Open-Ended Coaxial Exposure Device for Applying RF/Microwave Fields to Very Small Biological Preparations

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***Abstract*—An easily fabricated open-ended coaxial exposure device for applying RF/microwave energy to very small biological preparations is described. The device utilizes the fringing fields of a coaxial cable opening into a ground plane. Operation of the device is easily integrated into standard laboratory procedures to observe a biological specimen; monitor temperature; regulate temperature, pH, and pO_2; and record cellular membrane potentials. The electromagnetic field configuration of the device leads to elimination of detectable interaction with microelectrodes. Measured patterns of electric field and specific absorption rate (SAR) are given for a device built with quarter-inch semirigid coaxial cable and operating at 2450 MHz. Comparison is made with previous exposure devices for small biological preparations.**

I. Introduction

A SMALL preparation is often useful in investigations of RF/microwave radiation effects on biological tissues, as well as in many other kinds of studies, at the cellular and molecular level. This is especially true for a preparation comprising a biological specimen and an aqueous medium which imitates the normal chemical environment of the specimen used in studies on mechanisms of interactions. For example, cells isolated from neural and cardiac tissues can be used to study interactions of RF/microwave fields with excitable cells and components of their membranes. The biological specimen may be obtained by surgery, by cell culture, or by a combination of these methods. In some instances the specimen may consist of cell suspensions or unicellular organisms. Although the type and the size of a particular preparation depend on several factors, specimens can have volumes of a cubic millimeter or less. The primary advantage of an isolated preparation is the elimination of the influences of surrounding tissue and other systems of an organism which may serve to mask, or otherwise complicate, responses to the stimulus under study. In other words, a small, isolated preparation simplifies execution of controlled experiments on the tissue being studied.

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There are two essential requirements for an exposure device to be used in a well-designed RF/microwave experiment. One is the delivery of accurately known amounts of RF/microwave energy to the preparation. In biological effects research the amount of absorbed RF/microwave energy is usually expressed in terms of specific absorption rate (*SAR*), in mW/g, which is a measure of energy delivered per unit time per unit mass, or power delivered per unit mass. The second essential requirement is that the RF/microwave fields not directly interact with experimental probes in or near the specimen. Probe interaction could possibly lead directly to artifactual data. Worse, probe interaction could induce an electrical current or cause a temperature increase which may change experimental conditions.

Other requirements of an exposure device for isolated preparations vary with the type of preparation and the type of experiment; however, several general desirable features can be listed. Physical access to the preparation is often required for mechanical and temperature probes, electrodes or microelectrodes, and reference electrodes. Illumination of and observation of a preparation is also often required to measure a biological endpoint or to place microelectrodes in the specimen. Control of parameters such as temperature, pH, and pO_2 necessary for the maintained physiological integrity of the preparation must be provided.

In this paper, we describe an RF/microwave exposure device which utilizes the nonradiating fringing fields of an open-ended coaxial cable for experiments on small and very small biological preparations. The paper is an updated expansion of material which has been given in technical reports [1]–[4] and presentations. Being readily adapted to common laboratory methods for maintaining temperature, pH, and pO_2, the device provides straightforward means for observation of, electrical recording from, and access to a preparation. The device is described in the

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context of its application to aggregates of cultured cardiac cells; however, it should be useful in many applications requiring controlled amounts of RF/microwave energy delivered to very small preparations.

II. Review of Previous Exposure Devices

As background for the development of the open-ended exposure device, other selected exposure devices and their uses are briefly reviewed. The review is a collection of applications to isolated and small preparations. Representative and readily available original references are given for the reader's benefit. The advantages as well as the limitations of the devices for electrophysiological experimentation are noted. Exposure systems in biological effects research have recently been reviewed [5].

Many devices such as waveguides and horn antennas operating at RF/microwave frequencies, say 100 MHz to 30 GHz for discussion, are designed to propagate or to radiate energy by having dimensions similar to a free-space wavelength, 300 to 1 cm. For frequencies in the lower part of this range the devices are fairly large and pose three potential disadvantages for use as exposure devices for biological experiments. First, a power source is required to supply many times the power delivered to the preparation since the preparation occupies only a small fraction of the total exposed volume. Second, there may be undesirable coupling to probes of the preparation since access to the preparation is gained only through the RF/microwave fields. Thirdly, if the device is radiating RF/microwave energy, interference with nearby electronic instruments may occur. Despite the general disadvantage of large RF/microwave exposure devices, they have proven useful in some studies of small preparations.

Having self-contained electromagnetic fields, waveguide, primarily that for *S*- and *X*-bands, has frequently been adapted for use as an exposure device. *S*-band waveguide terminated with an appropriate physiological medium has been used to study rabbit superior cervical canglion [6]; frog sciatic nerve, cat saphenous nerve, and rat diaphragm muscle [7]; frog sciatic nerve [8]; frog heart [9]; alga internodal cells [10]; and Chinese hamster somatic cells [11]. In many cases, an elongate preparation geometry was taken advantage of (1) to align the preparation parallel to the RF/microwave electric field and (2) to stimulate and/or record electrical potentials from portions of the preparation outside the waveguide. Several other studies have used a glass test tube or a pipette inserted into *S*-band waveguide for exposure of, for example, red blood cells [12], [13]. Contraction force and rate have been measured using a strain gauge connected to isolated rat heart atria exposed in specially designed tubes inserted into *S*-band waveguide [14]. Also, *S*-band waveguide with a tapered termination including a sample cuvette has been designed for spectrophotometry of a solution or a cell suspension [15]. Since studies using the latter device did not require electrophysiology, access by electrodes was not provided.

