Effects of Electromagnetic Fields on Isolated Nerve and Muscle Preparations

CHUNG-KWANG CHOU, MEMBER, IEEE, AND ARTHUR W. GUY, FELLOW, IEEE

Abstract—**An S-band waveguide exposure system was designed to study the electromagnetic fields on the isolated tissues. The temperature of the exposed tissue was maintained at a constant temperature by circulating temperature controlled Ringer's solution through the waveguide. Isolated frog sciatic nerves, cat saphenous nerves, rabbit vagus nerves and superior cervical ganglia, as well as rat diaphragm muscles were placed in the waveguide either parallel or perpendicular to the electric field of the TE_{10} mode. Compound action potentials of nerves or contractile tensions of muscles were recorded before, during and after the 2450-MHz microwave irradiation. Results showed no significant change in characteristics of nerves or muscles exposed to CW specific absorption rate (SAR) of 0.3–1500 W/kg and pulsed peak SAR of 0.3–220 kW/kg. The effects observed during high-power radiation were reproducible by changing the solution temperature. No direct field stimulation of nerves or muscles was observed during microwave irradiation.**

I. Introduction

ELECTROPHYSIOLOGICAL experiments with proper engineering measurements can provide a method for directly quantifying the reactions of the nervous system exposed to electromagnetic fields and also appear to be useful for establishing thresholds for the EM radiation effect. Johnson and Guy [1] have investigated the effect of 918-MHz EM fields on the thalamic evoked response in cats. The measurable effect of the microwave exposure appeared to be an induced temperature rise in the thalamus region with an associated decrease in latency of the evoked responses. This effect was also reproduced using a heat exchanger with circulating heated fluid, embedded at the base of the cat skull [2]. The effect of EM radiation on the cat spinal cord was also studied [3]. The results showed the same heating effect of the EM radiation.

The above *in vivo* experiments involved exposures of whole animal heads or parts of the body to EM fields. These experiments cannot give a clear indication of what tissues or portions of the nervous system are being affected when exposed to EM fields. Therefore, experiments exposing isolated nervous tissues to EM fields are desired to clarify the effects of EM fields on the nervous system.

McAfee [4] demonstrated thermal stimulation of peripheral nerves using 3-cm and 12.2-cm waves. Romero-Sierra *et al.* [5] reported structural changes in rat sciatic nerve exposed to 27-MHz fields at unknown field intensity. Kamenskii [6], [7] and Rothmeier [8] claimed some low-level effects on frog sciatic nerves. Since the nerves in the above work were isolated in the air during irradiation, it was difficult to determine what the actual temperature changes were in these nerves. Under such conditions SAR could be much higher than for the normal situation in which the nerve is buried in other tissue, and the temperature inside the nerve could be higher than that measured at the surface. The aperture source which Kamenskii used [9] could cause hot spots in the irradiated nerve due to the discontinuities at the edge of the aperture [10].

The effects of microwaves on isolated muscle cells have been studied by Portela *et al.* [11]. They exposed isolated frog sartorious muscles to 10 mW/cm^2, 2.88-GHz microwaves for two hours and found transient changes in muscle membrane resistance, capacitance, ion conductances, velocity of action potential, water permeability, space constant, rise time and fall time of action potential, etc. They also found that the effects on winter frog muscles were more profound than on summer frog muscles. However, no permanent effect was observed. Although the effects observed in Portela's work were related to an incident power density, the actual fields or SAR within the muscle preparation was difficult to predict because of the complexity of the chambers containing the preparation. Also, the temperature of the muscle was not measured, which is critical for this study.

To avoid or minimize the problems in the above studies we have designed a system to study the effects of EM fields on isolated nerves and muscles in the waveguide where the fields could be precisely specified and the temperature of the tissue could be controlled. Since the functions of the nerves and muscles are to transmit nerve impulses and to contract, the compound action potentials of nerves and the contractile tensions of muscles were measured for studying the effects of EM fields on these tissues. Some of the studies have been briefly or partially described in previous publications [2], [12] or presented in symposia [13]–[15]. More details on methods and discussions are presented here.

