is more than 38 times greater than the SARs for enhanced efflux of calcium ions from chick brain tissue *in vitro*, the low-frequency response pattern was similar to that reported by Bawin *et al.* (1975) and Blackman *et al.* (1979). The relation of enhanced efflux with unmodulated fields at 1.0 W/kg with the effects of modulated fields is not known at this time; however, it is not due to a temperature increase in the sample because enhancement was not found at SARs of 2.0 and 5.0 W/kg.

The effect of modulated RF fields on the EEG was investigated by Takashima *et al.* (1979). Rabbits were exposed 2 h daily for 6 weeks to 1.2 MHz, amplitude modulated at 15 Hz, or 5 MHz amplitude modulated at 14 Hz. Following exposure, the EEG was recorded with scalp electrodes and, when compared to the pretreatment EEG pattern, was found to be altered with enhanced low-frequency components and decreased high-frequency components. The EEG pattern returned to the pretreatment pattern after

several weeks postexposure. Although the electric field intensity was given as 500 V/m, with an error factor as large as 2, the important aspect of the results was that unmodulated fields of similar intensity had no effect on the EEG pattern. The absence of metallic electrodes in the animal during exposure avoids the major criticism of earlier studies that reported field-induced changes in EEG patterns (Gavalas *et al.* 1970; Bawin *et al.* 1973).

Sagan and Medici (1979) studied the influence of 450-MHz fields, sinusoidally amplitude modulated at either 3 or 16 Hz, on locomotor activity in young chickens. The experiments were performed in a plastic, modified Skinner box with light beams to monitor activity; the complete apparatus was placed in an anechoic chamber and exposed in the far field. The authors found no statistically significant change in performance during or immediately after a 23-min exposure at 1 or 5 mW/cm<sup>2</sup> (SAR estimated at 0.2 and 1.0 W/kg). They concluded that the lack of a field-induced response could be due to the use of modulation frequencies not present in the chicken's EEG during performance on the particular (fixed-time schedule) task. An alternative possibility, based on the multiple-intensity ranges observed for field-induced calcium-ion efflux, is that the two intensities used in this study may have been outside the effective ranges.

In summary, four groups (Adey *et al.*; Blackman *et al.*; Dutta *et al.*; Takashima *et al.*) have shown that RF fields, sinusoidally modulated at sub-ELF frequencies, especially 16 Hz, cause CNS changes in different *in vitro* preparations and in the live animal. Many of these studies have been analyzed in reviews (Adey 1981; Blackman *et al.* 1981; Greengard *et al.* 1982; Myers and Ross 1981). It is generally agreed that both the mechanism of interaction and the physiological consequences of these changes are yet to be established.

#### 5.7.5.3 Non-CNS Studies

The effects of exposure of pancreatic tissue and T-lymphocytes to RF fields, sinusoidally amplitude modulated at low frequencies, have been examined. An increase of calcium-ion efflux from rat pancreatic tissue exposed *in vivo* at 2 mW/cm<sup>2</sup> for 1 to 2.5 h at 147 MHz, modulated at 16 Hz (estimated SAR < 0.075 W/kg), has been reported by Albert *et al.* (1980). However, the efflux was not accompanied by a change in protein secretion, which is normally associated with calcium mobilization in the pancreas. The authors attributed the lack of protein secretion to a limitation imposed by the exposure conditions, i.e., a relatively small volume of medium was available to the tissue for normal metabolic activity.

In another *in vitro* assay, the cytotoxic activity of mouse T-lymphocytes was suppressed by a 2-h exposure (1.5 mW/cm<sup>2</sup>) to 450-MHz fields, modulated

at frequencies between 16 and 100 Hz (Lyle *et al.* 1983). Peak suppression occurred at 60-Hz modulation, with smaller effects at 16, 40, 80, and 100 Hz. The exposed cells recovered full cytotoxic activity 12.5 h after the termination of exposure. This result demonstrated an inhibitory but reversible effect on a cell-mediated immune response by modulation frequencies.

#### 5.7.5.4 Sinusoidal ELF and Sub-ELF Signals

Most of the studies reviewed above demonstrate an absolute requirement for low-frequency sinusoidal modulation of the RF carrier wave in order for the signal to be effective biologically. For completeness, several reports are mentioned that describe biological effects of exposure to low frequencies in the absence of an RF carrier wave. Bawin and Adey (1976, 1977) exposed chick and cat cerebral tissue for 20 min to 1, 6, 16, 32 or 75 Hz at electric field gradients of 5, 10, 56, and 100 V<sub>p-p</sub>/m in air. Only two frequencies, 6 and 16 Hz, caused a reduction in calcium-ion efflux at 10 and 56 V/m for the chick tissue, and at 56 V/m for the cat tissue. Because all other combinations produced no field-induced responses, the authors described "amplitude and frequency windows" for calcium-ion efflux. Electric field gradients within the tissue were estimated to be 10<sup>-5</sup> V/m. The field-induced reduction in efflux is in contrast to the enhancement caused by modulated RF carrier waves. Nevertheless, the frequency dependence observed in the two studies was similar, which suggests an interaction with a common substrate as the site of interaction.