X-band waveguide has been used in studies of darkling beetle pupa, for which unmodified waveguide was intact during exposure [16], and in a study of crab upper leg nerve, for which pairs of inserted nonmetallic high-resistance conductors were used to stimulate and to record nerve potentials [17]. Slotted *X*-band waveguide has been used to expose a chamber containing a cell suspension [18]. In a slightly different use of waveguide, a culture dish containing cells has been placed on the open end of *E*- and *U*-band rectangular waveguides [19]. The approach gave very localized absorption patterns because of the high attenuation at frequencies of 70 GHz and 40 GHz, respectively.

Parallel plates have been used at 960 MHz and 1 GHz to expose isolated preparations placed in the capacitive fields between the plates. Contractions of turtle heart [20], rat heart [21], and rat gut [22], [23] have been observed before, during, and after exposure. A displacement or pressure transducer outside the plates was used to record contractions, and circulating fluid was used to control temperature. The use of Faraday cages to surround the experimental apparatus indicates stray RF/microwave radiation, which is undesirable for electrophysiology experiments.

There have been several RF/microwave exposure devices based on two-conductor transmission lines with cross-sectional dimensions smaller than a free-space wavelength. A microstrip exposure cell has been used to study electrical potentials of alga internodal cells with electrodes at locations where RF/microwave fields are negligible [24]. More often, coaxial transmission line, which offers the additional advantage of self-contained fields, has been used. Coaxial transmission line with circular cross section has been used in a closed configuration for study of EMP-like pulses on the morphology of bullfrog neural tissue [25]. Coaxial connectors have been modified to contain a cell suspension for measurement of dielectric properties [26] but could be used for experimental exposure with temperature control [27]. Cylindrical cavities based on transmission line [28] and reentrant cavity [29] designs have been developed for exposing cell suspensions to high *SAR* while preparation temperature is controlled. Because each device was enclosed for dosimetry and temperature control, access during exposure was not possible.

Stripline, coaxial transmission line with rectangular cross section, has been used to expose a number of different preparations. Designed to have a characteristic impedance of 50 Ω [30], the stripline forms a section of a transmission line circuit in which forward, reflected, and transmitted powers can be measured. Large stripline sections, also known as Crawford cells, have been used to expose chick brains in multiple test tubes [31], rat brain synaptosomes in a filter with holder [32], and pancreatic cancer cells in a tissue culture flask [33]. With the exception of a nonperturbing temperature probe in the last study, access during exposure was not provided.

Small stripline has been used to expose single invertebrate neural preparations during recording of intracellular potentials with microelectrodes [34]–[36]. A similar approach also seems to have been followed by another group

[37]. In the former systems a small chamber containing artificial seawater and a neural specimen was placed in the interconductor space. Electrode access, illumination, and observation were accomplished through holes in the short sidewalls so that standard electrophysiological techniques could be used. Microelectrode(s) (or suction electrode in unreported experiments) and metal reference electrode passed through the interconductor RF/microwave fields but were almost entirely oriented perpendicular to the electric field to minimize coupling. In addition, the reference electrode traveled along the internal surface of the outer conductor except at the preparation chamber. Small artificial potentials on the order of a millivolt referred to the electrode were attributed to the detection of fields by a high-impedance amplifier [34], [35] or a liquid-metal junction [36]. Passive temperature control was accomplished by circulating fluid around the preparation chamber.

III. Open-Ended Coaxial Device

Motivation for development of the open-ended coaxial device came from the need to deliver controlled amounts of RF/microwave energy to preparations of cardiac cells. More specifically, it was desired to expose aggregates of cultured chick embryo cardiac cells in culture medium or balanced salt solution. Since their introduction [38], [39], the aggregates have been found to offer numerous advantages over more traditional cardiac preparations. Cells in aggregates with diameters less than 200 μm are well coupled electrically, resulting in isopotential conditions for frequencies of 0 to 20 Hz [40]–[42]. Having a nonpropagating action potential, the cardiac cell aggregate provides a convenient specimen for studying membrane electrical properties under controlled experimental conditions. The goal was to obtain artifact-free recording of membrane potential from this very small (30–200 μm range), electrophysiologically advantageous preparation during RF/microwave exposure.

The open-ended coaxial exposure device implemented for exposure of cardiac cell aggregates is shown in Figs. 1 and 2. The RF/microwave exposure capability is provided by the open end of standard quarter-inch (nominal 6.4-mm OD) semirigid cable having copper conductors and Teflon dielectric. The center-conductor diameter of 1.8 mm and the outer-conductor inner diameter of 5.5 mm give an interconductor radial distance of 1.85 mm. The 50 Ω characteristic impedance of the cable allows efficient direct connection to other standard coaxial cables and devices.