II. Methods

A. Design of Waveguide Apparatus

Although the most obvious effects of microwaves are thermal in nature, which is desirable in most of the medical

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The authors are with the Bioelectromagnetics Research Laboratory, Department of Rehabilitation Medicine RJ-30, University of Washington School of Medicine, Seattle, WA 98195

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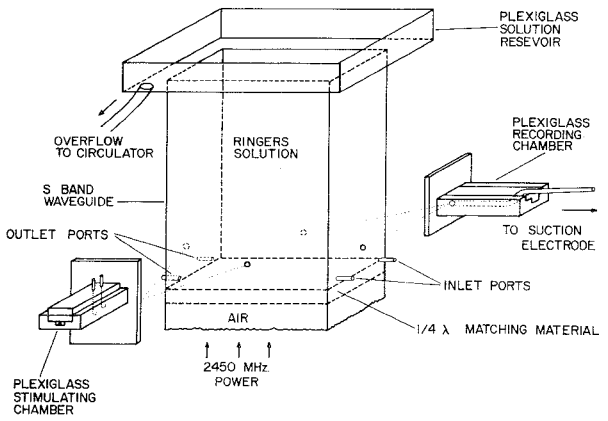

Fig. 1. Waveguide showing matching material, circulating ports, stimulating and recording assemblies.

applications, evidence is also being sought for the explanation of possible nonthermal effects.

The *S*-band waveguide apparatus for *in vitro* experiments was designed to study the effects of microwaves on isolated tissues. With isolated tissue preparations, it is possible to minimize the temperature effect by surrounding the exposed tissue with a temperature controlled bathing solution. Since only small amounts of isolated tissues are pulled through the waveguide in regions of known field configuration and magnitude, quantitative dosimetry is easily achieved by mathematical calculations. Also, with the stimulating and recording apparatus outside the waveguide, artifacts due to field enhancements at electrodes or EM field interaction with recording equipment can be eliminated. In addition, the well-matched waveguide exposure apparatus can simulate a whole range of low- to high-intensity exposure conditions with readily available low power sources, such as diathermy machine.

A silver-plated *S*-band WR 284 waveguide was equipped with inlet and outlet ports for circulating fluids, as shown in Fig. 1. The waveguide was pointed upward with a plexiglas solution reservoir on the top edge of the waveguide to prevent overflow. Four 3-mm diameter holes were drilled in the four walls of the waveguide at a distance 1 cm above the dielectric interface. Four plexiglas chambers were glued onto the outside walls of the waveguide to accommodate stimulating and recording electrodes for isolated tissues placed either parallel or perpendicular to the electric field of the TE_{10} mode. A quarter wavelength dielectric matching material was placed between the Ringer's solution and the air medium. The thickness and the dielectric constant of the material were determined first by measuring the complex dielectric constant ε^* of the Ringer's solution.

The dielectric constant ε' and conductivity σ of the solution can be obtained by measuring the amplitude attenuation and phase shift along a slotted line. The calculated values are $\varepsilon' = 74.3$ and $\sigma = 2.79$ mho/m at 2450 MHz. The corresponding penetration depth of the solution is 1.65 cm.

The above measured values were used to calculate the wave impedances and the guide wavelengths for the design of a quarter wavelength matching window. A half-inch thick (1.27 cm) Emerson Cuming Stycast Hi K 500F dielectric slab of dielectric constant 6 was milled to the size of the inner waveguide and pushed to the level of the circulatory ports. The upper portion of the waveguide was filled with Ringer's solution. Leakage was prevented by sealing it with vacuum grease at the contact between waveguide and the matching material. A voltage standing wave ratio (VSWR) value of 1.08 was measured at room temperature.

Since the 6-cm solution depth is three times greater than the field penetration depth, the solution can be considered as a medium of infinite depth. For small tissue preparations with dielectric properties close to that of the bathing solution, the maximum SAR in the isolated tissue can be calculated by the formula

$$P = \frac{4\alpha(P_I - P_R)}{\rho A} e^{-2\alpha x}$$

where P is the maximum SAR in W/kg, α is the field attenuation constant, P_I is the incident power in watts, P_R is the reflected power in watts, ρ is the density of tissue, x is the distance from the matching material interphase, and A is the cross-sectional area of waveguide.