Blackman *et al.* (1982) used chick brain to study the influence of 16-Hz signals at 15 intensities between 1 and 70 V<sub>p-p</sub>/m on the efflux of calcium ions. Two intensity regions that included 5, 6, and 7.5 V/m and 35, 40, 45, and 50 V/m caused enhanced efflux. No field-induced effects were seen below (1, 2, and 3.5 V/m), between (10, 20, and 30 V/m), or above (60 and 70 V/m) the two effective intensity regions. Moreover, 1- and 30-Hz signals at 40 V/m caused no change in efflux. This finding is consistent with the reports of multiple-intensity regions of enhanced efflux caused by modulated RF radiation (Blackman *et al.* 1980b, 1981). In addition to the intensity response, the frequency dependence corroborated reports by Bawin and Adey (1976) for low-frequency signals, and by Bawin *et al.* (1975) and Blackman *et al.* (1979) for modulated RF fields.

In these two low-frequency studies, the cause of the slight difference in effective intensities is unknown. The major disagreement in the results of Bawin and Adey (1976) and Blackman *et al.* (1982) is the direction of the change in efflux; the latter authors state that the "cause may be found in the slightly different preparations and procedures used in the two laboratories."

Several research groups have reported biological changes induced by low-frequency, sinusoidally oscillating magnetic fields. The myxomycete *Physarum polycephalum* has a longer mitotic cycle and reduced respiration rate after chronic exposure to 2.0-gauss magnetic fields at 75 Hz (Goodman *et al.* 1979). Human fibroblasts in culture exposed to sinusoidally varying magnetic fields for a wide range of frequencies (15 Hz to 4 kHz) and amplitudes (0.25 to 5.6 gauss) exhibit enhanced DNA synthesis (Liboff *et al.* 1984). Fruit flies (*Drosophila melanogaster*) preferred not to deposit eggs in a 10-gauss, sinusoidally varying 50-Hz magnetic field; similar exposure during development of the egg produced less viable eggs and pupae in the exposed samples than in controls (Ramirez *et al.* 1983). These results suggest that low-frequency, sinusoidally varying fields may alter fundamental biological processes.

Low-frequency, pulsed magnetic fields have also been reported to produce alterations in diverse biological systems. These systems include the developing chick embryo (Delgado *et al.* 1982; Ubeda *et al.* 1983), *Drosophila* egg laying and mortality (Ramirez *et al.* 1983), the de-differentiating amphibian red blood cell (Chiabrera *et al.* 1979), transcription in the *Dipteran* chromosome (Goodman *et al.* 1983), nerve cells in culture (Dixey and Rein 1982), and mouse bone cells in culture (Luben *et al.* 1982). Many of these studies used an intricate pulsed waveform, which has been used in therapeutic devices for bone nonunions. All the studies used pulse repetition rates below 500 Hz, with most below 100 Hz. Recently, Liboff *et al.* (1984) questioned the need for the particular wave shapes because it appears that the essential element is the low-frequency field.

#### 5.7.5.5 Summary

Many reports of effects of RF fields that are amplitude modulated at very low frequencies have not been independently corroborated. The major exception is calcium-ion efflux from chick brain tissue *in vitro* at intensity levels far below those that cause heating. This exception, combined with the results of studies of brain biochemistry and EEGs in animals and with synaptosomes and human neuroblastoma cells in culture, provides evidence that CNS tissue from several species, including human beings, is affected by low-intensity RF fields sinusoidally amplitude modulated at specific low frequencies (Table 5-23). The physiological significance of these field-induced effects is not established.

#### 5.7.6 Unresolved Issues

In addition to the CNS-related changes, amplitude-modulated RF fields have been reported to alter an immune response and a pancreatic tissue function. These reports with diverse biological systems are without apparent connection to each other except for

the physical agent causing the change. The biological effects of frequency-modulated RF radiation, e.g., FM radio signals, are not known. The reports cited above of Merritt and co-workers indicate that pulsed square-wave modulation may not cause calcium-ion efflux, whereas data from the Bawin *et al.* and Blackman *et al.* studies show that sine wave modulation is effective.

No report has yet described a mechanism of action in sufficient detail to identify the conditions necessary and sufficient to explain unequivocally calcium-ion efflux in the brain or the other biological changes caused by modulated RF fields. The response to specific frequencies and intensities is unusual and at present unexplained. This response to amplitude-modulated RF radiation or to sub-ELF signals alone may be a true field effect at a very low SAR and at biologically relevant frequencies, i.e., in the range of frequencies normally present in the EEG. The frequency-specific nature of the responses provides evidence against heat as the underlying cause. The unusual, multiple-intensity-range response challenges standard dose-response analyses, and by its very nature, may prohibit the invocation of threshold levels.