The semirigid cable opens into a ground plane consisting of the cut surface of the outer conductor, a circular brass hub, and part of the upper surface of a circular brass heating plate. An aluminum ring attached to the heating plate supports a hollow ring put in place at the beginning of an experiment. Holes in the ring allow gas to flow over the preparation for control of pH and pO_2. The temperature of the heating plate and thus of the preparation and the aluminum ring is electronically sensed and controlled. Heating of the plate is accomplished with direct current

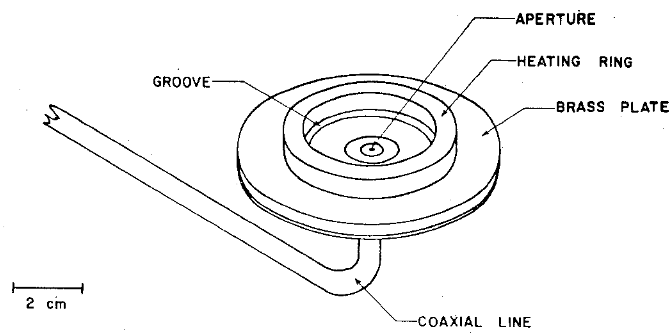

Fig. 1. Isometric projection of open-ended coaxial exposure device. Gas ring, plastic dish, and cable connector are not shown for clarity.

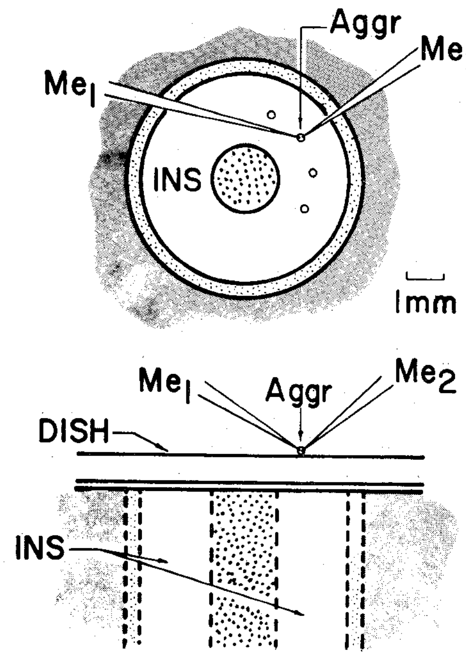

Fig. 2. Top and cross-sectional views of the specimen region. A typical arrangement of cardiac-cell aggregates (Aggr) and microelectrodes (Me_1 and Me_2) is shown over the interconductor dielectric insulator (INS) of the coaxial cable. Each conductor of the coaxial cable and the brass hub are shaded similarly in both views. The ground plane consists of the coaxial cable outer conductor and the brass hub in this region.

passing through high-resistance wires embedded in epoxy in a groove outside the aluminum ring. The heating plate, aluminum ring, and hollow ring along with the temperature-control circuitry makes up a system which has been successfully used to study aggregates of cardiac cells under controlled temperature and chemical conditions. A hubbed brass plate was added to the system to accept the open end of the semirigid cable. The hub fit tightly into an existing 5/8 in (15.9 mm) diameter hole which had been normally used for transillumination of the preparation.

As already done routinely, a 35-mm plastic dish (Falcon® Petri or culture) is placed directly on the heating plate to prepare for an experiment. A groove milled in the heating plate accommodates the ridge on the bottom of the dish and allows the dish to rest directly on the ground plane and the cut cable surface. This arrangement also provides

TABLE I
DIELECTRIC PROPERTIES OF AGGREGATE CULTURE MEDIUM 818A MEASURED AT 2450 MHZ

Temperature (°C)	Relative Dielectric Constant	Conductivity (mmho/cm)	Loss Tangent
24	75.3	30.4	0.296
26	74.7	30.3	0.297
28	73.9	30.1	0.298
30	72.4	29.7	0.301
32	70.5	29.2	0.304
34	67.7	28.0	0.303
36	64.6	27.3	0.310
38	61.3	26.0	0.312
40	58.8	25.0	0.312

TABLE II
DIELECTRIC PROPERTIES OF BALANCED SALT SOLUTION MEASURED AT 2450 MHZ

Temperature (°C)	Relative Dielectric Constant	Conductivity (mmho/cm)	Loss Tangent
24	73.8	30.2	0.301
26	72.3	29.8	0.303
28	68.9	29.0	0.309
30	65.9	28.3	0.315
32	62.8	27.3	0.319
34	58.5	25.9	0.325
36	53.9	24.0	0.327
38	49.1	22.2	0.332
40	45.2	20.8	0.337

additional mechanical stability of the dish. Spheroidal aggregates obtained from tissue culture are at the dish bottom and are bathed in either the medium in which they are cultured or a salt solution. The total volume of aggregates and bathing medium is 4 ml. To eliminate evaporation from the preparation, oil[1] is added to the preparation. Care is taken to add just enough oil to cover the top surface of the bathing medium in order to maintain the layered, planar geometry of dish, medium, and oil.