Experimental measurements using the techniques of implanted probe and thermocouple combination [1] and liquid crystal fiber optic temperature probe [16] were also performed. Because of the rapid heat convection of Ringer's solution, a jelly-simulated Ringer's solution (which is a mixture of 1.06-percent salt, 9.95-percent TX 150, and 88.9-percent water) was used instead. The dielectric constant of the simulated Ringer's solution was controlled by the amount of polyethylene powder and the conductivity by the concentration of salt. The measured SAR values, based on temperature rise, were 10 percent lower than the theoretical values. This may be due to the error in the dielectric properties of the simulated material and the high diffusion gradient in the material. Despite the experimental error, the theoretical calculation gives reasonable SAR values.

B. Isolated Nerve Studies

The effects of EM fields on the compound action potentials of both myelinated and unmyelinated nerves were investigated. Frog sciatic nerve and cat saphenous nerve, which contain mainly myelinated fibers, were used at the beginning of the study. The rabbit vagus nerve was used later since it consists of both myelinated and unmyelinated fibers. Its easy accessibility and lack of branches at the neck region of the animal donor also made it a convenient preparation to use in the experiments.

To investigate the effects of EM fields on the nervous system, the study of the effects on nerve fibers, i.e., axons, is not enough. It is also important to study the effects of EM fields on nerve cell bodies and synapses. The ganglion is a cluster of nerve cell bodies with numerous synaptic junctions in it. Also, the monosynaptic connections, the simple input-output relations and easy accessibility make the ganglion a favored tissue for studying the effects of EM fields on the nervous system.

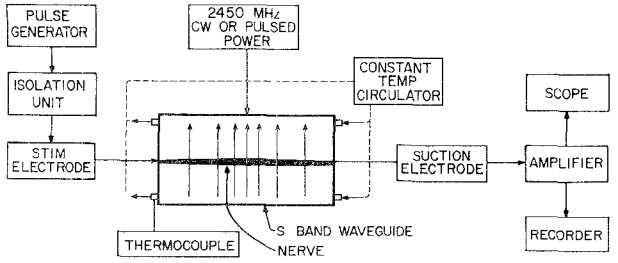

Fig. 2. Apparatus for microwave radiation of isolated nerve *in vitro*.

The superior cervical ganglion is the largest ganglion of the sympathetic trunk. There are both cholinergic and adrenergic nerves releasing acetylcholine and norepinephrine, respectively, at the synapses in the ganglion. The effect of EM fields on the electrical characteristics of the neuron cell body and two types of synaptic transmission can be conveniently studied by using the superior cervical ganglion.

In the study either the vagus or the superior cervical ganglion nerve was pulled through a hole in the waveguide into a stimulation chamber exterior to the waveguide containing a pair of platinum electrodes. The recording electrodes were 0.9-mm polyethylene tubule suction electrodes (Transidyne 1316) in the waveguide, but the platinum wire in the suction tube was always at the outside of the waveguide. Stimulation current pulses of 0.3 ms and 0.3–30 mA were provided to the nerves once every 2 s by a pulse generator (Hewlett-Packard 8005) through an isolation unit (Tektronix 2620).

The temperature of the Ringer's solution was held at 37°C within ± 0.02°C by a constant temperature circulator (Brinkman k-2/RD) with 1.3-l/min circulation rate (Fig. 2). The temperature of the solution was monitored at the outlet of the waveguide with a thermocouple and a digital temperature meter (DORIC DS-100-T3). The temperature at the center of the waveguide 1 cm above the dielectric material during various radiation levels was calibrated by a liquid crystal fiber optic temperature probe [16]. A maximum temperature rise of 1°C occurred during irradiation due to the limited maximum pumping rate of the circulator.

CW (Burdick MW-1 diathermy unit) and pulsed power (Applied Microwave Laboratory AML PH40K) sources, operating at 2450 MHz with incident and reflected powers measured by means of a directional coupler (Microlab FXR 30 dB), two bolometers (Hewlett-Packard 477) and two power meters (Hewlett-Packard 430 C), were used to feed the waveguide. The waveguide, suction electrode, bolometers, and direction coupler were enclosed in a grounded screened chamber to eliminate the interference from power lines and nearby equipment.