Other areas of unresolved issues include comparisons of CW vs. PW microwaves under identical exposure conditions. Such studies would help determine if the differences seen by Wangemann and Cleary (1976) were due to different exposure conditions or to the irradiation parameters (CW or PW). There is also a paucity of information on the effects of RF radiation at different frequencies, particularly at frequencies of environmental importance. Studies at different frequencies would help to determine the reasons for differences in effects at similar SARs. Such studies might help explain why Wangemann and Cleary (1976) reported serum chemistry changes in rabbits at 0.8 W/kg (2450 MHz), and why Lovely *et al.* (1977) reported no change in serum chemistry values in rats at 1 W/kg (918 MHz).

There are also data such as those reported by Boggs *et al.* (1972) where the results from microwave heating to a predetermined temperature are different from those resulting from the same temperature produced by other means of heating. Perhaps there are differences in the uniformity of heating or in the rate of heating which would account for these differences. In addition, a study by Deficis *et al.* (1979) reported elevated serum triglyceride and  $\beta$ -lipoprotein levels in mice exposed to 2450 MHz at 1.5, 3.3, or 4 mW/cm<sup>2</sup>, but not at 1 mW/cm<sup>2</sup>. Because the exposures were conducted in a multimodal cavity, SAR values were not reported and cannot be predicted. If this study is repeated, particular attention should be given to dosimetry. An alternative is to



make or report dosimetric measurements in the exposure system used.

The reported effects on thyroid function at 3.75 W/kg for 60 h contrasted with no effect at 6.25 W/kg for 16 h (Parker 1973) suggests that the total amount of energy absorbed may also be an important consideration. Additional studies could define further the relative importance of dose rate compared with total dose.

Table 5-23. Summary of Studies Concerning Biological Effects of Low Frequency Modulation of RF-Radiation

Effects	Species	RF (MHz)	Modulation (Hz)	Intensity (mW/cm <sup>2</sup> )	Time (min)	SAR (W/kg)	Reference
Altered calcium-ion efflux in brain tissue <i>in vitro</i>							
frequency specificity	Chicken	147	6-20	1-2	20	0.002*	Bawin <i>et al.</i> (1975)
influence of pH and lanthanum	Chicken	450	16	0.75	20	0.0035*	Bawin <i>et al.</i> (1978)
frequency and intensity specificity	Chicken	147	16	0.83	20	0.0014*	Blackman <i>et al.</i> (1979)
intensity specificity and sample spacing	Chicken	147	9, 16	0.83	20	0.0014*	Blackman <i>et al.</i> (1980a)
theoretical analysis of sample spacing	Chicken	147	16	0.83	20	0.0014	Joines <i>et al.</i> (1981)
intensity specificity	Chicken	450	16	0.1-1	20	0.0005-0.005*	Sheppard <i>et al.</i> (1979)
two intensity ranges	Chicken	50	16	1.5 3.6	20	0.0013 0.0035	Blackman <i>et al.</i> (1980b)
theoretical analysis of RF dependence	Chicken	50 147 450	16	—	20	~0.001	Joines and Blackman (1980); Athey (1981); Joines and Blackman (1981)
test of predictions of theoretical analyses	Chicken	147	16	0.37 0.49	20	0.0006 0.0008	Blackman <i>et al.</i> (1981)
no effect for pulse modulation	Rat	1000	16', 32'	0.5-15	20	0.15-4.35	Shelton and Merritt (1981)
no effect for pulse modulation	Rat	1000 2450	16' 8', 16', 32'	1, 10 1	20	0.29-2.9 0.3	Merritt <i>et al.</i> (1982)
change in calcium efflux kinetics in synaptosomes	Rat	450	16	0.5	10	—	Lin-Liu and Adey (1982)
frequency and intensity specificity in cultured neuroblastoma cells	Human being	915	16	—	30	0.05	Dutta <i>et al.</i> (1984)
Altered calcium-ion efflux in brain tissue <i>in vivo</i>							
no effect for pulse modulation	Rat	2060	8', 16', 32'	0.5-10	20	0.12-2.4	Merritt <i>et al.</i> (1982)
change in efflux kinetics from awake animal	Cat	450	16	3	60	0.29	Adey <i>et al.</i> (1982)
Changed EEG patterns	Rabbit	1.2 5.0	15 14	500 V/m 500 V/m	120 x 6wk 120 x 6wk	— —	Takashima <i>et al.</i> (1979)
No change in behavior	Chicken	450	3, 16	1, 5	23	0.2, 1.0*	Sagan and Medici (1979)
Suppressed T-lymphocyte activity	Mouse	450	16-100	1.5	120	—	Lyle <i>et al.</i> (1983)
Altered calcium ion efflux in pancreatic slices <i>in vitro</i>	Rat	147	16	2	60-150	<0.075	Albert <i>et al.</i> (1980)

\*Est. SAR.

'Square wave.

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