Two minor modifications of the existing laboratory microscope were required to carry out electrophysiological experiments with the open-ended coaxial exposure device. The exposure device was mounted first to a Plexiglas sheet, which was then fastened to the microscope stage $x-y$ positioning apparatus. Thus, the preparation could be positioned in the microscope field of view by moving the exposure device with the regular stage controls. Second, since transillumination of the preparation was no longer possible because of the coaxial cable, illumination of the field of view was accomplished through the microscope objective lens using an available epi-illumination option. Neither modification interfered with electrophysiological or other experiments on the aggregates.

The deposition of RF/microwave energy as a function of position in the preparation was characterized by measurements of electric field and by measurements of temperature change to derive *SAR*. Measurements were performed using dishes containing either 818A culture medium or balanced salt solution. The dielectric properties of the two bathing media were measured at 2450 MHz for temperatures between 24 and 40°C in separate experiments using a probe method [43]. As shown in Tables I and II, the properties are very similar, with the relative dielectric constant of the 818A culture medium being slightly larger because of its organic content. Because the dielectric properties are so similar and because preliminary field and temperature measurements made in both bathing media were within measurement error, the two bathing media were used interchangeably in subsequent field and temperature measurements.

Two types of probe antennas were used to measure electric fields in the bathing media. A monopole probe antenna, always oriented vertically for measurements, was fashioned from the end of a 0.047 in (1.2 mm) semirigid coaxial cable by removing a portion of the outer conductor and dielectric to expose 2.6 mm of center conductor. A dipole probe antenna, always oriented horizontally for measurements, was made in a similar way from the same semirigid cable stock. The exposed center conductor approximately 4 mm long was bent so that a 1.0 mm length extended directly from the cable and a 3.0 mm length formed a right angle with the cable axis. A short length of extracted center conductor wire was then soldered to the end of the outer conductor in such a way to form a 2.8 mm length in line with the 3.0 mm length of extended center conductor. The distance between the two dipole arms was approximately 0.5 mm. About 6 cm of semirigid cable was left intact on each probe antenna. A male SMA connector was mounted on the end of each cable for connection through low-loss flexible cable to an HP 435A power meter. The semirigid cable section and connector of each probe antenna were held in a vertical position by a micromanipulator for accurate positioning within the bathing medium.

Measurements of RF/microwave electric fields using the two probe antennas were performed at 2450 MHz. The open-ended coaxial exposure device was connected to a power source through a bidirectional coupler used to sample forward and reflected powers and through a double-stub tuner at the device used to minimize reflected power. The exposure device and the micromanipulator arm holding the antenna were carefully leveled to ensure that horizontal movements through the dish was at a constant distance from the dish bottom. All measurements were made when the temperature in the medium had stabilized at 37.0 ± 0.3°C. Horizontal sweeps were made with both antennas, and vertical sweeps were made with the dipole antennas. Horizontal sweeps were along or parallel to either an x axis or an orthogonal y axis corresponding to horizontal movement directions of the micromanipulator. The origin of the axes was directly above the center of the open-ended coaxial cable in the exposure device. For horizontal sweeps, the analog output of the power meter connected to the antenna probe and an analog position signal from a linear potentiometer were connected to an $x-y$ pen plotter to provide plots of received power versus position. For vertical sweeps of the dipole, readings were

[1]Klearol, Witco Chemical Corporation.

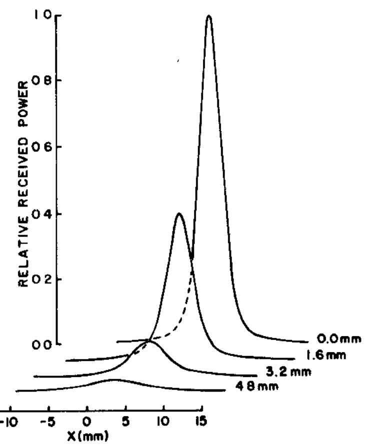

Fig. 3. Relative received power by monopole probe antenna. The monopole was vertical during horizontal sweeps parallel to a diametrical x axis. Number of millimeters beside each plot is the distance of sweep from the x axis. The largest received power over the x axis is arbitrarily assigned the value of one.

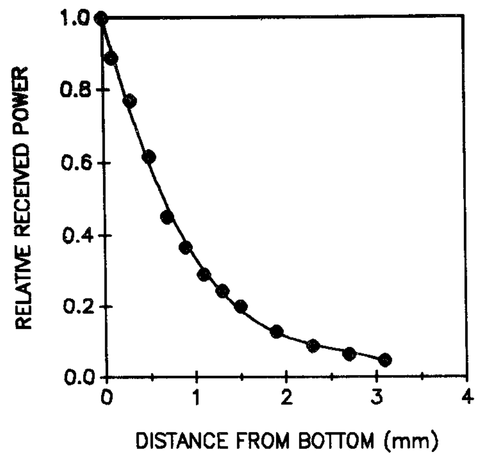

Fig. 5. Relative received power by dipole probe antenna, vertical sweep. The dipole was horizontal at a radial distance of 1.6 mm and parallel to and over a diametrical x axis. The largest received power at the dish bottom is arbitrarily assigned the value of one. The curve is a third-order polynomial fitted to the data.