The vagus nerves were exposed to each power level for 10-min periods separated by 5-min intervals with no applied power. The superior cervical ganglia were exposed for only 5 min at a time since their viability had a shorter lifetime. In one series of experiments, the isolated preparation was exposed to pulsed power producing a calculated average SAR of 0.3, 3, 30, and 220 W/kg in the tissue. The pulsewidths were 1 and 10 μs with recurrence rates of 1000 and 100 pulses per second (pps), respectively, producing peak SAR in the nerves of 0.3, 3, 30, and 220 kW/kg. The preparations were also exposed to continuous waves producing SAR of 0.3, 3, 30, 300, and 1500 W/kg. Compound action potentials were recorded on a tape recorder (Vetter A) and reduced offline by a computer of average transients (Technical Measurement Corp 400C).

Tests for possible direct stimulation of nerves and ganglia by microwave fields were also performed using maximum available source power, producing a SAR of 1500 W/kg average for CW and 220 kW/kg peak for pulsed fields. The electrical stimulation used for the previous experiments was removed during these tests.

The experiments using frog sciatic nerve and cat saphenous nerve at the beginning phase of the study also followed the same procedure described above, except that the frog sciatic nerve was immersed in amphibian Ringer's solution, which differs slightly in ion content from mammalian Ringer's solution, and the temperature of the solution was set to room temperature instead of 37°C.

C. Isolated Muscle Study

To study the effects of EM fields on muscle contraction, rat diaphragm muscle was used. The diaphragm muscle is a very thin striated muscle, so that glucose and oxygen can diffuse into the muscle very easily to maintain its viability while stable recordings of contractions are obtained. Since the muscle is very thin, the temperature of the muscle can easily be maintained to that of the bathing solution. In addition, the long and easily dissectable phrenic nerve makes the diaphragm muscle a convenient preparation to use.

A tension transducer was constructed to measure the isometric contractile tensions of muscle. A schematic diagram of the device is shown in Fig. 3. The transducer was designed so that during muscle contraction a shutter between a light emitter diode (TIL 23) and a photoresistor (1N 2175) would move so that the infrared light transmission between them would change so as to produce a voltage proportional to tension.

Diaphragm muscle with both a right and left phrenic nerve was isolated from the rat anesthetized with pentobarbital sodium (40 mg/kg, IP). A section of about 4 cm^2 of the sternocostal portion of the diaphragm muscle was dissected while immersed in the Ringer's solution. Strings were sutured on the central tendon and residual intercostal muscles. The strings were then pulled through small holes on the opposite walls of the waveguide perpendicular to the electric field of the TE_{10} mode. Two plexiglas chambers attached to the exterior walls of the waveguide were used to support the muscle under tension, and a third provided an access port for stimulation of the phrenic nerve.

The muscle was fixed at one end and connected to the tension transducer at the other end by the strings which were passed through vaseline filled chambers sealed with vacuum grease (Fig. 4). Current pulses 0.3 ms in width and 0.3–30 mA in amplitude were applied to stimulate the phrenic nerve once every 5 s for single twitch experiments and 15–30 per s for tetanus experiments. The diaphragm muscles were

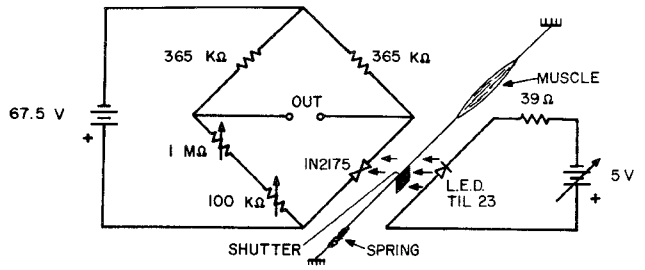

Fig. 3. Tension transducer using light emitter diode (TIL 23) and photo resistor (1N2175) for contractile tension measurements.

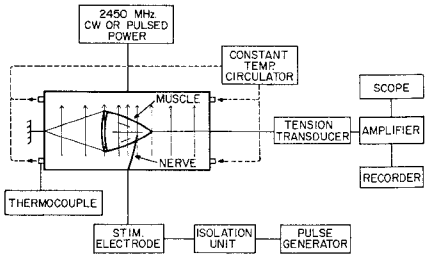

Fig. 4. Apparatus for microwave radiation of isolated muscle *in vitro*.

exposed to each power level for 5 min followed by 5–10-min intervals with no applied power. Tension of 10 single twitches averaged by a computer of average transients and tensions of tetanic contractions were recorded on a *x-y* plotter.