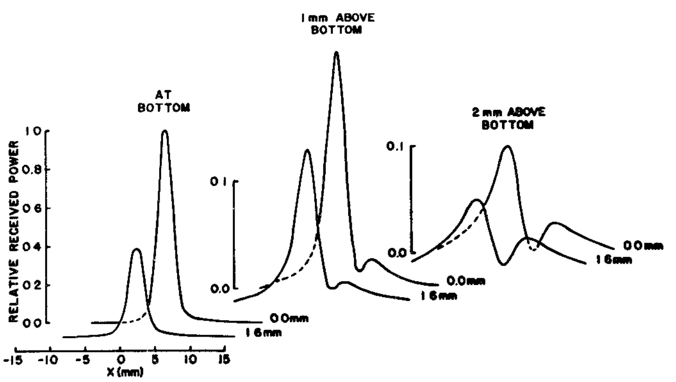

Fig. 4. Relative received power by dipole probe antenna, horizontal sweeps. Note that different scales are used. The dipole was horizontal during sweeps parallel to a diametrical x axis and was parallel to the x axis for all sweeps. Number of millimeters beside each plot is the distance of sweep from the x axis. The largest received power over the x axis at the dish bottom is arbitrarily assigned the value of one.

taken from the power meter scale and the micromanipulator vernier scale.

Typical results of horizontal sweeps with the monopole probe antenna are shown in Fig. 3. Sweeps were made over the x axis and at 1.6, 3.2, and 4.8 mm from the x axis with the tip of the probe antenna 1 mm above the dish bottom. The central peak in received antenna power seen in the sweep over the x axis shows the expected radial symmetry. The same respective power peaks and profiles were seen in sweeps of the monopole probe antenna parallel to the y axis. The spatial resolution of the antenna was apparently not fine enough to detect the central null, which occurs over the exact center of the coaxial cable. The broad central power peak seen in the vertical monopole sweep over the x axis is consistent with the nearly vertical electric field lines over the center conductor.

Typical results of horizontal sweeps with the dipole probe antenna are shown in Fig. 4. Sweeps were made with the dipole at the dish bottom, 1 mm above the bottom and 2 mm above the bottom, as indicated in the respective figure panels. At each depth, sweeps with the dipole arms parallel to the x axis were made over the x axis at 1.6 mm from the x axis. At the dish bottom, a single peak occurs about 1.5 mm to the left of center. At 1 mm from the bottom, there are two peaks: one about 2.1 mm to the left and one about 2.8 mm to the right of center. At 2 mm from the bottom, there are also two peaks: one about 2.7 mm to the left and one about 3.9 mm to the right of center. When two peaks are present, the right peak is smaller.

Again, because of symmetry, we expect the profile of received power from the dipole probe antenna to be the same in all radial directions. As seen in Fig. 4, total symmetry did not occur in sweeps over the x axis. Since the power profile obtained when the antenna was rotated 180° was the mirror image of the respective profile in Fig. 4, the asymmetry was due to imbalance of the dipole probe and not to device asymmetry. The dipole arm soldered to the outer conductor seems to have effectively shielded the input when it was the arm closer to the device center.

Vertical sweeps with the dipole probe antenna gave results consistent with the findings from horizontal sweeps. Fig. 5 shows the results from a vertical sweep of the dipole probe antenna at 1.6 mm from the center. Received power decreases with height, with 50 percent of the maximum at about 0.7 mm above the dish bottom.

Temperature-change measurements for SAR calculations were made using two different temperature probes. The temperature probe used for the majority of measurements consisted of a small thermistor affixed to high-resistivity leads (approximately 80 kΩ/m). The leads were connected to a bridge circuit to provide an analog signal for recording. Later, a Vitek model 101 electrothermia monitor with an RF/microwave-transparent probe was also used. For both probes a chart recording of the temperature was analyzed to determine the rate of temperature rise at the onset of a known level of RF/microwave power

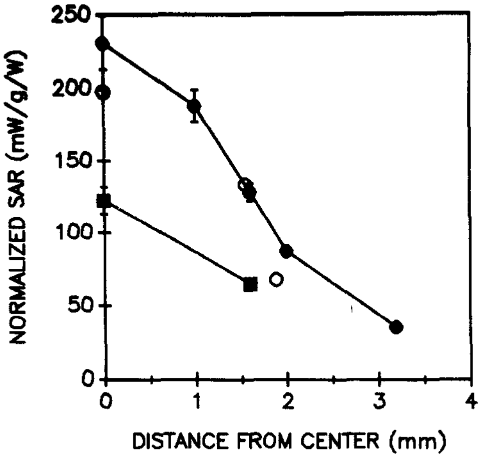

Fig. 6. Specific Absorption Rate (*SAR*) as a function of radial distance. Solid circles represent values at the dish bottom obtained from a custom thermistor circuit; solid squares, at 1.2 mm above the dish bottom. Open circles represent values at the dish bottom obtained from a Vitek model 101. *SAR* has been normalized to the net input power. Bars show standard deviations around an average of at least three separate measurements at each point.