Tests for possible direct influence of EM fields on muscle tension were also performed using maximum available power, producing SAR of 1500 W/kg average for CW and 220 kW/kg peak (1- or 10-μs pulsewidth) for pulsed fields. Electrical stimulation was removed during these tests.

III. Results

A. Effects on Nerve Action Potential

Exposure of the frog sciatic nerve and cat saphenous nerve, as well as rabbit vagus nerve and superior cervical ganglion, did not result in either amplitude or conduction velocity charges of compound action potentials during the time that the temperature was held constant for either CW or pulsed irradiation (Figs. 5–7). At high-applied power, the action potential showed a slight increase in conduction velocity consistent with the 1°C bathing solution temperature rise. This effect could be reproduced by raising the temperature of the solution. A much greater increase in conduction velocity was observed when the circulator was shut off and the temperature was allowed to increase due to radiation [2].

The conduction characteristics of the nerve cells, including axon and cell body, were independent of the electric field polarization. Also, no direct stimulation of nerve cells by either CW (1500 W/kg) or pulsed (220 kW/kg) fields was observed.

B. Effects on Muscle Contraction

Exposure of isolated rat diaphragm muscle to either CW or pulsed 2450-MHz EM fields did not result in either

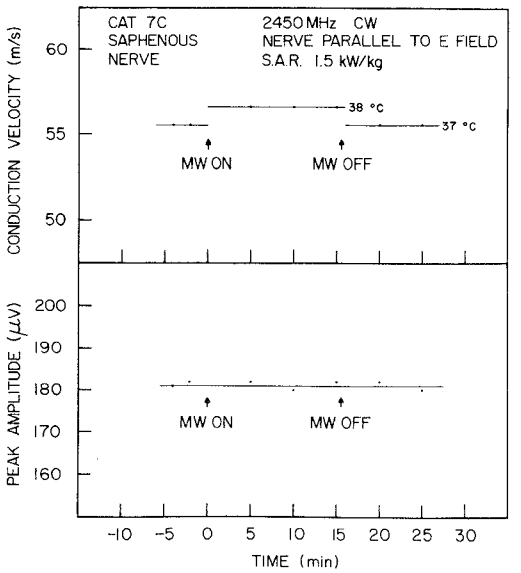

Fig. 5. Amplitude of compound action potential and conduction velocity of cat saphenous nerve exposed to 2450-MHz CW fields.

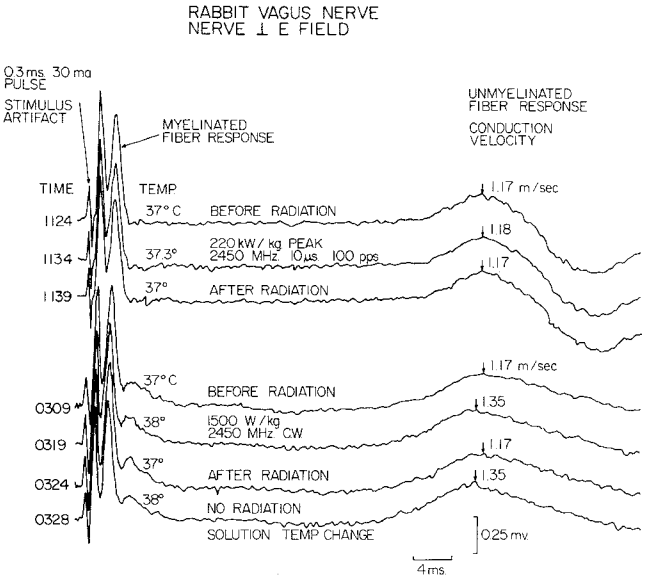

Fig. 6. Compound action potentials of isolated rabbit vagus nerve exposed to 2450-MHz CW and pulsed fields.

amplitude or time course change of contractile tensions when the temperature of the solution was held constant. The top trace of Fig. 8 shows very little change in single twitch tension, even at 220-kW/kg peak SAR. The associated temperature rise was only 0.2°C. During CW 1500-W/kg radiation as shown in the middle trace of Fig. 8, the muscle tensions were smaller in amplitude and shorter in latency. The temperature rise of the solution was 1°C, due to the limited heat removal rate of the circulator. The same effect was also reproduced by changing solution temperature. In both cases, after the temperature was returned to 37°C, the time courses of the twitches were restored, but a complete recovery of the amplitude was not observed.

During the tetanic contraction, the tension usually decreases, probably because of depletion of acetylcholine or

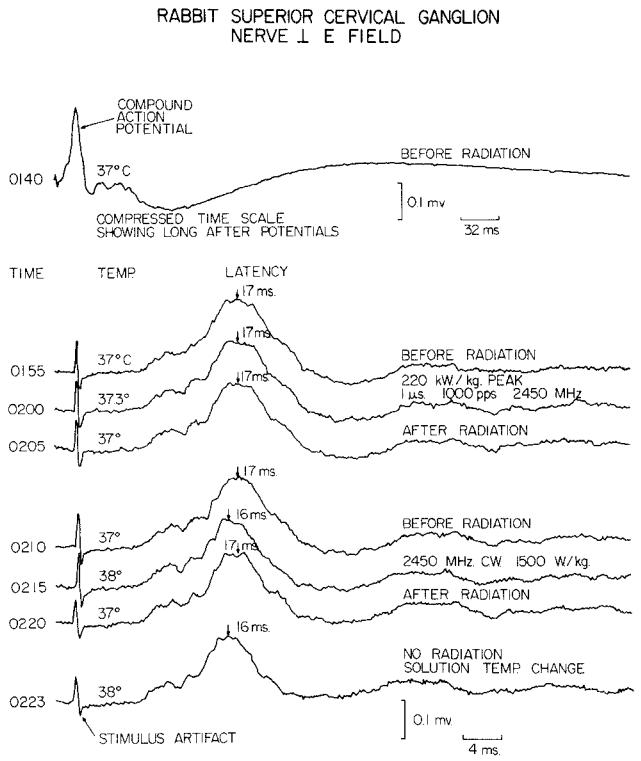

Fig. 7. Compound action potentials of isolated rabbit superior cervical ganglion exposed to 2450-MHz CW and pulsed fields.

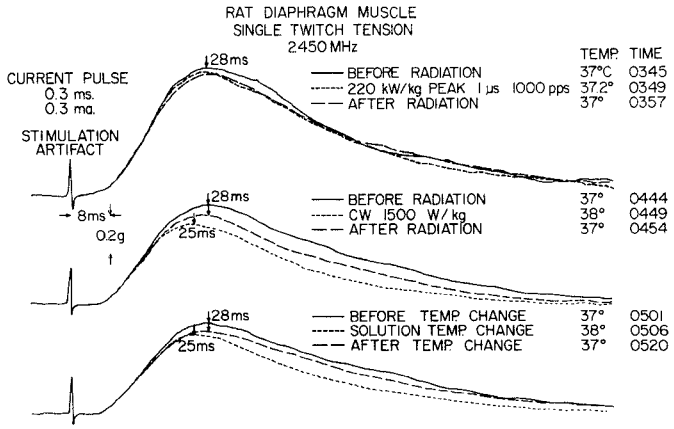

Fig. 8. Single twitch tensions of isolated rat diaphragm muscle exposed to 2450-MHz CW and pulsed fields as compared to the effect of temperature.

fatigue of the muscle. Fig. 9 illustrates that exposure of the isolated diaphragm muscle to either CW or pulsed EM fields at maximum available power during the decreasing phase of the tetanic contraction did not cause any increased or decreased decay of tension.

Tests for possible direct stimulation of muscle by EM fields also gave negative results. No tension change was observed during these tests.