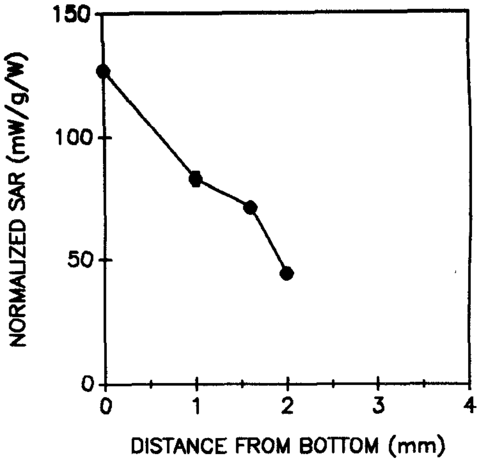

Fig. 7. Specific Absorption Rate (*SAR*) as a function of vertical distance at a radial distance of 1.6 mm. *SAR* has been normalized to the net input power. Each point represents an average of three measurements. Bars show standard deviations, which are smaller than half-a-symbol width for all but the point at 1 mm.

to the exposure device. The *SAR* was calculated from this rate by using a standard formula [44] and normalizing to the net input power.

The *SAR* was determined for a number of points in the bathing medium to provide radial and vertical profiles, as shown in Figs. 6 and 7, respectively. The solid symbols in the two figures represent *SAR*'s derived from thermistor-circuit data; the open circles in Fig. 7 denote values from the Vitek model 101. The generally lower *SAR* values derived from the Vitek model 101 are consistent with its larger probe size, about 1.1 mm. The results from the Vitek probe, which has proven RF/microwave-field insensitivity, showed that the thermistor-circuit measurements were reliable despite some practical problems with electrical noise from the unprotected submerged thermistors.

Figs. 6 and 7 show the expected declines in *SAR* with radial distance and distance above the bottom. In Fig. 6 we see that the *SAR* at the dish bottom decreases from 231 mW/g per watt of net input power at the center to 35 mW/g per watt at 3.2 mm from the center. At 1.2 mm above the bottom, the *SAR* decreases from 122 mW/g per watt of net input power at the center to 65 mW/g per watt at 1.6 mm from the center. At the latter height, the *SAR* at 3.2 mm from the center was not determined because temperature increases were too small to be measured reliably. In Fig. 7 we see that *SAR* at a radial distance of 1.6 mm decreased from 127 mW/g per watt of net input power at the dish bottom to 44 mW/g per watt at 2 mm from the bottom.

Microelectrodes pulled from glass micropipettes [45], [46] have been used in studies of RF/microwave biological effects in isolated [9], [24], [34]–[37] and whole-animal [47]–[49] preparations. In many studies, especially those using isolated preparations, precautions have been taken to record electrical potentials at sites outside the RF/microwave fields. However, the strategy is not always possible and induced potentials have been observed in microelectrodes in RF/microwave fields [24], [34]–[36]. Glass microelectrodes have been shown not to disturb RF/microwave heating patterns in tissue [44], but effects of RF/microwave fields on microelectrodes have not been extensively studied.

The localized fields of the open-ended coaxial exposure device provided the opportunity to observe the electrical potential of a microelectrode during exposure of its tip. Microelectrodes filled with 2.5 M KCl and having dc resistances of 37 to 67 MΩ were positioned in such a way that their tips were within 50 μm of the bottom of the dish containing 4 ml of bathing medium. Two microelectrode tips were positioned approximately orthogonal to each other over the edge of the center conductor of the coaxial cable, a site selected because of its clear definition and larger RF/microwave fields. The arrangement resulted in one microelectrode tip being approximately parallel to the local electric field lines and the other tip being approximately orthogonal to them.

For experiments on microelectrodes, power to the exposure device was continuous wave (292 mW/g *SAR*) or modulated either as 12.5 μs pulses at a repetition rate of 1000/s (200 mW/g peak, 2.5 mW/g average) or as a 300 Hz square wave with a 50 percent on–off ratio (274 mW/g peak, 137 mW/g average). High-gain recordings from microelectrodes were analyzed on an HP model 5420 digital signal analyzer. For each microelectrode tip orientation, no additional signal at either the modulation frequency or at respective harmonics was detected in triggered oscilloscope traces or in auto- and cross-correlation power spectra from the signal analyzer. Besides the inherent noise of the microelectrode and the preamplifier [50], a major component of microelectrode noise was the expected power line frequency of 60 Hz. Fig. 8 illustrates signal analyzer results for both microelectrode orientations for 300 Hz modulation. Although there is a 300 Hz component in the parallel microelectrode signal, it was present at