IV. Discussion

When the temperature of the nerves or muscles exposed to EM fields was kept constant, no change in conduction

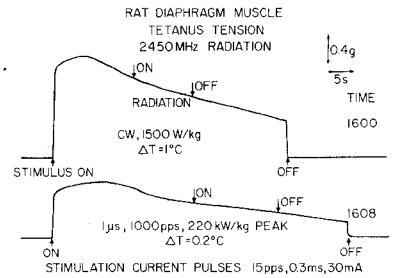

Fig. 9. Tetanus tensions of isolated rat diaphragm muscle exposed to 2450-MHz CW and pulsed fields.

characteristics or contraction was observed. This result is different from the low-level effects reported by Kamenskii [6], [7], Rothmeier [8], and Portela *et al.* [11]. According to Rothmeier, the power measurements conclusively showed that 52 percent of the incident energy was absorbed by the nerve which was suspended in air. In the X-band waveguide with 1-mW power input, calculations may be made to show that the SAR would be as high as 66 W/kg for a 1-mm diameter nerve. This could certainly cause thermal effects when no temperature control was employed. Kamenskii has measured that the temperature of the nerves was increased by 2°C in 30 min, which led to a 16 ± 4.5-percent conduction velocity increase. Our temperature effect study showed a 2.5-percent increase of conduction velocity per degree celsius [13]. As a matter of fact, it is very difficult to measure accurately the temperature of small objects, such as a 1–2-mm diameter nerve, by a thermocouple. For Kamenskii's and Rothemeier's preparation the nerves were also exposed in air so SAR could be very high even though the incident power densities were low. The effect of 1°C temperature change on muscle contraction is shown in Fig. 8. This sensitive temperature dependence may explain the transient effect which Portola *et al.* observed.

Kritikos *et al.* [17] have studied the threshold voltage of nerve excitation by AC fields at 10–30 MHz. The threshold is lowest around 100–200 Hz, and then increases exponentially with the increasing frequency. No nerve was excited by ac fields above 20 kHz. They also observed no attenuation of action potentials and no elevation of threshold voltage in the presence of pulsed ac fields. A dc voltage shift was measured by the intracellular electrode during the presence of ac fields. They referred to this effect as a rectification due to nonlinear electrode processes. This may also explain the microwave induced changes in regular firing rhythms of isolated ganglia [18].

In this study, no direct electric field stimulation of nerve axons, ganglia, or muscles was observed during microwave irradiation. It is proposed by Frey [19] that the RF hearing effect may be due to the direct nerve stimulation based on his failure of observing a cochlear microphonic response from the experimental animals. Recently Chou *et al.* [20], [21] have demonstrated that with the proper recording techniques microwave-induced cochlear microphonics can be recorded from guinea pigs and cats. This finding does not support the possibility of direct nerve stimulation by microwaves. It is also hypothesized [22] that microwave radiation

causes neurotransmitter release by excitation of the nerve remnants in the heart which induced a decrease in heart rate. Based on the compound action potential measurement, we did not observe any action potential from the nerves during both CW and pulsed microwave radiation. The experiments of Clapman and Cain [23] and Liu *et al.* [24] also did not find any effect of pulse microwave radiation on heart rate either *in vitro* or *in vivo*.

A sequence of events occurs before the muscle contracts. Therefore, if differences of contraction had existed with and without irradiation, step by step examination of each individual possibility would have been needed. The first step would have been the removal of nerve stimulation and the substitution of mechanical stimulation on the muscle *per se*. This would have eliminated any change during the synaptic transmission. If changes still persisted, skinned fiber (muscle fiber without sarcoplasmic reticulum) experiments would have been performed to identify the mechanism [25]. A more sophisticated method with better control of ion concentrations would have been needed. Fortunately, no EM field effect on the whole muscle contraction process was observed. There is no need to study the effect of EM fields on each individual process.

At the beginning phase of this investigation, a complete bundle of frog gastrocnemic muscle with innervated tibial and sciatic nerve was isolated and suspended in the *S*-band waveguide with amphibian Ringer's solution circulating at room temperature. The calcaneal tendon was fixed and the other tendon was connected to a 50-g strain gauge. No muscle tension change during the irradiation was observed when the nerve was not stimulated. The tension changed drastically during the CW high-power irradiation since there was no way to control the temperature rise at the center of the muscle bundle. A number of problems were all related to the large size of the gastrocnemic muscle bundle. It made the calculation of muscle power absorption and the measurement of muscle temperature difficult. This bulky muscle mass also restricted the diffusion of glucose and oxygen to the center of the muscle. The insufficient nutrient and oxygen transport caused the decay of tension in several minutes, in contrast to the 3–5-h stable recording of rat diaphragm muscle. Mammalian muscle preparations are more desirable than the poikilothermic muscle preparations because their observed biological effects can be more easily extrapolated to humans.

The muscle was exposed to perpendicular electric fields only, since the length of the stretched muscle was longer than the narrow width of the waveguide. Because of the triangular shape of the dissected diaphragm, only the fibers in the center portion are really perpendicular to the electric fields and other fibers intersect at various angles with the electric fields.

The disadvantage of this exposure apparatus is the narrow operable frequency band. The low limit is the cutoff frequency of the waveguide which is about 2.1 GHz for this size of waveguide. The upper frequency limit is 4.2 GHz, above which TE_{01} and TE_{20} modes can also be excited in the waveguide. Furthermore, the quarter wavelength matching window was designed for 2450 MHz. Deviations from this frequency decrease the energy transmitted into the solution. This apparatus can only be used to study the acute effect of EM fields on isolated tissues, since the isolated tissues are deteriorating preparations. Because the stimulating electrodes are not inside the waveguide, the excitability of the nervous tissue cannot be studied using this waveguide system.

V. Conclusion

CW and pulsed 2450-MHz electromagnetic fields produce no effects other than thermal effect on the conduction characteristics of isolated nerves and ganglia or on the contraction of isolated muscles.

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On the Matching of Transmission Cavity Stabilized Microwave Oscillators

KLAUS SCHÜNEMANN, MEMBER, IEEE, AND REINHARD KNÖCHEL

Abstract—**A matching condition is derived for a transmission cavity stabilized microwave oscillator, which takes account for the power loss in the diode mounting structure. In addition, the power dissipated in the damping resistor—which is commonly used in order to eliminate mode jumping problems—is minimized, thus leading to a useful improvement in both output power and loaded Q-factor of the compound oscillator structure. The effectiveness of the design procedure is finally demonstrated by applying it to a Gunn oscillator realization: at 15 GHz a loaded Q-factor of 6500 could be achieved at the sacrifice of only 2.4-dB overall power loss.**

I. Introduction

COUPLING an oscillator to a transmission cavity of a high unloaded Q-factor is well known as an efficient and simple means of improving the frequency stability. The general features involved in this method have first been discussed by Shelton [1], who introduced a damping resistor in the middle of the half-wavelength long intermediate transmission line in order to suppress unwanted modes of oscillation. The coupling line between original oscillator (diode mounting structure) and stabilizing cavity can otherwise operate as a resonator which introduces two additional potential modes of oscillation.

A theory of cavity stabilization of a microwave oscillator has been given by Ashley and Searles [2] who for the first time developed an IMPATT diode oscillator stabilized by a transmission cavity. Their theory led to very simple and efficient design formulas which can easily be applied in practice. The requirement is that the oscillator sees a matched load, which means the input reflection coefficient of the cavity has to be zero. By further taking the transmission loss of the cavity into consideration as a design objective, the input and output coupling coefficients β_1 and β_2 can be calculated. The obtainable stabilization factor is then related to the transmission loss in a simple and evident way.

The investigation of [2] leaves two problems unsolved.

1) No quantitative instruction has been given concerning the amount of damping required. Following the intentions of [2] (matching of the oscillator by putting the reflection coefficient of the cavity input to zero), one can suppose, however, that the damping resistor should present zero reflection at both ports of the intermediate transmission line.

2) It is not clear whether or not the neglect of the circuit losses of the diode mounting structure is justified.

In this present work emphasis is therefore paid to the solution of these problems.

In a recent study of Nagano and Ohnaka [3] a transmission cavity stabilized oscillator has been presented which violates the design principles of [2] in that the input impedance at the diode port of the cavity has not been matched to the characteristic impedance of the intermediate transmission line. Instead, it has been adjusted for maximum generated power. This, in our opinion, is an unnecessary and unrealistic assumption. It seems to be more adequate to suppose, for a general oscillator structure, that the active

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The authors are with the Institut für Hochfrequentechnik, Technische Universität Braunschweig, Post Box 3329, D-3300 Braunschweig, Germany

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