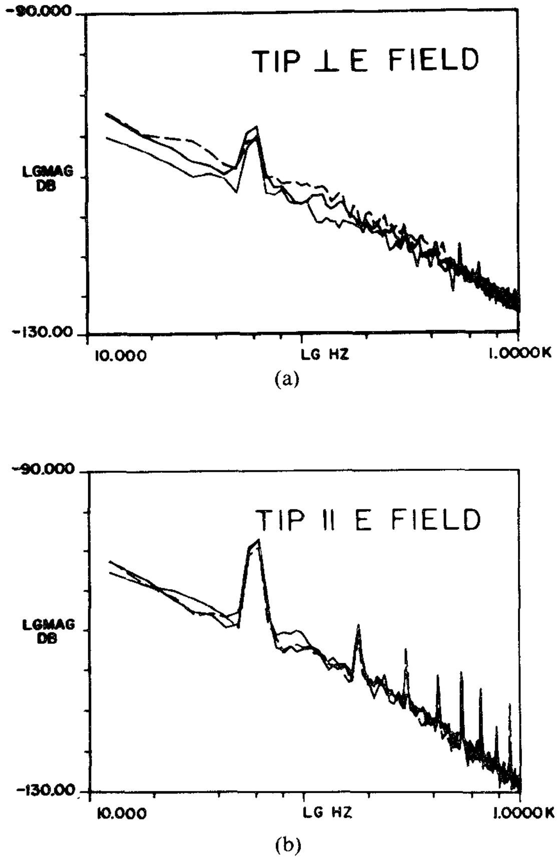

Fig. 8. Voltage noise spectra in microelectrodes with tip regions simultaneously exposed to RF/microwave fields modulated at 300 Hz. Each panel shows noise power magnitude (dB V^2/Hz) versus frequency as plotted by the digital signal analyzer. The local SAR was 274 mW/g peak and 137 mW/g average. Dashed traces represent voltages recorded during exposure; solid traces, before exposure; and dash–dot traces, after exposure. (a) The tip is approximately perpendicular to the electric field lines; $R_{DC} = 38$ MΩ. (b) The tip is approximately parallel to the electric field lines; $R_{DC} = 47$ MΩ.

the same level with and without RF/microwave power applied and was one of several odd-numbered harmonics of 60 Hz in the particular recording. The lack of an RF/microwave signal in the microelectrode experiments was consistent with the absence of artifact in recordings of membrane potential made during several dozen experiments on cardiac-cell aggregates.

The open-ended coaxial exposure device has additional advantages over alternative exposure methods which were assessed using probe antenna measurements. For the same net input power and the same dish configuration, there was more energy delivered to the preparation by the open-ended coaxial exposure device than by slots in WR-284 waveguide or RG-8 coaxial cable. In addition, the slot sizes required to achieve delivered power levels comparable to those attained in the open-ended coaxial exposure device resulted in RF/microwave fields beyond the preparation. The extended field configuration of the slotted devices was not desirable for two reasons. First, expected coupling to microelectrodes and to sensitive preamplifiers was undesirable for the reasons described in the introduction. Secondly, the RF/microwave fields in the preparation were found to be substantially influenced by the surroundings and could have become difficult to control and might have led to discrepancies in the determination of effective levels of the RF/microwave energy.

IV. Discussion

The open-ended coaxial exposure device provides a way in which to monitor physiological processes of a very small biological specimen during RF/microwave exposure. Observation of the specimen with a microscope and recording of biopotentials with microelectrodes are easily accomplished using the device. The essentially horizontal radial electric field lines over the dielectric of the coaxial cable provide a method to orient the specimen and the probes of the specimen to the experimenter's advantage. The device is especially applicable to biological specimens much smaller than a wavelength and is suitable for specimens as small as a single 10 μm diameter cell. Once the energy deposition has been characterized for a particular device and an RF/microwave frequency, exposing a small specimen with a known amount of energy requires only placement of the specimen at a specific site in the medium. Accurate positioning of the specimen on the dish bottom at a selected site over the coaxial cable dielectric is easily accomplished with the aid of a standard graticule in the microscope eyepiece. The bathing medium absorbs power mainly in the region over the coax opening and acts as a thermal mass to minimize specimen temperature changes.

The lack of detectable electric potentials in standard high-impedance glass-pipette microelectrodes during exposure is a particular advantage in making electrophysiological measurements at the cellular and membrane levels. Although not all modes of interaction between the RF/microwave fields and the microelectrodes were tested, the absence of induced potentials during exposure is a strong indication that any interaction is inconsequential in electrophysiological experiments. Since no dc offset was seen during exposure, it appears that the open-ended coaxial exposure device provides greater isolation between RF/microwave fields and microelectrode recording equipment than does a stripline device. The difference in isolation is probably due to the fields of the open-ended coaxial device being more localized to the microelectrode tip region, possibly indicating a very minimal effect on the electrolyte-to-electrolyte junction at the tip. The localized fields also allow less restricted access to the preparation and greater control of preparation temperature. The absence of RF/microwave fields beyond the preparation is always a distinct advantage in any study utilizing electronic instrumentation.

Although the device built and tested was based on quarter-inch coaxial cable, the principles of operation remain the same within the normal frequency range of a particular cable of any size. Thus, smaller cables could be used to expose smaller specimens; larger cables, larger specimens. However, there are practical limitations based on known cable characteristics [51], [52]. For smaller cables, there is greater attenuation of propagated energy. The higher insertion loss would cause additional heating of the cable and would require higher source power for the same normalized *SAR*. Power rating and electrical breakdown may become restrictive. For larger cables, there are upper frequency limitations for only the TEM mode to propagate. Although propagation of higher modes may not decrease coupling to the preparation, fields within the preparation may not be as easily analyzed or measured. A more important consideration in this case may be the deviation from a 50 Ω impedance which allows the connection to standard coaxial devices.

In general, standard laboratory equipment and experimental procedures do not have to be altered to accommodate the addition of RF/microwave exposure capability provided by the open-ended coaxial exposure device. Examples of minor equipment modifications required in our specific application are described above. Two precautionary procedural measures, stemming from observations made with the probe antennas, were taken. Received powers from the probe antennas were affected by the amounts of bathing medium and oil in the dish, with the maximum effect of either fluid about 20-percent for volumes which might be used in experiments. The differences in received power could have been due to changes in antenna characteristics and not to changes in the fields themselves. However, to reduce the potential variability of delivered RF/microwave energy introduced by these factors to a low level, 4 ml of bathing medium and enough oil to cover only the top of the medium were always used in experiments. The medium depth of approximately 4 mm, more than four times the inner-conductor radius of 0.9 mm, should result in any small depth variations having a negligible effect on the RF/microwave fields [53].

The profiles of received powers from monopole and dipole probe antennas agree well with profiles of *SAR* determined from localized temperature increases. All three measurements are related directly to the square of the RF/microwave electric field: monopole power ideally to vertical component, dipole power ideally to horizontal component, and *SAR* to resultant field. Of course, sensitivities of actual antennas and temperature probes differed from the ideals, and their spatial resolutions were limited. The squared vertical electric field (Fig. 3) and the *SAR* (Fig. 6) fell off as a function of radial distance, with the largest gradient 1 to 2 mm from the center. The squared horizontal electric field (Fig. 2) was largest over the interconductor space of the coaxial cable and then fell off as a function of radial distance. Over the center conductor of the coaxial cable, the smaller gradients of the squared vertical electric field as well as the smaller values of the squared horizontal electric field are consistent with the essentially vertical electric field over the center conductor.

It is interesting to compare results of the RF/microwave-field and *SAR* measurements of the open-ended coaxial exposure device with published calculated values. One analysis is of a coaxial line which opens into an infinite ground plane contacting a half-space of tissue [54]. The majority of results are for 2450 MHz and material with dielectric properties similar to the bathing media used here. In planes 0.1 mm from the coaxial opening, a peak in the squared electric field is calculated to exist over the edge of the inner conductor. For planes further from the opening the peak is smaller, becomes less sharp, and shifts to locations closer to the center of the opening. For all cases investigated, the calculations show that 90-percent of the power delivered by the coaxial opening is absorbed within a hemisphere with a radius equal to the inner radius of the outer conductor. Another analysis is of the open end of a quarter-inch coaxial line immersed in distilled water [53]. The resultant electric field on the aperture has its major peak at 1.2 times the inner-conductor radius. Fields are found to be negligible beyond the outer conductor. Dielectric discontinuities farther away than six times the inner-conductor radius change capacitance less than 1 percent.

The calculated fields of the two analyses are consistent with the peaks and gradients measured for the open-ended coaxial exposure device. The measured peaks at radial distances of 0 and 1.5–2.1 mm using monopole and dipole antennas, respectively, would likely have a resultant peak in the 0.9–1.1 mm range that the analyses predict. The declines of received antenna power and *SAR* with increasing radius and height are qualitatively the same as calculated. Although the plastic dish used in the exposure device is not represented in either analytical model, the similarities in results constitute a reassuring check on both approaches.

We note studies using specialized RF/microwave exposure devices which have some of the features of the open-ended coaxial exposure device. Although not designed for electrophysiological studies, the open-ended waveguide used to expose monolayer cell cultures resembles the basic configuration of the exposure device [19]. In another type of application, a 3.58-mm semirigid coaxial cable in contact with the tissue has been used to deliver energy to the pacemaker region of isolated and *in situ* frog hearts [55]. Microelectrode recordings have recently been made from chick embryo hearts during RF/microwave exposure [56]. The exposure device consisted of a flared, open end of coaxial cable over which a copper heating plate with a rectangular slot was placed. RF/microwave fields delivered by both coaxial devices were isolated from electrodes, instruments, and other experimental probes. The latter coaxial apparatus is similar to the exposure device presented here also in using the open end of a coaxial cable, in containing the preparation in a plastic dish placed over the open end, and in using a heating plate under the dish to maintain a physiological temperature. However, the apparatus differs in that the dish is farther from the coax opening and that there is an intervening slot. These two differences probably cause a lower coupling efficiency of fringing fields to the preparation and certainly increase the difficulty in analyzing the RF/microwave fields in the bathing medium.

V. Conclusions

The open-ended coaxial exposure device meets all of the requirements for a device to expose a small, or very small, biological preparation in a controlled manner. The particular advantages over other previously used devices are the easy access to the preparation during exposure and the minimal, if any, coupling of RF/microwave fields to microelectrodes used in electrophysiological studies. The device should be generally applicable to studies of biological effects of RF/microwave energy. By having an easily determined pattern of energy within the preparation, use of the device aids in the determination of the RF/microwave "dose" applied. Since the device is usable over a wide RF/microwave frequency range, the same preparation can be exposed at various frequencies without disruption of delicate experimental arrangements.

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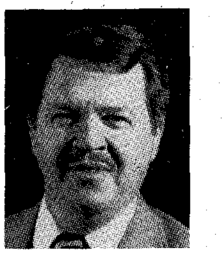

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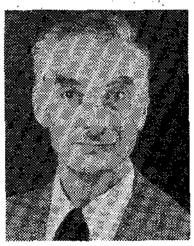

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